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CONFEZIONAMENTO ALIMENTARE

Primo Convegno
Nazionale

Shelf-life degli alimenti confezionati

Milano 11-13 giugno 2003

a cura di
LUCIANO PIERGIOVANNI e SARA LIMBO

Special Issue

ITALIAN JOURNAL
OF
FOOD SCIENCE

SIRAP-GEMA CHIRIOTTI EDITORI SIPA

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CONTENTS

AUTHOR INDEX X

INTRODUCTION ... XV

SESSION I: SHELF LIFE MODELLING

Main lecture: Prof. Kit K.L. Yam - Rutgers University, NJ - USA

An overview of shelf life models for packaged foods 3
K.K.L. Yam

LECTURES

The sensory and consumer approach to the shelf life of foods 5
S. Porretta

Effect of composition and viscosity on carotenoid oxidation rate 7
L. Manzocco, E. Venir, M. Anese, M.C. Nicoli, E. Maltini

Effect of the physical state of lipids on shelf life of frozen foods 9
S. Calligaris, L. Manzocco, M. Munari, M.C. Nicoli

The prediction of shelf life of cheese on the basis of storage temperature 11
S. Parisi

Effects of storage temperature, oxygen level and lightness on shelf-life of brown parboiled rice 20
M. Zardi, S. Limbo, G. Aletti

Shelf-life prediction of sliced fresh apples 29
P. Rocculi, M.A. Del Nobile, A. Bacci, M. Dalla Rosa

Instrumental texture determination of Ricotta cheese during storage 41
L. Piazza, M. Bartoccini, S. Barzaghi

Modelling the barrier properties of nylon film destined for food packaging applications 53
G.G. Buonocore, M.A. Del Nobile

POSTERS

A ready-to-eat food: steamed carrots in protective atmosphere packages 64
A. Langella, F. Villani, P. Masi

Time-temperature exposure of fresh milk during commercial life 70
M. Riva, V. Crepaldi

Accurate determination of pressure, composition and amount of unfilled volume (UFV) in packaged foods. Set up of a new quasi-automatic device 79
C. Spreafico, M. Squarzoni, L. Piergiovanni, P. Maiocchi

Accelerated shelf life testing: possible applications of a new instrument (Oxitest) to oxygen sensitive food products 85
L. Indino, L. Piergiovanni, P. Maiocchi

SESSION II: NEW TECHNOLOGIES FOR EXTENDING SHELF-LIFE

Main lecture: Prof. Joseph H. Hotchkiss - Cornell University, NY - USA

Current and future packaging approaches to extended shelf life of foods 97
J.H. Hotchkiss

LECTURES

Product innovation in food science: ready to eat products 98
V. Nicolais, F. Villani, P. Masi

Behaviour of film wrapped Ponkan mandarins treated with imazalil and sodium carbonate 109
S. D'Aquino, A. Palma, G. Lanza

Biopreservation of fresh vegetables: microbiology and ecology 121
G. Scolari, M. Vescovo

Polysaccharide-lipid edible coating as water vapour barrier: application to bakery products 130
A. Sensidoni, B. Bravin, D. Peressini

The influence of using different packaging on the quality decay kinetics of "Cuccìa" 132
G. Muratore, C.M. Lanza, M.A. Del Nobile, M. Leonardi, P. Tamagnone, C. Nicolosi Asmundo

Effectiveness of pasteurization on *Alicyclobacillus acidoterrestris* spores in the presence of low molecular weight chitosan 142
P.M. Falcone, D. Campaniello, C. Altieri, M. Sinigaglia, M.R. Corbo, M. Anese, M.A. Del Nobile

Superficial treatment using plasma processes on polymer films used for packaging 152
L. Laguardia, A. Cremona, E. Vassallo, R. De Mitri

Study and development of an antimicrobial packaging system based on the release of silver ions 166
M. Cannarsi, C. Altieri, M.A. Del Nobile, P. Favia, G. Iacoviello, R. D'Agostino

Potentiality of PEEKWC as a new material in food packaging 173
A.M. Torchia, G. Clarizia, A. Figoli, E. Drioli

Study of an innovative PET (Polyethylene terephthalate) packaging for mayonnaise and evaluation of product shelf-life 185
A. Sensidoni, M. Leonardi, A. Possamai, P. Tamagnone, D. Peressini

POSTERS

Prediction of water permeability of flexible multilayer films intended for food packaging applications 196
G.G. Buonocore, D. Dainelli, M.A. Del Nobile

The influence of using packaging films with different permeabilities on the quality decay kinetic of plum tomato (Pomodorino Datterino) 199
G. Muratore, M.A. Del Nobile, L. Bongiovanni, G.G. Buonocore, C.M. Lanza, C. Nicolosi Asmundo

Study of apple slice preservation by combined methods technology 204
P. Rocculi, S. Romani, C. Lisi, M. Dalla Rosa

Quality evaluation of pastries with an almond paste base 212
A. Baiano, G.G. Buonocore, V. Marchitelli, M.A. Del Nobile

Controlled release of active compounds from antimicrobial films intended for food packaging applications 216
A. Conte, G.G. Buonocore, L. Nicolais, M.A. Del Nobile

Biological oxygen scavengers for the maintenance of brief maturation dairy products 219
M. Cannarsi, C. Altieri, M.R. Corbo, M. Sinigaglia, M.A. Del Nobile

Antimicrobial and antioxidative packaging material incorporating nisin and α-tocopherol to extend shelf life of perishable foods 221
C.H. Lee, D.S. An, S.C. Lee, H.J. Park, D.S. Lee

Performance comparison of PVC and PE cling film by means of shelf-life evaluation tests ... 223
S. Colli, S. Pozzo, M. Piana

Performance evaluation of active EPS tray for fresh bass fillet 228
F. Mostardini, M. Brazzoli

SESSION III: SHELF-LIFE TESTING

Main lecture: Prof. H. Hofstra - TNO Netherlands - AJ Zeist - The Netherlands

An overview of methods and procedures for shelf life testing 235
S. Notermans, M. de Nijs, H. Hofstra

LECTURES

Shelf life monitoring and modelling by e-nose and image-analysis 237
M. Riva, S. Mannino

Shelf life study of packed industrial Ricotta cheese 252
P.M. Toppino, L. Campagnol, D. Carminati, G. Mucchetti, M. Povolo, S. Benedetti, M. Riva

Volatile compounds as indicators of microbial spoilage 267
M.L. Puglisi, M. Gullo, L. De Vero, P. Fava

Influence of the oxygen barrier properties of the package on the shelf life of extra virgin olive oil 269
G. Gambacorta, M.A. Del Nobile, P. Tamagnone, M. Leonardi, E. La Notte

Water vapour barrier properties of biodegradable films 279
C. Giardi, G.G. Buonocore, L. Nicolais, M.A. Del Nobile

Shelf-life of *Brassica Rapa* L. var. *Silvestris* in protective atmosphere packaging 287
V. Nicolais, T. Maturi, A. Langella, A. Romano, F. Villani, G. Barbieri, P. Masi

Blood orange slices minimally transformed: chemical, microbiological and sensory studies ... 298
C. Caggia, P. Rapisarda, C.M. Lanza, S.E. Bellomo, P. Pannuzzo, M. Lo Bianco, C. Restuccia, C. Spampinato, A.G. Sciuto

Combined technologies to improve quality of reconstituted apple cubes during processing and storage 315
P. Pittia, G. Sacchetti, D. Mastrocola

Specific spoilage organisms and shelf life of green olives directly fermented in ready-to-sell packages 327
A.D. Romano, G. Muratore, C.L. Randazzo, M. Di Salvo, C. Caggia

Influence of temperature on the quality factors of shredded carrots 336
E. Torrieri, M.J. Sousa, A. Horta, P. Masi, J. Kerry, F.A.R. Oliveira

POSTERS

Evaluation of HMF as a marker of the shelf-life of honey 349
B. Fallico, M. Zappalà, E. Arena, A. Verzera

Non-conventional analytical indices to evaluate the quality of the covering oil during the shelf-life of preserved vegetables 353
T. Gomes, A. Baiano, F. Caponio

Effect of superheated water cooking on some textural characteristics of cuttlefish (*Sepia officinalis*) 357
D. Barbanti, R. Massini, E. Chiavaro, M. Rinaldi

The influence of water activity on physico-chemical characteristics of edible coatings 361
A. Conte, C. Giardi, G.G. Buonocore, M.A. Del Nobile

Influence of packaging material on bread characteristics 365
M.A. Pagani, M. Lucisano, M. Mariotti, S. Limbo

Use of recent analytical parameters to evaluate the quality of refined oils used as a covering medium for canned fish 369
F. Caponio, A. Pasqualone, T. Gomes

Potential use of "Ponkan" and "Page" mandarins as minimally processed fruit 373
A. Palma, S. D'Aquino, V. Astone, P. Rapisarda, M. Agabbio

Method for evaluating the barrier properties of food packaging versus external pollutants 377
M. Baronciani, M. Amicabile, L. Tinelli, V. Rocchelli

Shelf-life study of Taleggio cheese packed with paper or in modified atmosphere and comparison of analytical methods for ammonia detection 381
P.M. Toppino, M. Riva, E. Cori, L. Campagnol, L. Passolungo, C. Pinelli

Shelf-life extension of minimally processed artichokes 385
A.G. Fiore, M. Anese, M. Sinigaglia, T. De Pilli, A. Derossi

Study on sorption of flavor compounds from wine by polyethylene film 390
G. Muratore, N. Guarrera, M.A. Del Nobile, P. Fava, C. Nicolosi Asmundo

Preliminary study for the extension of the shelf-life of a typical Sardinian product, "Pardulas", by means of active packaging 394
P. De Regibus, G. Vestrucci, M. Zappa

Microbiological aspects of horsemeat packed in modified atmosphere 396
L. Franzetti, M. Pompei, A. Posata, A. Galli

SIRAP-GEMA 404

SIPA 405

AUTHOR INDEX

Agabbio M. 373
Aletti G. 20
Altieri C. 142-166-219
Amicabile M. 377
An D.S. 221
Anese M. .. 7-142-385
Arena E. 349
Astone V. T. 373
Bacci A. 29
Baiano A. 212-353
Barbanti D. 357
Barbieri G. 287
Baronciani M. 377
Bartoccini M. 41
Barzaghi S. 41
Bellomo S.E. 298
Benedetti S. 252
Bongiovanni L. 199
Bravin B. 130
Brazzoli M. 228
Buonocore G.G. 53-196-199-212-216-279-361
Caggia C. 298-327
Calligaris S. 9
Campagnol L. 252-381
Campaniello D. ... 142
Cannarsi M. 166-219
Caponio F. ... 353-369
Carminati D. 252
Chiavaro E. 357
Clarizia G. 173
Colli S. 223
Conte A. 216-361
Corbo M.R. . 142-219
Cori E. 381
Cremona A. 152
Crepaldi V. 70
D'Agostino R. 166
D'Aquino S. 109-373
Dainelli D. 196
Dalla Rosa M. 29-204
De Mitri R. 152
De Nijs M. 235

De Pilli T. 385
De Regibus P. 394
De Vero L. 267
Del Nobile M.A. 29-53-132-142-166-196-199-212-216-219-269-279-361-390
Derossi A. 385
Di Salvo M. 327
Drioli E. 173
Falcone P.M. 142
Fallico B. 349
Fava P. 267-390
Favia P. 166
Figoli A. 173
Fiore A.G. 385
Franzetti L. 396
Galli A. 396
Gambacorta G. 269
Giardi C. 279-361
Gomes T. 353-369
Guarrera N. 390
Gullo M. 267
Hofstra H. 235
Horta A. 336
Hotchkiss J.H. 97
Iacoviello G. 166
Indino L. 85
Kerry J. 336
La Notte E. 269
Laguardia L. 152
Langella A. 64-287
Lanza C.M. 132-199-298
Lanza G. 109
Lee C.H. 221
Lee D.S. 221
Lee S.C. 221
Leonardi M. 132-185-269
Limbo S. 20-365
Lisi C. 204
Lo Bianco M. 298
Lucisano M. 365
Maiocchi P. 79-85
Maltini E. 7
Mannino S. 237
Manzocco L. 7-9
Marchitelli V. 212
Mariotti M. 365
Masi P. 64-98-287-336

Massini R. 357
Mastrocola D. 315
Maturi T. 287
Mostardini F. 228
Mucchetti G. 252
Munari M. 9
Muratore G. 132-199-327-390
Nicolais L. ... 216-279
Nicolais V. 98-287
Nicoli M.C. 7-9
Nicolosi Asmundo C. 132-199-390
Notermans S. 235
Oliveira F.A.R. 336
Pagani M.A. 365
Palma A. 109-373
Pannuzzo P. 298
Parisi S. 11
Park H.J. 221
Pasqualone A. 369
Passolungo L. 381
Peressini D. 130-185
Piana M. 223
Piazza L. 41
Piergiovanni L. 79-85
Pinelli C. 381
Pittia P. 315
Pompei M. 396
Porretta S. 5
Posata A. 396
Possamai A. 185
Povolo M. 252
Pozzo S. 223
Puglisi M.L. 267
Randazzo C.L. 327
Rapisarda P. 298-373
Restuccia C. 298
Rinaldi M. 357
Riva M. 70-237-252-381
Rocchelli V. 377
Rocculi P. 29-204
Romani S. 204
Romano A. 287
Romano A.D. 327
Sacchetti G. 315
Sciuto A.G. 298
Scolari G. 121

Sensidoni A. 130-185
Sinigaglia M. 142-219-385
Sousa M.J. 336
Spampinato C. 298
Spreafico C. 79
Squarzoni M. 79
Tamagnone P. 132-185-269
Tinelli L. 377
Toppino P.M. 252-381
Torchia A.M. 173
Torrieri E. 336
Vassallo E. 152
Venir E. 7
Verzera A. 349
Vescovo M. 121
Vestrucci G. 394
Villani F. ... 64-98-287
Yam K.K.L. 3
Zappa M. 394
Zappalà M. 349
Zardi M. 20

INTRODUCTION

L'espressione "shelf life" indica, come noto, il periodo di tempo che corrisponde alla vita commerciale di un alimento confezionato, quel periodo di tempo che va dal momento in cui il prodotto alimentare confezionato lascia la linea produttiva, fino all'ultimo giorno considerato utile per il suo migliore consumo. È quindi in questo intervallo di tempo che l'alimento inevitabilmente perde le sue migliori ed originali caratteristiche ed è in questo intervallo di tempo che le tecnologie di stabilizzazione, le misure di prevenzione del rischio igienico, le scelte di packaging e praticamente tutte le azioni della tecnologia alimentare hanno modo di dimostrare la loro efficacia. Al di là dell'esigenza prettamente economica di estendere il più possibile la vita commerciale degli alimenti confezionati, lo studio, la previsione e la conoscenza della "shelf life" di un prodotto rappresentano un tema di ricerca tra i più attuali e certamente tra i più multidisciplinari ed affascinanti, implicando le competenze di tutti gli specialisti delle scienze e delle tecnologie alimentari. Non sempre, comunque, la finalità degli studi di shelf life è quella di allungare il tempo di commercializzazione dei prodotti confezionati, perché non sempre ciò risulta necessario, conveniente o praticabile; sempre, però, vale la pena di operare perché gli alimenti giungano al consumo nelle migliori condizioni igieniche e sensoriali possibili. Anche se non serve aggiungere "giorni alla vita commerciale" è sempre utile e positivo aggiungere "vita ai giorni commerciali".

Il Gruppo Scientifico Italiano di Confezionamento Alimentare (GSICA) ha promosso a Milano, dall'11 al 13 giugno 2003, un Convegno Nazionale, con il preciso intento di coinvolgere tutte le competenze necessarie a questo specialistico tema. Il convegno si è articolato in tre sessioni:

- *Shelf-life modelling*, aperta dalla Relazione del Prof. Kit L. Yam – Rutger University, NJ-USA – e che ha raccolto i contributi relativi a: previsione della SL di alimenti confezionati, previsione delle proprietà di barriera dei materiali, cinetiche di degradazione dei prodotti alimentari, previsione del rischio alimentare…

- *New technologies for extending Shelf-life*, aperta dalla Relazione del Prof. Joseph H. Hotchkiss – Cornell University, NY-USA – e proseguita con presentazioni relative a nuovi materiali, nuovi dispositivi di packaging, nuove tecnologie di stabilizzazione degli alimenti, nuove tecniche di riduzione del rischio…

- *Shelf-life testing*, aperta dalla Relazione del Prof. H. Hofstra – TNO Netherlands – e che ha riunito i contributi pertinenti a tecniche non invasive di analisi, indicatori di qualità, valutazione delle proprietà funzionali degli imballaggi, nuove procedure di misura del rischio…

Il Convegno ha complessivamente raccolto, tra poster e relazioni orali, 58 contributi da Università Centri del CNR, Stazioni Sperimentali e Aziende ed è stato seguito da circa 300 persone nelle tre giornate.

L'interesse dimostrato dall'evento ha suggerito di programmare una seconda edizione del Convegno cha avrà luogo nel giugno 2006 a Catania. Il 2° Convegno Nazionale "Shelf Life degli Alimenti Confezionati" sarà promosso dal GSICA e organizzato dalla Sezione di Tecnologie Agroalimentari del D.O.F.A.T.A[1]. (Dipartimento di Orto-Floro-Arboricoltura e Tecnologie Agroalimentari) dell'Università degli Studi di Catania.

Luciano Piergiovanni

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SESSION I

SHELF-LIFE MODELLING

Main lecture: Prof. Kit K.L. Yam
Department of Food Science, Rutgers University, NJ - USA

AN OVERVIEW OF SHELF-LIFE MODELS FOR PACKAGED FOODS

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ABSTRACT

Shelf life models can be an effective tool for food packaging scientists and technologists who have the technical knowledge and experience to apply them properly. Although not intended to be a replacement for laboratory experiments, shelf life models can greatly facilitate research and development by providing predictions for what-if scenarios, reducing the number of necessary experiments, and allowing experimental data to be managed efficiently. This presentation provides an overview of the basic concepts, scientific principles, and applications of shelf life models for packaged foods.

A typical shelf life model consists of two elements: assumptions and a model equation (or a set of model equations). The assumptions govern the applicability and limitations of the model. Inexperienced users sometimes incorrectly apply a model because they fail to check or understand its assumptions. The model equation may take the form of a rate equation or a shelf life equation, depending on the specific application. The rate equation describes a quality factor as a function of time, as well as package and environmental variables. If the critical limit of quality factor (above or below which the product is no longer acceptable) is specified, the rate equation can be reduced to the shelf life equation, in which shelf life is expressed as a function of package and environmental variables (where time is no longer a variable). Both equations succinctly describe the relationships or interactions between the food, package, and environments.

Selecting the quality factor and its critical limit is often a prerequisite to solving a shelf life model equation. Food products can deteriorate due to microbial spoilage, loss in nutrients and pigments, production of undesirable components, physical changes, and so on. Although several deterioration modes may occur simultaneously, it is the most sensitive one that limits shelf life. The selected quality factor should be a good indicator of this sensitive deterioration mode, and its critical limit can be determined based on legal and marketing considerations.

The scientific principles commonly used in developing shelf life models are chemical reaction kinetics, microbial growth kinetics, gas permeation, and diffusion theory. In chemical reaction kinetics, the familiar rate equation $d[A]/dt = -kA^n$ is applied to describe the changes in concentration of chemical compounds

such as pigment and nutrient. The Arrhenius equation $k = k_o \exp(-E_a R^{-1}T^{-1})$ is used to describe the temperature dependence of reaction rate. Combining these two equations yields the shelf life plot, which is a plot of logarithm of shelf life versus temperature that is often used in accelerated shelf life testing. The empirical parameter Q_{10} is sometimes used instead of the Arrhenius equation.

The Gompertz equation and logical equation are frequently used to describe microbial growth rate. The gas permeation equation is used to describe the oxygen transmission rate and water vapor transmission rate through the package. The Fick's law of diffusion is used to describe the migration of substances from or to the package.

Various shelf life models have been developed, including those for oxygen sensitive foods, moisture sensitive foods, microbial growth, modified atmosphere packaging for fresh produce, and migration of fat or moisture. When properly applied, these models have been shown to provide good or reasonable results. An obstacle in the past was that the mathematics of these models was rather complicated. Today, shelf life models are available in computer programs or Excel spreadsheets. While this encourages more people to use shelf life models, this also increases the chances of misuses. To avoid the pitfall of garbage-in-garbage-out when using computer programs, it is important to understand the assumptions and limitations of a shelf life model before using it, and whenever possible, experiments should be conducted to verify the model predictions.

THE SENSORY AND CONSUMER APPROACH TO THE SHELF-LIFE OF FOODS

L'APPROCCIO SENSORIALE E DI "CONSUMER" ALLA SHELF LIFE DEGLI ALIMENTI

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ABSTRACT

Sensory evaluation is the key factor for determining the shelf life of many food products. Microbiologically stable foods, such as canned ones and biscuits, have a shelf life defined by the changes in their sensory properties, but many fresh foods as well, such as yogurt or pasta, after relatively prolonged storage may be microbiologically safe to eat but may be rejected due to changes in sensory attributes.

Traditionally, the shelf life of foods has centered on the product, for example, the shelf life of minimally processed strawberry stored at 4°C is reported to be 3d, based on flavour and texture changes measured with a trained panel. Thus, the hazard or the risk is focused on the fruit. But some strawberry consumers would probably accept the same strawberry stored for 3 d, and it is also probable that another group of consumers would reject the fruit stored for only 1 d. Thus from a sensory point of view, food products do not have shelf lives of their own, rather they will depend on the interaction of the food with the consumer.

Survival analysis is a branch of statistics used extensively in clinical studies, epidemiology, biology, sociology and reliability studies; the Weilbull model, derived from survival analysis has been successfully used in shelf life studies.

In survival analysis, the survival function S(t) is defined as the probability of an individual surviving beyond time t. Referring this definition to the sensory shelf life, the "individual" would not be the fruit itself, but rather the consumer, that is the survival function would be defined as the probability of a consumer accepting a product stored beyond time t. The hazard would not be focused on the product deteriorating, but rather on the consumer rejecting the product.

The length of the shelf life is normally obtained from simple averages of age to failure, on the assumption that the failure distribution is symmetrical. If the distribution is skewed, the estimates of the mean time to failure based on simple averages is biased by the inclusion of unfailed data.

The Weibull distribution is characterized by an increasing or a decreasing failure rate, while in the lognormal distribution the failure rate is zero at time zero, increases with time to a maximum, and then decreases back to zero with increasing

time. By definition, the lognormal model seems biologically inadequate since the failure rate in a deteriorating food product should increase with time rather than decrease after a maximum rate.

RIASSUNTO

La valutazione sensoriale è un fattore strategico nella determinazione della shelf life di numerosi prodotti alimentari.

Prodotti anche stabili microbiologicamente, come le conserve e i biscotti, possiedono una loro shelf life basata sulle proprietà sensoriali, ma numerosi prodotti freschi, come lo yogurt o la pasta, a seguito di un periodo relativamente prolungato, pur microbiologicamente sicuri da consumare, possono essere rifiutati per la modificazione di qualche attributo sensoriale.

Per tradizione, la shelf life degli alimenti è concentrata sul prodotto; ad esempio la shelf life di una fragola trasformata in modo light e conservata a 4°C può essere di tre giorni se ci basiamo sulle modifiche di sapore e di consistenza determinate da un panel.

Come detto, il rischio è incentrato sul prodotto, tuttavia, un gruppo di consumatori di fragole potrebbe, in realtà, accettare le stesse fragole anche dopo tre giorni, mentre un altro gruppo potrebbe rifiutarle dopo un solo giorno. Da un punto di vista esclusivamente sensoriale, gli alimenti non hanno una propria shelf life, ma la stessa dipende dall'interazione con i consumatori.

L'analisi di sopravvivenza è un settore della statistica impiegata in modo massiccio su studi clinici, epidemiologici, biologici e sociologici; il modello di Weibull è di derivazione dell'analisi di sopravvivenza ed è stato impiegato con successo su studi di shelf life applicati agli alimenti.

La funzione di sopravvivenza (St) è definita come la probabilità di un oggetto di resistere oltre un tempo (t).

Riferendo tale definizione alla shelf life sensoriale, l'oggetto non è tanto il frutto di per sé, quanto il consumatore, ovvero la funzione si ridefinisce come la probabilità di un consumatore di accettare un prodotto conservato per un certo tempo (t).

Il rischio non si concentra sul deterioramento/invecchiamento del prodotto, quanto sul rifiuto dello stesso da parte del consumatore.

La durata della shelf life è normalmente ottenuta con semplici medie dei periodi di decadimento, assumendo che la distribuzione dei dati sia simmetrica e, nel caso quest'ultima ipotesi non sia verificata le medie risultano affette da distorsioni.

La distribuzione di Weibull è caratterizzata da un andamento crescente o decrescente del decadimento sensoriale, mentre quella logaritmica (normale) assume che il decadimento valga zero al tempo zero, aumenti con il tempo fino a un massimo e quindi decresca ancora fino a zero. Tale modello sembra biologicamente inadeguato, in quanto il decadimento dei prodotti alimentari dovrebbe solo aumentare col tempo.

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LECTURE

EFFECT OF COMPOSITION AND VISCOSITY ON CAROTENOID OXIDATION RATE

INFLUENZA DELLA VISCOSITÀ E DI ALCUNE VARIABILI COMPOSITIVE SULLE CINETICHE DI OSSIDAZIONE DI UN CAROTENOIDE

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ABSTRACT

Colour changes due to pigment degradation are often responsible for considerable quality depletion of foods. Actually, the shelf-life of several foods is related to the fate of their pigments.

The most common feature in all food pigments, including carotenoids, is the presence of chromophores with electron withdrawing groups which increase resonance through conjugation. Being highly unsaturated, these molecules are particularly prone to isomerisation and oxidation. The latter are major cause of colour fading of carotenoid-containing plant materials but may also promote losses of nutritional value and development of off-flavours.

Although pigment oxidation is often an unavoidable event, the rate at which it proceeds may depend on several factors. It has been observed that oxidation rate is affected by both environmental (light, oxygen, temperature, pressure) and endogenous variables which characterise the pigment-containing matrix (composition, water activity, physical state of pigment and other food components, viscosity, molecular mobility).

For these reasons, oxidation mechanisms, which are generally applied to pure lipids, hardly allow the development of pigment degradation in complex systems, such as foods to be predicted. For instance, water soluble components, such as sugars, have been claimed to decrease oxidative reactions by modifying water activity, viscosity and reactant diffusivity. However, systematic approaches to the study of the role of these variables on pigment oxidation are missing.

In the light of these considerations, this work was addressed to study the effect of compositional variables and viscosity on the oxidation rate of a natural occurring pigment. To this aim, a water soluble carotenoid, crocin, was chosen as an example. In particular, crocin oxidation was evaluated while changing media composition, viscosity and/or water activity. The latter were modified by adding increasing amounts of solutes (sucrose, glycerol, maltodextrins and polyvinylpyr-

rolidone) to an aqueous solution containing a constant amount of crocin. Crocin oxidation was followed at room temperature for up to six months.

Results indicate that crocin oxidation is affected by solute nature and concentration and, in the case of polymers, by polymerisation degree. For each polymer, the increase in viscosity and hence in polymerisation degree, was associated to a decrease in crocin oxidation rate. However, the effect of specific interactions between polymer and reactants in determining crocin oxidation rate was not negligible. Crocin oxidation rate in sucrose and glycerol media seemed to be associated to their capability to modify both water activity and oxygen availability. In particular, the decrease in oxidation rate in glycerol systems was mainly associated to the decrease in oxygen solubility while, in sucrose-containing solutions, it was mostly associated to the water activity depletion.

RIASSUNTO

Lo scadimento qualitativo di molti alimenti, e quindi la loro shelf-life, sono spesso dovuti alla degradazione dei pigmenti che conferiscono loro la tipica colorazione. Molti pigmenti ampiamente presenti negli alimenti, quali ad esempio i carotenoidi, devono il loro colore a gruppi cromofori caratterizzati da sistemi coniugati. L'elevato livello di insaturazione rende queste molecole particolarmente propense a reazioni di isomerizzazione e di ossidazione. Oltre alla modificazione del colore dell'alimento, queste reazioni possono anche causare perdite di valore nutritivo, sviluppo di off-flavours o, più semplicemente, modificazioni della percezione da parte del consumatore di altre caratteristiche sensoriali, quali dolcezza, sapidità e aroma.

Se è vero che l'ossidazione di molti pigmenti è un evento spesso ineluttabile, le cinetiche di reazione dipendono da numerosi fattori. Vari autori hanno infatti rilevato che la velocità di ossidazione di queste molecole può dipendere sia da variabili ambientali (temperatura, presenza di ossigeno, luce), che da variabili endogene che caratterizzano la matrice nella quale il pigmento si trova (composizione, stato fisico e mobilità molecolare). Nonostante le informazioni circa l'effetto di queste variabili siano numerose, limitati sono i dati sul ruolo della mobilità molecolare, così come rappresentata dalla viscosità (η), nel modificare le cinetiche di ossidazione.

Lo scopo di questo lavoro è stato quindi quello di studiare le cinetiche di ossidazione di un pigmento naturale al variare della viscosità della matrice nella quale si trova. A tale scopo, è stato scelto, come esempio, un carotenoide idrosolubile quale la crocina. In particolare, sono state determinate le cinetiche di ossidazione della crocina in sistemi acquosi caratterizzati da diversi valori di η. La modulazione di questa variabile è stata ottenuta addizionando alla soluzione di crocina quantitativi crescenti di saccarosio, glicerolo, maltodestrine o polivinilpirrolidone. Sono state quindi valutate le cinetiche di decolorazione del carotenoide durante la conservazione a temperatura ambiente fino a sei mesi.

I risultati ottenuti indicano che, in alcuni sistemi, la cinetica di ossidazione della crocina è significativamente influenzata dalla modificazione dei valori η. Va comunque rilevato che i soluti impiegati per modulare η causano anche modificazioni di altre proprietà del sistema, quale ad esempio l'attività dell'acqua e la concentrazione di ossigeno. Mentre in alcuni casi è stata osservata la prevalenza di un'unica variabile nel determinare le cinetiche di ossidazione, in altri è stato notato un effetto combinato di a_w e concentrazione di ossigeno. Al fine di prevedere la cinetica di decolorazione del carotenoide nelle diverse condizioni, è stata proposta una funzione matematica che descrive i cambiamenti di colore al variare delle caratteristiche della matrice.

LECTURE

EFFECT OF THE PHYSICAL STATE OF LIPIDS ON SHELF-LIFE OF FROZEN FOODS

INFLUENZA DELLO STATO FISICO DEI LIPIDI
SULLA PREVISIONE DELLA SHELF-LIFE DI PRODOTTI CONGELATI

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ABSTRACT

Shelf-life determination for frozen foods is a time consuming process, because of the slow deterioration rates occurring in foods at subzero storage temperatures. For this reason, a rapid procedure to estimate the shelf-life of frozen foods is highly desired to meet market demand. However, the rates of deterioration reactions in frozen foods are often hardly predictable due to the occurrence of a variety of temperature-dependent changes such as phase transitions of crystallising components (i.e. water, sugar, lipids). In fact, as the temperature decreases, chemical, physico-chemical and structural changes can occur influencing the kinetics of chemical and biochemical reactions. Thus, in most cases, Arrhenius and Williams-Landel-Ferry (WLF) kinetic models cannot be used to describe the temperature dependence of reaction rates in frozen foods.

On the basis of these observations, the aim of the present research represents a first attempt to investigate the relationships between the physical state of food and its stability. Since lipid oxidation is one of the most important chemical reactions leading to quality loss during frozen storage, this study was focused on the evaluation of the influence of lipid physical state on oxidation rate. At the moment, while a great number of results are available on the mechanisms of lipid oxidation, few researches have been performed on this area.

In the present research, the physical state and the oxidation kinetics of sunflower oil were evaluated as a function of storage temperatures from -30° to 60 °C. In this temperature range, sunflower oil presents different solid-liquid ratios due to crystallisation phenomena. In addition, since in many frozen foods lipids are present in a dispersed phase, water-in-oil emulsions were also considered. Results indicate that the physical state of lipids significantly affects the kinetics of oxidative reactions. In particular, being slightly affected by oil physical state, the rate of the propagation phase was well described by the Arrhenius model. On the contrary, the formation of secondary products was

strongly dependent on phase transitions occurring as temperature decreased. In fact, the Arrhenius equation fitted the temperature dependence of hexanal formation only at temperatures above 0°C. A mathematical model accounting for both temperature and physical state was proposed to predict the kinetics of oxidation in the entire temperature range.

RIASSUNTO

La determinazione della shelf-life di un alimento congelato in tempi economicamente accettabili per l'industria alimentare rappresenta, ancora oggi, un problema di non facile soluzione. Infatti, le metodologie ed i modelli matematici predittivi, che vengono utilizzati abitualmente per la stima della shelf-life di alimenti conservati a temperature superiori allo zero, non possono essere facilmente applicati ai prodotti congelati. Le ragioni di questa difficoltà sono da attribuire al fatto che, al diminuire della temperatura, alcuni componenti dell'alimento (come acqua, zuccheri e lipidi) possono andare incontro a transizioni di fase di primo e secondo ordine. Ne deriva che, a seconda della temperatura di conservazione, lo stesso alimento risulterà "diverso" per composizione, caratteristiche chimico-fisiche e stabilità. Pertanto, la dipendenza dalla temperatura della shelf-life di alimenti congelati non è spesso prevedibile utilizzando i modelli di Arrhenius e Williams-Landel-Ferry (WLF).

Il presente studio ha avuto come obiettivo quello di studiare le relazioni esistenti tra lo stato fisico dei componenti alimentari e la loro stabilità. Poiché nei prodotti congelati l'ossidazione dei lipidi rappresenta la principale causa di scadimento qualitativo, il lavoro si è focalizzato sulla valutazione dell'influenza dello stato fisico dei lipidi sulla velocità di sviluppo delle reazioni ossidative. Ad oggi, nonostante l'ossidazione lipidica sia un campo ampiamente indagato, poche e frammentarie sono le notizie riguardanti la relazione tra stabilità e stato fisico dei lipidi. La comprensione di questa relazione è indispensabile per la messa a punto di modelli matematici predittivi per il calcolo della shelf-life dei prodotti congelati, dove la frazione lipidica può trovarsi in diversi rapporti solido/liquido.

La sperimentazione è stata condotta su olio di girasole e, al fine di simulare un formulato alimentare, emulsioni olio di girasole/acqua. Su questi campioni sono state valutate caratteristiche fisiche, quali temperatura di fusione, velocità di cristallizzazione, viscosità e rapporto solido/liquido alle diverse temperature. Inoltre, è stato seguito lo sviluppo delle reazioni ossidative in funzione della temperatura di conservazione. In particolare, le temperature scelte sono state tali da poter disporre di campioni allo stato solido, liquido e semisolido.

I risultati ottenuti sembrano indicare che lo stato fisico in cui si trovano i lipidi influenza in modo complesso le cinetiche di formazione dei prodotti di ossidazione. In particolare, mentre lo stato fisico non sembra influenzare in modo significativo le prime fasi dell'ossidazione, la formazione dei prodotti secondari è risultata essere fortemente dipendente dalle transizioni di fase a cui può andare incontro la frazione lipidica. Al fine di descrivere le cinetiche ossidative nell'intero *range* di temperature considerate è stato proposto un modello matematico che tiene conto sia dell'effetto dalla temperatura che dello stato fisico dei lipidi. Questi risultati appaiono di considerevole interesse per l'industria alimentare in quanto potrebbero consentire di ridurre i tempi di stima della vita commerciale di alimenti congelati contenenti lipidi.

THE PREDICTION OF SHELF-LIFE OF CHEESE ON THE BASIS OF STORAGE TEMPERATURE

LA PREDIZIONE DELLA DATA DI SCADENZA
NEI FORMAGGI IN FUNZIONE DELLA TEMPERATURA DI STOCCAGGIO

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ABSTRACT

The aim of this work was to show a practical procedure for predicting the shelf-life of foods on the basis of storage temperature.

So, some Italian dairy products – soft and semi-hard cheese (typical names: mozzarella & scamorza cheese) in particular – were considered.

It is possible to calculate the so-called "Temperature-dependent Shelf-Life" of foods, if basic principles of shelf-life prediction are known (certain durability values at any determined temperature; storage conditions).

"Temperature-dependent Shelf-Life" depends on the particular type of food, on the production formula and on temperature conditions, as well as "Degradation Rate" (an other quantity directly related to food durability).

Relation between Temperature-dependent Shelf-Life and absolute temperature has to be naturally fixed.

So, a useful equation forecasting food durability on the basis of thermal values was established.

The relation between Temperature-dependent Shelf-Life and absolute temperature, according to Arrhenius' equation is known. This relation was defined.

In addition, a second linear equation was written (for small increases in temperature): the aim of this procedure was to check calculated values (with exponential and linear methods) in real experiments and to evaluate eventual failures.

Obtained data confirm that the exponential equation is correct, while linear approximation could not furnish the same good results.

This work goes into some aspect of the calculation of food durability, a lecture formerly given at the 2nd University Master Course in Food Hygiene & Food Legislation (IA.L.A.) – Messina, Italy, A.Y. 2002-03.

- Key words: food durability, shelf-life, degradation rate, theoretical minimum shelf-life, conversion temperature -

RIASSUNTO

Scopo di questo lavoro è stato quello di presentare un'applicazione pratica del calcolo previsionale della data di scadenza degli alimenti in funzione della temperatura di stoccaggio.

Per questo scopo sono stati presi in esame alcuni prodotti lattiero-caseari italiani, nella fattispecie formaggi molli e semiduri a pasta filata (mozzarelle e scamorze).

Una volta noti i presupposti operativi che stanno alla base della previsione della data di scadenza degli alimenti, descritti in un lavoro dello stesso Autore e conoscendo in maniera certa la data di scadenza di taluni prodotti, fatte salve le condizioni di stoccaggio, è possibile calcolare la cosiddetta "Shelf-Life in funzione della temperatura" del prodotto, la quale è dipendente dal tipo di alimento, dalla formulazione e dalla temperatura di stoccaggio, così come la "Velocità di Degradazione" (un'altra quantità direttamente correlata alla durabilità alimentare).

In questo lavoro vengono richiamate le formulazioni già elaborate per i formaggi, distinte per 2 temperature differenti: 2±2°C e 10±2°C; a questo scopo l'Autore si è avvalso delle risultanze di 2 lavori realizzati in seno ad un'industria alimentare operante nel campo dei formaggi freschi a pasta filata.

Una volta noti i valori di durabilità rielaborati in base ai dati di cui sopra, e distinti per temperatura di stoccaggio, si può procedere alla costruzione della curva previsionale della Shelf-Life in funzione dei valori termici.

Si va così a costruire 2 relazioni tra durabilità e temperatura, tenendo presente l'equazione di Arrhenius tramite una dimostrazione matematica che viene qui riportata per intero e la possibilità di interpolare linearmente i risultati sperimentali.

Si valutano criticamente i risultati, confrontando infine le risultanze di quanto emerso con una piccola serie di esperimenti condotti su 2 campioni di mozzarelle e di formaggi semiduri, suddivisi in 3 aliquote conservate a 3 temperature differenti.

I risultati confermano la bontà dell'equazione esponenziale e la riuscita del modello previsionale, suggerendo altresì qualche considerazione sull'approssimazione lineare.

Questo lavoro approfondisce qualche aspetto del calcolo della durabilità alimentare, tematica già trattata nell'ambito dell'omonima docenza al Master Universitario di 2° livello in Igiene degli Alimenti e Legislazione Alimentare (I.A.L.A.) di Messina, A.A. 2002-03.

INTRODUCTION

The calculation of the shelf-life of food obliges, as it should be obvious, an exhaustive analysis of the entire food production system, involving different aspects – chemistry, microbiology, legislation (enacted laws and related different interpretations), marketing strategies (consumer science), until the comprehension of distributive structures, the last ring of the food industrial chain.

In order to discuss shelf-life dependance on storage temperatures, some important premises should be done, first of all the correct definition (Parisi, 2002a) of "food durability" (Best Before end? Expiration Date? Sell-by-Date?) and "Food

degrading", in other words that non-synergic sum of modifications that is able to turn a particular food into another highly different product and/or not in conformity with claimed or tacit properties.

In fact, a generic edible product could be harmful (uncontrolled spreading of *E. coli* O157:H7, etc.) without certain alteration signs (bad colour, odour, taste); on the other hand, many unpleasant situations highlight themselves with non-biological or chromatic alterations: so, a pasteurized Italian salame (salt pork) is not acceptable if its texture is too soft, because of thermal stress; the same thing frequently occurs with some soft cheese showing important texture defects, or with some melted cheese sticky slices (excess fat, in other words greas).

The above-mentioned examples can naturally derive from process failures or erroneus raw material selection, but food durability and degrading are (Parisi, 2002a) anyway intended as a complex function of all process factors, and not of some important causes: so, a correct analysis should be based on the knowledge of synergic interaction of intrinsic (inherent to food materials) and extrinsic (not inherent) degrading causes.

Some previous paper have shown (Parisi, 2002b, 2003) how shelf-life values are calculable about cheese by means of a complete analysis of the food system; in other words, a correct formula able to fix a minimum durability in function of chemical & microbiological features of "true" and "processed" cheese was established at 2 different storage temperatures.

Subsequently, the author expressed a new question: could minimum food durability be calculated knowing chemical & biological parameters and also storage temperature values?

After some reflection on the concept of durability a new mathematical equation about food durability was established as solution of our problem:

Temperature-dependent Shelf-Life, $[SL]^{T°K}$: it is that time period – assumed as food minimum durability – directly dependent on the storage temperature value (T°K) and – indirectly – on chemical, microbiological and structural food features.

In other words, minimum durability of a particular food is fully estimable at a determined storage temperature (°K) if:

1) minimum shelf-life values are known (or fully estimated) for that food at any temperature;

2) $[SL]^{T°K}$ dependence on temperature values (°K) is mathematically known.

Known minimum durability at 2 fixed temperatures, exact $[SL]^{T°K}$ expression has to be necessarily calculated.

A correct estimation of $[SL]^{T°K}$ and of the so-called "Degradation Rate" about foods is certainly possible knowing all problem features and drawing – on the basis of these data – a plan of food durability classified (Parisi, 2002a) in 14 steps, from the 1st: definition of food and durability genre until the last: analysis of commercial complaints by reconstructing the thermal history in a mathematical way.

Our "Shelf-Life" depends naturally on (A) the particular type of food or beverage, (B) chemical, microbiological and structural food features, and (C) the storage temperature.

Mathematical expression basically depends on temperature values, as well as Arrhenius' Law suggests:

$$[SL]^{T°K} = SL_{MIN} \times \exp [T_{CONV} / T°K].$$

Please note:

a) storage temperature is here correctly defined as °Kelvin value;

b) storage temperature is mentioned as denominator (as well as Arrhenius' Law);

c) SL_{MIN} and T_{CONV} numbers are a couple of numbers theoretically fixed for one and only one kind of product (2 different or similar foods should not have the same coefficients);

d) $[SL]^{T°K}$ tends to SL_{MIN} when T°K tends to infinite positive values ($\lim_{T°K \to +\infty} [SL]^{T°K} = SL_{MIN}$); so, SL_{MIN} is the theoretical minimum $[SL]^{T°K;}$ please note this number is a time period, as Shelf-Life experimental results;

e) T_{CONV} - measure unit: °K - is named conversion temperature because of this relation:

$$\ln ([SL]^{T1°K} / [SL]^{T2°K)} = T_{CONV} \times (1 / T2°K - 1 / T1°K) =$$
$$= T_{CONV} \times (T1°K - T2°K) / (T1°K \times T2°K).$$

In other words, it is possible to calculate the quotient between 2 different Shelf-Life values if related temperatures and T_{CONV} are known; so, T_{CONV} is really the one data we need to define a durability if another [SL] is known.

Please note T_{CONV} is not constant: in fact, some differences were evaluated in different calculations, but differences are insignificant, so this number could be considered approximatively constant for our purposes.

$[SL]^{T°K}$ is a very useful mathematical tool: it is always possible to know exact shelf-life values for a particular food knowing:

1) correct shelf-life values at a standard temperature: these data must be assumed as constant values, and best conclusions should be obtained with a previsional mathematical calculation (Parisi, 2002A, 2002b, 2003);

2) SL_{MIN} and T_{CONV} coefficients: these data have to be set;

3) temperature values (measure unit: °Kelvin scale).

So, a well-advised researcher aiming to define correctly durabilities in a defined field should perform 2 different series of degrading tests on the same foods using 2 storage temperatures; data processing will give shelf-life values by establishing a related formula (Parisi, 2002a).

Finally, a and b are calculated using our Arrhenius-based formula for $[SL]^{T°K}$.

This paper recalls here all announced (Parisi, 2002a, 2003) formulas for soft and semi-hard Italian cheese, found on-the-ground by the Author, relating to 2 different temperature storage: 2±2°C and 10±2°C.

Knowing all durability values, it is possible to draw a predictive graph of shelf-life values as a function of temperature values.

A relation between durability and storage temperature is so established, considering Arrhenius' approach (Parisi, 2002a), by means of a mathematical demonstration completely displayed below.

The next operation consists in writing a second linear equation (if temperature increase is little) using a simpler approach: our goal is to determine whether durability may be linearly (not exponentially) calculated without big errors.

Subsequently, total results are critically evaluated and compared with a little series of experiments about 2 mozzarella and semi-hard (like Italian scamorza) cheese, subdivided in 3 stored subsamples at 3 different temperatures.

Results confirm that the exponential equation is the best way to predict for our intentions, but some interesting reflections about the linear approximation approach should be done.

Table 1.

Type of cheese and weight	Ingredients
mozzarella cheese (like Italian "Fior di Latte") - 1,000 g	Cow's Milk, salt, rennet, citric acid
semi-hard cheese (like Italian Scamorza) - ≅ 700 g	Cow's Milk, salt, rennet, citric acid

Numerous analyses and evaluations were conducted (Parisi, 2002b, 2003) within an industrial study aiming to fix necessary chemical & microbiological parameters for calculating minimum shelf-life a priori in dairy products, considering 2 different storage temperatures: 2±2°C and 10±2°C.

Some features of these cheese are displayed in Table 1.

All products were subdivided in several sub-samples; each sub-sample was packed into transparent plastic films, aiming to see chromatic variations and/or undoubted bacterial spreadings.

Supposing that initial (just produced) and final (expired) conditions of a determined dairy product are function of a group of strictly correlated variables (Parisi, 2002b, 2003), a methodic study on the following parameters was conducted:

1) moisture content (as grams/100 grams of dairy product);
2) fat matter (as grams/100 grams of dairy product);
3) fat matter on dry content (not dimensional);
4) Moisture on Free Fat Basis, or M.F.F.B. (not dimensional);
5) pH value;
6) Total Viable Count without Lactic Bacteria (C.F.U./gram);
7) Coliform count (C.F.U./gram);
8) Yeast & Mould count (C.F.U./gram).

2 mathematical expressions were established on the basis of data analyses for calculating minimum durability about the above -mentioned cheese (all coefficients are measured in days):

$$\text{Shelf-Life (storage temperature: } 2\pm2^{\circ}\text{C)} = -1.6 \times \text{MFFB} + 29.9 \times \text{pH} - 10.9 \times \text{Log MIC};$$

$$\text{Shelf-Life (storage temperature: } 10\pm2^{\circ}\text{C)} = -0.7 \times \text{MFFB} + 11.6 \times \text{pH} - 2.6 \times \text{Log MIC};$$

where

a) Shelf-Life is for: experimental minimum durability;
b) MFFB is for: Moisture on Free Fat Basis;
c) pH is for: pH value;
d) Log MIC is for: Yeasts & Moulds count, expressed as 10-based logarithm.

These similar equations were written solving a system as displayed below:

$$\begin{cases} x = a \times A_x + b \times B_x + c \times C_x + d \times D_x + \ldots \\ y = a \times A_y + b \times B_y + c \times C_y + d \times D_y + \ldots \\ \\ z = a \times A_z + b \times B_z + c \times C_z + d \times D_z + \ldots \\ j = a \times A_j + b \times B_j + c \times C_j + d \times D_j + \ldots \\ \ldots\ldots \\ \ldots\ldots \end{cases};$$

This system is obtained attributing minimum durability of all products (1st, 2nd, 3rd,,... = x, y, z,...) to the 1st member of every equation, and related initial values of useful parameters (A = MFFB, B = pH, C = Log MIC,...) to the 2nd member.

MATERIALS AND METHODS

Table 2 shows all details about the materials and related methods.

Table 2.

Parameter	Method / references	Materials (suppliers)
% Moisture	FIL-IDF 4:1998 Norm	AD - 4714 A Thermobalance (A&D Instruments)
% Fat Matter	Gerber method (Giuliano and Stein, 1973)	Butirrometro Van Gulik Butyrrometer, scale: 0-40%, accuracy: 0.5% (Dr. N. Gerber)
pH value	Zaffino and Vivaldi, 1996	pH-meter: pH 538 (WTW); electrode: Sentix 41(WTW)
T.V.C. without lactic bacteria	FIL-IDF 153 (1991) Norm	Culture media: Gelose Eugon Agar
Coliform count	AOAC 991.14: 1991 Norm	Culture media: Petrifilm E.Coli / Coliform Count Plate (3M)
Yeast & Mould count	ISO 7954: 1987 Norm	Culture media: Oxytetracycline-Glucose-Yeast extract (OGYE) agar

RESULTS AND CONCLUSIONS

$[SL]^{T°K}$ is estimable in accordance with the following expression:

$$[SL]^{T°K} = SL_{MIN} \times \exp [T_{CONV} / T°K];$$

where SL_{MAX} and T_{CONV} have to be determined for every type or sub-type of cheese, and T°K is for: storage temperature values, scale: °Kelvin.

SL_{MAX} and T_{CONV} are obtained solving this system:

$$\begin{cases} [SL]^{275.16°K} = SL_{MIN} \times \exp (T_{CONV} / 275.16\ °K) \\ [SL]^{283.16°K} = SL_{MIN} \times \exp (T_{CONV} / 283.16\ °K) \end{cases};$$

An alternative arrangement could be possible if our temperature field is very limited (≈10°C);

$[SL]^{T°K}$ is now calculable with this simplified equation:

$$[SL]^{T°K} = \alpha + \beta \times T°K;$$

α and β are obtained solving this system:

$$\begin{cases} [SL]^{275.16°K} = \alpha + \beta \times 275.16\ °K \\ [SL]^{283.16°K} = \alpha + \beta \times 283.16\ °K \end{cases};$$

Where $[SL]^{275.16°K}$ and $[SL]^{283.16°K}$ are medium Shelf-Life values of mozzarella and semihard cheese, and temperature values (°K) correspond to 2±2°C (standard value) and 10±2°C respectively.

The following sections show both mathematical approaches for determining $[SL]^{T°K}$, aiming to evaluate discrepancies and errors F&B technologists may commit employing linear interpolation instead of the exponential formula.

a) exponential approach

Mozzarella cheese showed these calculated minimum durabilities:

minimum durability at 2±2°C = 46.5 days
minimum durability at 10±2°C = 15.2 days

An exponential interpolation requires a solvable system, displayed below:

$$\begin{cases} [SL]^{275.16°K} = SL_{MIN} \times \exp [T_{CONV} / 275.16° K] \\ [SL]^{283.16°K} = SL_{MIN} \times \exp [T_{CONV} / 283.16°K] \end{cases};$$

Final resolution give us a and b couple (for mozzarella cheese only):

$$SL_{MIN} = 1.9 \times 10^{-16} \text{ days};$$
$$T_{CONV} = 11{,}019.0 \text{ °K (the one necessary data).}$$

Finally, the equation that is able to calculate $[SL]^{T°K}$ – for mozzarella cheese – as function of T°K is:

$$[SL]^{T°K}(\text{days}) = 1.9 \times 10^{-16} \times \exp [11{,}019.0 \text{ °K} / T°K].$$

Please remember this formula is valuable for mozzarella cheese only because of SL_{MIN} and T_{CONV} specificity.

For semi-hard cheese, minimum durabilities at 2±2°C and 10±2°C are 58.3 and 19.9 days respectively;

if exponential approach is used in this case also, final formula – for semi-hard cheese – is:

$$[SL]^{T°K}(\text{days}) = 2.5 \times 10^{-15} \times \exp [10{,}369.5 \text{ °K} / T°K].$$

One more time, a reflection should be done about SL_{MIN} and T_{CONV} coefficients: semi-hard cheese related values are different from those calculated for mozzarella cheese, so tacit meaning is each dairy product (every food) owns an potentially exclusive couple of numbers (minimum durability and conversion temperature) if compared with other products.

b) Linear approach

If a linear approximation would be used for $[SL]^{T°K}$ calculus on the basis of storage temperatures, F&B technologists should solve a system as this:

$$\begin{cases} [SL]^{275.16° K} = \alpha + \beta \times 275{,}16°K \\ [SL]^{283.16°K} = \alpha + \beta \times 283{,}16°K \end{cases};$$

Final resolution, undoubtedly simpler than previous exponential process, give us 2 constants, named α e β: mozzarella cheese samples own these values:

$$\alpha = 1119.6 \text{ days};$$

$$\beta = -3.9 \text{ days} / °K.$$

So, the simplified equation that is able to calculate $[SL]^{T°K}$ – for mozzarella cheese – as function of T°K is:

$$[SL]^{T°K}(\text{days}) = 1119.6 - 3.9 / °K \times T°K;$$

An analogous reasoning furnishes a useful formula for semi-hard cheese:

$$[SL]^{T°K}(\text{days}) = 1379.1 - 4.8 / °K \times T°K.$$

c) Critical comparison between 2 methods

A correct validation of the 2nd method in respect of the exponential approach requires the comparison of theoretical results with some experimental data.

So, 1 mozzarella and 1 scamorza cheese were each subdivided in 3 subsamples; every subsample was packed into transparent plastic films and stored at a different temperature, namely: 2±2°C; 6±2°C; 10±2°C.

This technique allowed us to realize whether a generic dairy product showed predictable minimum durabilities according with a linear approximation, as well as exponential procedures.

Table 3 shows all experimental results and related comparisons: please note all subsamples were inspected after 2 × n days from production date, with n ≥ 1 (e.g., 2, 4, 6,... days) until declared unacceptability.

Our comparison between true and calculated $[SL]^{T°K}$ (2 mathematical approaches) demonstrated that:

1) exponential method allows to calculate $[SL]^{T°K}$ without important differences: mozzarella and scamorza cheese show little variances between true and extrapolated data in our temperature range; the only incorrect result (32.5 days, error: 1.5) does not overestimate minimum durability;

2) linear approximation appears to be correct when storage temperature is

Table 3.

...Subsample, stored at...	Experimental minimum shelf-life (days)	Calculated shelf-life after exponential interpolation	Calculated shelf-life after linear interpolation	Differences (days)	
				true - expon.	true - linear
mozzarella cheese 2±2°C	42	41.7	42.0	0.3	0.0
mozzarella cheese 6±2°C	24	23.9	28.0	0.1	-4.0
mozzarella cheese 10±2°C	14	13.9	14.0	0.1	0.0
scamorza cheese 2±2°C	54	53.8	54.0	0.2	0.0
scamorza cheese 6±2°C	34	32.5	37.0	1.5	-3.0
scamorza cheese 10±2°C	20	19.9	20.0	0.1	0.0

fixed at 2±2°C or 10±2°C only (higher performance than exponential method): on the other hand, calculated $[SL]^{T°K}$ data show more important errors (differences are -4.0 and -3.0) at 6±2°C in respect of exponential results, and this situation occurs in all 2 cheese series.

Examined differences allowed to realize that:

a) the 1st (exponential) method is very reliable: minimum shelf-life values were well anticipated when 0°C ≤ storage temperatures ≤ 12°C and, in general, a similar performance has to be expected for every temperature; calculated shelf-life has never had higher than true data;

b) same reflections cannot be stated when the 2nd (linear) approximation is preferred;

c) linear calculation overestimates minimum durabilities when T°K is included between 0°C and 12°C, therefore it could be inferred that such system, surely easier than the 1st, allows to acquire unacceptable data because higher than true values: all cheese (or food) could be released with excessive shelf-life values;

d) on the other hand, the linear method underestimates minimum durabilities when T°K is not included between 0°C and 12°C; all cheese (or food) could be released with lower shelf-life values than true durabilities.

Apart from the details, a correct previsional procedure for minimum durabilities in foods has to be based on an exponential approach: this argumentation can only rationally give us correct data in comparison with experimental results.

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EFFECTS OF STORAGE TEMPERATURE, OXYGEN LEVEL AND LIGHTNESS ON SHELF-LIFE OF BROWN PARBOILED RICE

INFLUENZA DI TEMPERATURA, OSSIGENO E LUCE SULLA SHELF LIFE DEL RISO INTEGRALE PARBOILED

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ABSTRACT

Brown parboiled rice (BPR) was stored at room temperature (25°C) in dark storage for 25 months and under exposure to light for 130 days to study the effect of light on the shelf-life of this product. The evolution of rancidity was evaluated measuring volatiles by the HS-GC method and recording the absorbance ratio at 234 and 270 nm.

In the second part of the work, BPR was stored under different conditions of temperature, oxygen concentrations and package transmittance, which were chosen according to a Central Composite Design in order to assess the simultaneous effects of these variables on the development of rancidity.

RIASSUNTO

La shelf-life del riso integrale parboiled, ed in particolare l'effetto che su di essa ha la luce, è stata studiata condizionando il prodotto a temperatura ambiente (25°C) per 25 mesi al buio, e per 130 giorni esposto ad una fonte luminosa. In questo periodo di shelf-life l'irrancidimento del prodotto è stato monitorato attraverso la determinazione delle componenti volatili mediante spazio di testa statico (HS-GC) e attraverso la valutazione degli idroperossidi mediante analisi spattrofotometrica (rapporto di assorbanze a 234 e 270 nm).

Nella seconda parte del lavoro il riso integrale parboiled è stato condizionato a diverse temperature, concentrazioni di ossigeno e differenti valori di trasmittanza alla luce dei materiali di confezionamento, stabilite in base alla matrice di un disegno sperimentale per la creazione di superfici di risposta, così da poter valutare l'effetto simultaneo di queste tre variabili sulla shelf-life del prodotto.

- Key words: accelerated shelf-life test, parboiled rice, rancidity -

INTRODUCTION

The main oxidative process involved in food deterioration is lipid oxidation, which results in the development of rancidity (unpleasant tastes and odours), colour changes and potential formation of toxic compounds. Lipid oxidation is influenced by several factors strictly dependent on storage conditions and the chemical composition of the food.

In particular, foods susceptible to lipid oxidation are usually stored in packages with low permeability to oxygen. Under these conditions, the oxygen level in the headspace is the limiting factor controlling the rate of lipid oxidation. Relative humidity of the immediate environment directly affects the water activity and moisture content of a food. Generally, many deterioration reactions increase exponentially in rate with increasing water activity (a_w), but for lipid oxidation the rate increases as the a_w decreases below the monolayer value (Singh, 1994).

It is also well known that exposure of fat foods to ultraviolet radiation and visible light accelerates oxidative deterioration. The effects of light can be explained by both photolytic autoxidation which correspond to the production of free radicals (primarily from lipids) during exposure to UV light, and photosensitised oxidation which occurs in the presence of photosensitisers and visible light (Min and Boff, 2002). The storage temperature is also an important variable because of its role of catalyst of oxidation reaction: generally the reaction rate increases with increasing temperature.

Development of rancidity, with resultant loss of quality and acceptability, can occur in cereal products due to degradation reactions at various points along the chain from grain at harvest and storage, through the different technological processes, to the final products.

The packaging technologies may slow down the effect of these variables by reducing residual oxygen inside a package or slowing the oxygen and water vapour permeability through the surface; they may also reflect or absorb a fraction of incident light and various packaging materials have been shown to differ markedly in their transmission of light (Turhan et Sahbaz, 2001).

In general, rancidity is a great problem in cereal products derived from whole grain or those containing bran and germ components because these tissues are rich in unsaturated lipids. Also palatability factors in brown parboiled rice are deteriorated gradually when it is stored at room temperature. In fact, rice parboiling is a hydrothermic process that involves three essential steps: soaking of raw rice, steam heat treating and drying to a level of moisture suitable for subsequent processing. In these last years parboiled rice has shown a positive trend in spite of common white rice because it is easier to cook and the consumer is sure about plate success (Zecca, 2000).

On the other hand, brown parboiled rice is very susceptible to rancidity. In fact, during the parboiling process the antioxidants, present in external teguments, are severely reduced and the fats move towards the external surface with the consequence of a greater susceptibility to oxygen attack (Sowbhagagya and Bhattachardrya, 1976).

In a former work (Zardi, 2002) it the accelerating effect of temperature on brown parboiled rice deterioration was studied. An amount of hexanal equal to about 1 ppm, which made the rice sensorially unacceptable by the consumer, was noticed after 11 weeks of storage at 25°C and after 10 weeks at 35°C. This means that the shelf-life of brown parboiled rice decreased only one week increasing the temperature from 25° to 35°C.

The first aim of this experiment was to investigate the effect of light on the oxidative deterioration of brown parboiled rice during a simulated storage. Secondly, the synergic effect of different variables, i.e. the levels of oxygen in package headspace, the storage temperature, and the intensity of light through the packaging was also evaluated to suggest the optimal range of these variables in which to carry out an accelerated shelf-life test.

MATERIALS AND METHODS

Rice conditioning and experimental design

Brown parboiled rice (BPR), variety Ariete cultivar Ribe was used. In the first part of the study samples were packed in printed prelined cartons and stored for 25 months at 25°C in air, in dark conditions. To test the effect of light, 35 g of samples were put as a thin layer in Petri dishes (diameter equal to 140 mm) and packed in pouches (20 x 20 cm) made of a multilayer film (PET/PE+EVA/EVOH/PE+EVA, 84 µm) with a very low oxygen transmission rate (0.4 cm^3 m^2 $24h^{-1}$, 38°C and 90%UR). These samples were stored for 130 days, in presence of oxygen (20.9% O_2), at 25°C under exposure to light. In order to avoid a transmittance value of the film at 330 nm equal to about 35%, three layers of the film were placed one upon another. In the last part of the experiment, temperature, oxygen and light values were modulated according to a Central Composite Design that consisted of points of factorial design (2^3) augmented with the star points located at +1.68179 and -1.68179 coded units from the centre of the experimental domain. Coded and natural values of each variable are summarised in Table 1.

This Central Composite Design studied the effects of three factors in 18 runs (Table 2) and all runs were evaluated after 30 days. In this part of the experiment, the pouches were made as previously described and different light transmittances at 330 nm were obtained putting layer on layer of the same film. The illumination was made by a neon lamp, OSRAM (24W), placed at a distance of about 20 cm from the samples.

The different concentrations of oxygen inside the pouches were realized by means of a packaging machine, which substituted air inside the package with the anhydrous gaseous mixtures of oxygen and nitrogen. The zero-oxygen concentration (run 8, Table 2) was reached using oxygen absorber sachets based on iron powder (Ageless, type ZPT 50).

Variables studied were assorbance ratio at 234 nm and 270 nm to evaluate the first step of oxidation reaction (hydroperoxides development) and aldehydes

Table 1 - Coded and natural values of the variables.

Factors	Star point (-1.68179)	Low (-1)	High (+1)	Central Point (0)	Star point (+1.68179)
Temperature (°C)	5	13	37	25	45
Oxygen (%)	0	8	32	20.9	40
Transmittance (%)	10	20	50	35	50

production to follow the second step of oxidation (hydroperoxides break to smaller molecules).

Response surface methodology was employed to optimise the best combination of variables that had the most accelerating effect in brown parboiling rice storage. A second order polynomial equation was used to describe the interactive effects of the three independent variables:

$$Y = \beta_0 + \beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_4X_1^2+\beta_5X_2^2+\beta_6X_3^2+\beta_7X_1X_2+\beta_8X_1X_3+\beta_9X_2X_3$$

where Y is the response (absorbance ratio or aldehydes concentration), X_{1-3} are the independent variables (temperature, oxygen and packaging transmittance to light) and β_{0-9} the regression coefficients. The computation was performed with the aid of Statgraphics Plus version 4 software package (Statistical Graphic Corp.).

Table 2 - Central Composite Design consisting of 18 experiments.

Run	Oxygen (%)	Temperature (°C)	Transmittance at 330 nm (%)
1	40	25	35
2	20	25	35
3	32	37	50
4	20	25	35
5	8	37	50
6	20	25	35
7	8	13	50
8	0	25	35
9	32	13	50
10	20	25	35
11	20	5	35
12	20	25	10
13	32	13	20
14	8	37	20
15	8	13	20
16	32	37	50
17	20	25	60
18	20	45	35

Atmosphere control

Samples were submitted to atmosphere control using a gas chromatograph (Hewlett Packard HP 5890 series II) equipped with a thermoconducibility detector and a steel column (2 m x 6 mm, CTR I, Alltech, Milano).

Hexanal

1 g of BPR was put in a vial, closed with a silicon/teflon septum and aluminium crimp top and analysed by HS-CG (HS40 Perkin Elmer, GCHP 5890 series II, Hewlett Packard) using the conditions reported in Table 3.

Table 3 - Analytical conditions and equipment used for HS-GC analysis.

HS		GC	
Sample thermostation	30 min at 100°C	Analytical column	Carbowax, 20M; 25mx0.53 mm; 1 mm film thickness
Pressurisation time	2 min	GC program	50°C (0 min); 5°C/min to 100°C; 10°C/min to 200°C
Injection time	0.1 min	Injection mode	Splitless
Needle temperature	130°C	Injection temp.	150°C
		Detector	FID at 280°C
		Carrier gas flow	Helium (2.2 mL/min)

The hexanal concentration was determined by means of a previously prepared calibration curve.

Determination of rancidity development

The hydroperoxides and their secondary products were extracted by stirring 2 g of powdered rice (particles diameter not greater than 0.5 mm), with 20 mL of cyclohexane for 30 min. The solution was filtered, and a Perkin Elmer Lambda UV/Visible spectrophotometer was used to determine absorbance at 234 nm, corresponding at conjugated diene hydroperoxide and at 270 nm, corresponding to the products degradation of non volatile hydroperoxides (Drapron, 1997).

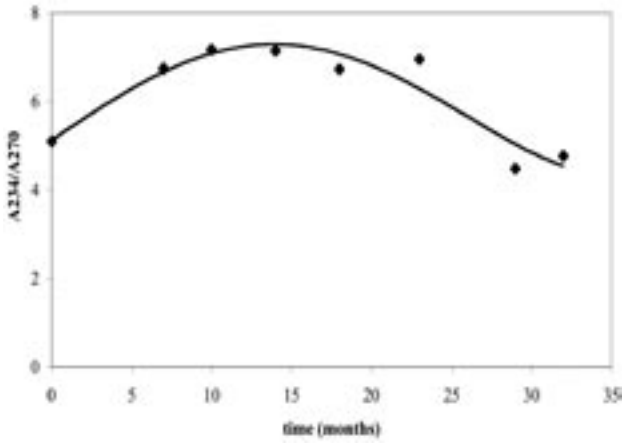


Fig. 1 - Rancidity development in dark storage.

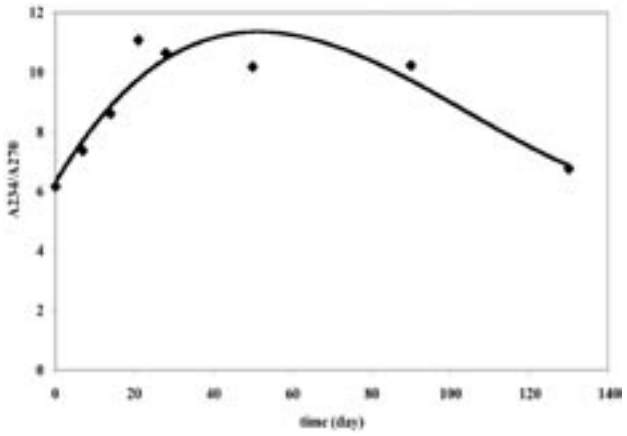


Fig. 2 - Rancidity development in light storage.

RESULTS AND DISCUSSION

The development of rancidity during dark and light storage, in presence of a constant oxygen concentration (20.9%), was measured recording absorbance ratio at 234 and 270 nm and the results are shown in Fig. 1 and Fig. 2, respectively. At the beginning of storage the ratio value increases until a maximum which corresponds to the highest hydroperoxide production. Progressively, the ratio decreases due to hydroperoxide degradation and volatile and non volatile compounds production.

The pronounced effect of light on fat rancidity of brown parboiled rice is evident. In fact, the time to reach a maximum accumulation of hydroperoxides was equal to 10 months in dark conditions while only to 40 days under light storage (Fig. 2).

Oxidation reactions can also generate off-flavour, especially aldehydes, which are originated from unsaturated fatty acids. Hexanal is the main off-flavour in cereals and it may arise from oxidative degradation of linoleic acid that accounts for

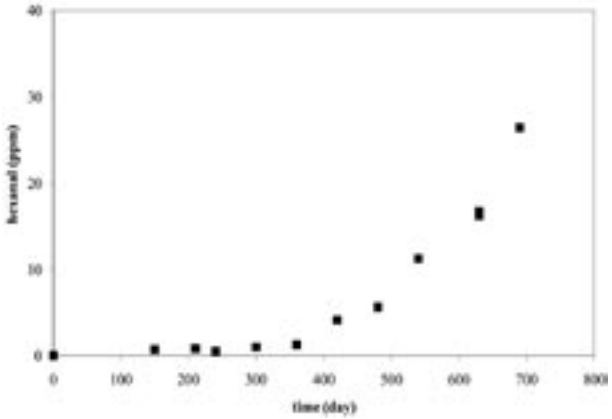


Fig. 3 - Hexanal evolution during dark storage.

about 34% of the total fatty acid in brown rice. The other aldehydes, like octanal and nonanal arise from oleic acid, generally increase less than hexanal that was considered as a rancidity index (Galliard, 1994).

Data concerning the evolution of hexanal concentration during 25 months of storage in printed prelined cartons and during light storage in plastic pouches had the same trend but it was evident that light accelerated the oxidation rate, as shown in Fig. 3 and 4.

In order to find the accelerating factor induced by light the statistical software SAS/STAT was used. The best model which described experimental data was significant at every level ($\alpha < 0.0001$) and the equations 1 and 2 represented the dark and light storage, respectively:

y= a*b (x)^0.5 (Eq. 1) dark storage

y= a*b* (x*C)^0.5 (Eq. 2) light storage

where $a=e^{-4.3}$ and $b=e^{0.2805}$ for both the equations.

As evident, factor C can be considered the accelerating factor which describes the hexanal production, induced by light exposure in brown parboiled rice.

The same equations written in logarithm form showed that there was a linear relationship between the logarithm of hexanal concentration and root square of time (Fig. 5) and the straight lines had the same intercept. In this form, it was easier to obtain the light accelerating factor C as the square ratio between the slopes of the two lines:

$$C = (\text{slope}_{\text{light}} / \text{slope}_{\text{dark}})^2 = (0.8142/0.2805)^2 = 8.4$$

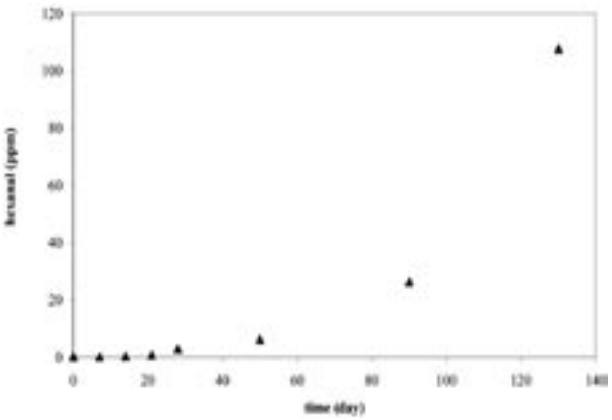


Fig. 4 - Hexanal evolution during light storage.

It is evident that light has a dangerous effect on the shelf-life of BPR. In fact the oxidation rate expressed as hexanal concentration is eight times greater than the rate of aldehyde production in BPR stored in dark conditions.

The simultaneous effect of oxygen, light and temperature on BPR was investigated in the second part of the experimental work. For this purpose a Central Composite Design was used to define the operating conditions. The absorbance ratio (A_{234}/A_{270})

and the hexanal production were evaluated after 30 days of storage. For each response variable, polynomial equations accounting for the effects of the modulation of the three variables, light, temperature and oxygen were obtained. The analysis of variance (ANOVA) was performed on the design to assess the significance of the model. The analysis of the results from ANOVA showed that the significant variables for absorbance ratio were temperature and oxygen in their quadratic terms. The pareto chart of effects is shown in Fig. 6, where the vertical line represents the 95% of confidence level and the bar lengths are proportional to the standardized effects.

At low temperatures and oxygen concentrations the absorbance ratio is low, but the highest value, which corresponded to the highest accumulation of hydroperoxides, was achieved at central point conditions of experimental design (25°C, 20.9% O_2, 35% transmittance) (Fig. 7).

The results showed that oxygen and temperature played a fundamental role in the first step of oxidation. The effect of packaging transmittance seemed to have a little effect on hydroperoxide production because the oxidation is probably promoted by wavelengths lower than 330 nm, at which the film used in this work showed a higher transmittance.

With regard to hexanal production, the ANOVA analysis showed that after 30 days of storage, the values of this response variable were principally affected by temperature in

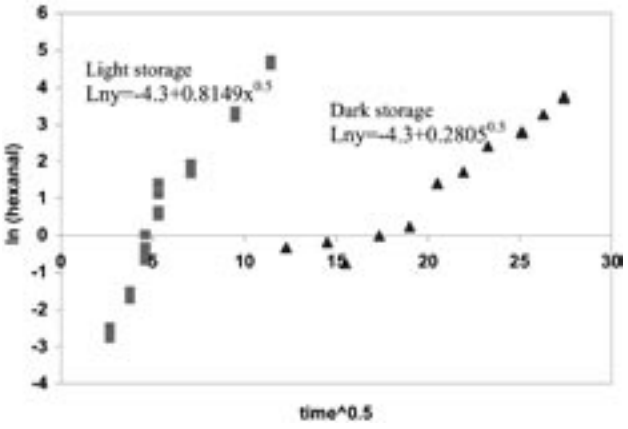


Fig. 5 - Linear relationship between Ln of hexanal concentration and square root of time.

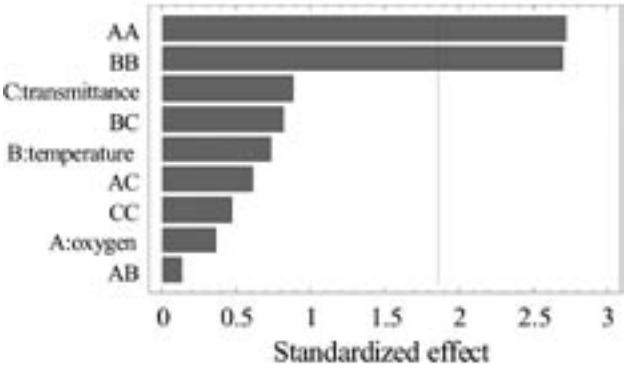


Fig. 6 - Standardized pareto chart for absorbance ratio.

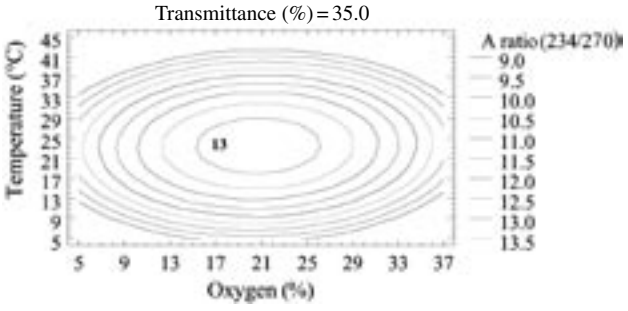


Fig. 7 - Iso response surface contour plots relative to the absorbance ratio (A_{234}/A_{270}) in BPR: interaction between Oxygen concentration and Temperature.

its linear and quadratic terms ($p \leq 0.05$). The regression model explained 79% of the total variation ($p \leq 0.01$). Figures 8-10 show the iso-response surface plots relative to the hexanal production.

According to Fig. 8, the interaction between transmittance and oxygen concentration, at the central value of temperature indicated that the hexanal concentration was maximal when the oxygen concentration was about 20-25% and transmittance was around 35-40%. Contour plot of the response surface as a function of temperature and oxygen (Fig. 9) presented a saddle shape: the oxidation reactions were reduced at lowest value of oxygen and temperature and the hexanal concentration tended to increase rapidly when temperature increased. In particular, the maximum hexanal concentration was reached after 30 days of storage at 45°C in presence of oxygen at 20.9% and using a plastic film with 35% of transmittance at 330 nm.

The hexanal concentration equal to 1 ppm, that makes the rice unacceptable for the consumer, was reached in the same conditions of transmittance but storing the product at low oxygen levels (<8-10%) and at 17°-20°C. The iso-response curve concerning the interactive effects of transmittance and temperature (Fig. 10) showed that package transmittance had a smaller effect on hexanal production than temperature.

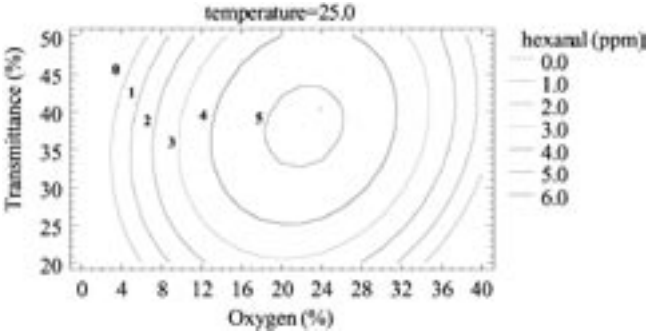


Fig. 8 - Iso response surface contour plots relative to the hexanal production in BPR: interaction between Transmittance (%) and Oxygen concentration (%).

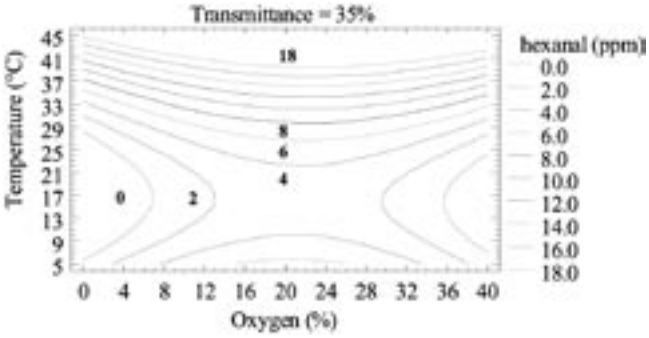


Fig. 9 - Iso response surface contour plots relative to the hexanal production in BPR: interaction between Temperature (%) and Oxygen concentration (%).

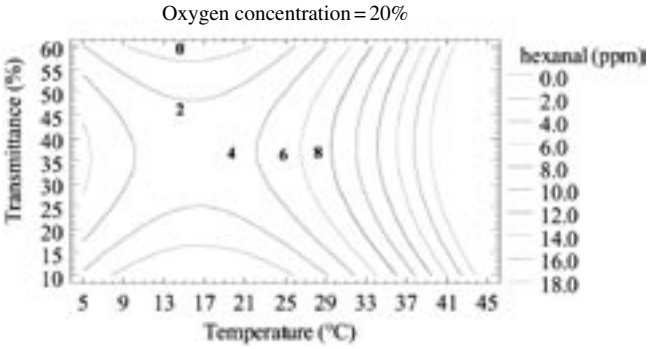


Fig. 10 - Iso response surface contour plots relative to the hexanal production in BPR: interaction between Transmittance (%) and Temperature (°C).

The degree of protection offered by the package to the light radiations is very important in increasing the shelf-life of BPR but during accelerated storage in which different factors are combined, temperature has the main role in catalysing the oxidative reaction.

CONCLUSIONS

Hexanal is really a good index of rancidity in BPR and it can be used in storage experiments to follow the rate of oxidation.

The effect of light on BPR degradation is evident and an accelerating factor can be calculated if temperature and oxygen concentration inside the package are kept constant during storage. In fact, the storage of BPR in a flexible package characterised by a transmittance value of 35%, in the presence of oxygen at 20.9% and at room temperature, accelerates BPR shelf-life about eight times.

The Central Composite Design used in this experimental work has been useful to understand the simultaneous effects of different variables on the oxidative status of BPR. This approach showed the important role of oxygen concentration in development of primary oxidation products and the significant effect of temperature in hexanal production. Increasing temperature and oxygen concentration, the effect of package transmittance at the chosen wavelength (330 nm) is less evident. Therefore, these preliminary results are useful to determine the optimal range of variables for an accelerated shelf-life test on brown parboiled rice.

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LECTURE

SHELF-LIFE PREDICTION OF SLICED FRESH APPLES

PREDIZIONE DELLA SHELF-LIFE DI MELE LAVORATE AL MINIMO

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ABSTRACT

The influence of dipping and modified atmosphere (MA) treatment on the decay of quality parameters of minimally processed apples packed in multilayer pouches during refrigerated storage was assessed. A model based on the principle of enzyme kinetics for predicting respiration rates of fresh produce was tested.

The model has successfully fitted the experimental data, corroborating the hypothesis used to derive it, both in the case of aerobic and anaerobic metabolism.

Quality parameter evaluation showed a significant effect of MA and particularly of dipping treatment to retard discoloration (evaluated with *tristimulus* colorimeter and image analysis), while texture was better maintained in non dipped samples.

The synergistic effect of MAP and dipping to maintain quality was much more evident in slowing down ripening and metabolism of the product.

- Key words: apples, dipping, enzyme kinetics, modified atmosphere packaging, respiration model -

RIASSUNTO

Nella ricerca oggetto di questo lavoro è stata valutata l'influenza di pretrattamenti (dipping) e del confezionamento in atmosfera modificata (AM) sul decadimento qualitativo di mele minimamente processate, confezionate in buste di film plastico multistrato, durante 8 giorni di conservazione refrigerata (4°C).

È stata inoltre testata l'efficacia di un modello, basato sui principi della cinetica enzimatica, per la previsione dei coefficienti di respirazione del prodotto.

Il modello è risultato approssimare bene i dati sperimentali relativi alle concentrazioni di O_2 e CO_2 nello spazio di testa delle confezioni per tutti i campioni realizzati (TQ: tal quale, confezionato in aria; A: tal quale, confezionato in AM; B: sottoposto a dipping, confezionato in aria; C: sottoposto a dipping, confezionato in AM), in condizioni di metabolismo sia aerobico che anaerobico.

I risultati relativi ai parametri qualitativi valutati hanno evidenziato un effetto positivo dell'AM utilizzata ed in particolare del trattamento di *dipping* adottato sull'inibizione dell'imbrunimento enzimatico (valutato con colorimetro tristimolo ed analisi d'immagine), mentre la consistenza del prodotto è risultata meglio preservata nei campioni non sottoposti a dipping.

L'effetto sinergico del trattamento di dipping e del confezionamento in AM è risultato maggiore nel rallentare la maturazione ed il metabolismo respiratorio di mele di IV gamma.

INTRODUCTION

Factors affecting the shelf-life of minimally processed fruit (MPF) are a result of a complex process concerning a number of physicochemical and biochemical modifications that mainly affect flavour, colour and texture (Mecarelli and Massantini, 1994).

Browning is a principal quality deterioration phenomena for MPF. Browning reactions in apples become evident when, for instance, the fruit is subjected to processing or to mechanical injury (LAURILA *et al.*, 1998).

Extensive studies have been performed to reduce undesirable browning and quality loss, by the application of antioxidants or reducing agents to substitute sulphites.

According to Ponting and Joslyn (1972), sliced Golden Delicious apples could be protected from browning by using a mixture of ascorbic acid (AA) (0.5%) and citric acid (CA) (0.05%) at pH 7. Sapers and Douglas (1987) showed that the treatment of cut apple surface with 1% CA solutions containing 0.4, 0.8, 1.6 or 3.2% of AA is effective in the inhibition of browning.

Modified atmosphere packaging (MAP) has become a widely used food preservation technique which minimally affects fresh product characteristics, and hence fits in well with the consumer trend for fresh, natural and additive-free food (DAY, 1996).

MAP reduces the rates of respiration and ethylene production, retards softening and changes occurring in the product, by creating and maintaining an optimum micro atmosphere (usually reduced O_2 and elevate CO_2 levels) (Lee, 1991).

Recently there has been great interest in the potential benefits of using nitrous

oxide (N_2O) for MAP applications. Nitrous oxide has shown a significant anti-ethylene activity by extending the lag phase of ethylene production in fresh fruits (Gouble *et al.*, 1995). Respiration rates of fresh-cuts generally were higher than the intact product (Watada *et al.*, 1996). Haykawa *et al.* (1975) obtained analytical solutions to model gas evolution during storage of fresh product as function of respiration rate and packaging envelope permeability.

LEE and co-worker have developed a simple, semi-empirical respiration model, based on enzyme kinetic principles, that adequately described the respiration of fresh produce in terms of O_2 consumption rate and CO_2 evolution rate (r_{O2} and r_{CO2}) as a function of O_2 and CO_2 concentrations. The model was confirmed with published data for a wide variety of commodities and with experimental data (Lee *et al.*, 1991).

This research is aimed at studying the influence of dipping and MA treatment on the decay of quality parameters (colour, texture, ripening index) of minimally processed apples, packed in multilayer pouches, as a result of enzymatic reactions. Another purpose of this work was to evaluate r_{O2} and r_{CO2} with kinetic equation proposed by Lee (1991), to evidence the metabolic effect of dipping and MA treatment.

MATERIALS AND METHODS

Model description

Respiration kinetics

Based on the similarity of fresh produce respiration and microbial respiration, Michaelis-Menten type enzyme kinetics was applied to model respiration of the fresh product (LEE *et al.*, 1991).

The dependence of respiration on CO_2 concentration was modelled on the basis of the uncompetitive inhibition, which has been used exclusively for product inhibition in reactions involving several substrate and products (Cornish-Bowden, 1979).

For O_2 consumption rate and CO_2 production rate, the uncompetitive inhibition mechanism due to CO_2 is expressed as:

Oxygen

$$r_{O_2} = \frac{V_m \cdot [O_2]}{K_m + \left(1 + \frac{[CO_2]}{K_i}\right) \cdot [O_2]} \quad (1)$$

Carbon dioxide

$$r_{CO_2} = (K_1 + K_2 \cdot CO_2) \cdot \frac{V_m \cdot [O_2]}{K_m + \left(1 + \frac{[CO_2]}{K_i}\right) \cdot [O_2]} + K_3 \cdot \exp(-K_4 \cdot [O_2]) \quad (2)$$

Mass balance equations

The mass balance on the oxygen and carbon dioxide in the package head space was carried out by solving the ordinary differential Eq. (3) and (4) derived previously by Hayakawa *et al.* (1975):

$$\frac{d\left(n_{O_2}(t)\right)}{dt} = S \cdot P_{O_2} \cdot \frac{\left[p_{O_2}^{est} - \frac{n_{O_2}(t) \cdot R \cdot T}{V_{st}}\right]}{l} + - m_p \cdot \frac{V_m \cdot [O_2]}{K_m + \left(1 + \frac{[CO_2]}{K_i}\right) \cdot [O_2]} \quad (3)$$

$$\frac{d\left(n_{CO_2}(t)\right)}{dt} = S \cdot P_{CO_2} \cdot \frac{\left[p_{CO_2}^{est} - \frac{n_{CO_2}(t) \cdot R \cdot T}{V_{st}}\right]}{l} + + m_p \cdot \left\{ \left(K_1 + K_2 \cdot [CO_2]\right) \cdot \frac{V_m \cdot [O_2]}{K_m + \left(1 + \frac{[CO_2]}{K_i}\right) \cdot [O_2]} + K_3 \cdot \exp\left(K_4 \cdot [O_2]\right) \right\} \quad (4)$$

It is worth noting that the term $K_3 \cdot \exp(-K_4 \cdot [O_2])$ takes into account the extra production of carbon dioxide related to anaerobic respiration.

Nomenclature

$[O_2]$	Oxygen concentration in the package head space (%)
$[CO_2]$	Carbon dioxide concentration in the package head space (%)
V_m, K_m, K_i, K_1, K_2, K_3 and K_4	Model's parameters obtained by fitting
r_{O2}	Oxygen consumption rate (moli/(kg·h))
r_{CO2}	Carbon dioxide production rate (moli/(kg·h))
n_{O2}	Mole of oxygen in the package head space
n_{CO2}	Mole of carbon dioxide in the package head space
S	Area of the package surface (cm^2)
P_{O2}	Package oxygen permeability ($mole \cdot cm \cdot cm^{-2} \cdot s^{-1} \cdot atm^{-1}$)
P_{CO2}	Package carbon dioxide permeability ($mole \cdot cm \cdot cm^{-2} \cdot s^{-1} \cdot atm^{-1}$)
p_{O2}^{est}	External oxygen partial pressure (atm)
p_{CO2}^{est}	External carbon dioxide partial pressure (atm)
m_p	Mass of the packed product (kg)
V_{st}	Volume of the package head space (cm^3)
T	Temperature (K)
R	Universal gas constant
l	Film thickness (cm)
t	Time (h)

The model's parameters were obtained by fitting equations (3) and (4) to the experimental data.

The permeability data of the plastic film and film thickness have been indicated by the supplier. Volume of the package head space was measured with destructive method.

Fitting procedures

To fit the experimental data with the proposed model the Robust method was adopted (Press *et al.*, 1989). There is a double reason for using the Robust method: firstly, the model is not a simple expression, but it consists of a set of ordinary differential equations that must be numerically solved; secondly, to properly validate the proposed model the two sets of available data (i.e., the oxygen and carbon dioxide concentration versus time) must be simultaneously fitted.

The set of differential equations was solved by using a fourth-order Runge-Kutta formula (Press *et al.*, 1989).

Sample preparation and modified atmosphere packaging

Fresh Golden Delicious (*Malus malus*) apples from Valtellina (Italy) were purchased from a local market at commercial maturity.

Golden Delicious variety was chosen, by preliminary trials, from other apple varieties on the basis of its lower susceptibility to browning.

After washing in tap water the apples were hand-peeled, cored and cut, with a sharp knife, into slices of 1 cm thickness.

For polyphenoloxydase (PPO) inhibition and maintenance of texture, apple slices were immediately dipped in an aqueous solution of 1% of Citric Acid (CA) and 1% Ascorbic Acid (AA) at 25°C for three minutes in a w/w proportion of 4 to 1.

All reagents used were of analytical grade.

After gently drying with blotting paper, 200 g of apple slices (about fifteen slices) were put into polypropylene boxes and packed in multilayer pouch (85 microns thick). Boxes and films were purchased by Hot Mould Sistem srl (Torino, Italy).

The permeability data of the plastic film at 23°C were the following, as indicated by the supplier: Water Vapour Transmission Rate (WVTR): 2.25 ($g/m^2/24h$) at 60% RH; O_2TR: 45 ($cm^3/m^2/24h$); CO_2TR: 20 ($cm^3/m^2/24h$).

The samples were packed using a ternary gas mixer (CVC, Milano, Italy) and a compensated vacuum-packing, Mod. Welding Machine (Food Basic, Vigevano, Italy).

An alternative protective atmosphere (MA) composed by 85% of nitrous oxide (N_2O), 10% of carbon dioxide (CO_2), 5% of oxygen (O_2) compared with air was used.

Four different samples were set-up:

TQ (undipped, packed in air);
A (undipped, packed in MA);
B (dipped, packed in air);
C (dipped, packed in MA).

The samples were stored in a refrigerated cell at 4 (±1) °C for 8 days.

Gas levels inside the pack were analysed every hour during the first day of storage and approximately on the first, second, fourth, sixth and eighth day of storage.

Other physico-chemical determinations were carried out on the fresh product and after dipping just before storage (zero time) and on the first, second, fourth, sixth and eighth day of storage.

Five sub-sample pouches per sample and storage time were prepared.

Physico-chemical determinations

The soluble solids (°Brix) content (SS) was determined at 20°C by refractometry using a digital refractometer (PR1, Atago, Japan) on the pulp obtained from apple slice samples, after filtering through Whatman #1 filter paper.

The titrable acidity (TA) was determined at 20° C through AOAC n. 22.058 (1990).

SS and TA were carried out for each sample on the pulp obtained from ten apple slices, two from each sub-sample box.

The ratio between SS and TA was used as ripening index (Sweeney *et al.*, 1970).

The texture analysis was achieved at room temperature (20 ± 2°C) by measuring the energy necessary for penetration of a 6 mm diameter stainless steel cylinder for 6 mm in apple slice tissues, using a Texture analyser mod. HD500 (Stable Micro Systems, Surrey, UK) equipped with a 50 kg load cell. Test speed was performed at 0.5 mm per sec and data are expressed as Kg sec.

Ten measurements were obtained for each sample from the five replicates with two measurements for each replicate. Average data for each sample was obtained from penetration tests carried out on ten slices.

The CO_2 - O_2 level measurements inside all sample boxes during storage were performed with a Check Point O_2/CO_2 (PBI Dansensor, Milano, Italy). The apparatus is based on an electrochemical sensor able to record O_2 content and on a mini-IR spectrophotometer able to record CO_2 in the pack atmosphere (accuracy: 0.1% O_2; 2% CO_2). The instrument was calibrated in ordinary atmosphere with O_2 and CO_2 air percentages.

Colour measurements

Tristimulus reflectance colorimeter

Surface colour was measured using a tristimulus reflectance colorimeter (Chromameter-2 Reflectance, Minolta, Japan), equipped with a CR-300 measuring head. Standard C.I.E. conditions, with illuminant "C" (6774K) were used. Parameters were expressed as L* and a* (Skrede, 1985; Sapers and Douglas, 1987) where L* corresponds to lightness, a* to red/green chromaticity. The instrument was calibrated with a white tile (L* = 98.03, a* = -0.23, b* = 2.05) before the measurements.

Ten readings were obtained for each sample from the five replicates with two measurements for each replicate. Colour was recorded using the CIE – L* a* b* scale.

All the measurements were carried out in quadruplicate.

Image analysis

The tristimulus approach to colour measurements was compared with an image analysis that was used to evaluate the colour because the fruits do not always go brown homogeneously (RUSS, 1995).

Images of apple samples were obtained by digitalisation using a colour

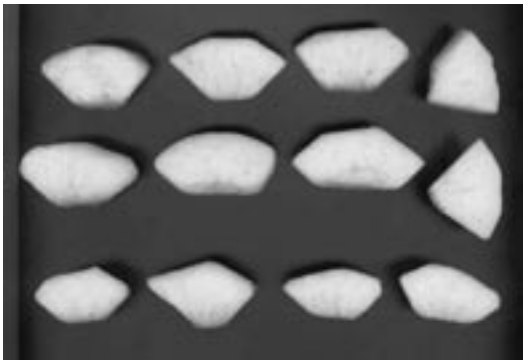


Fig. 1a.

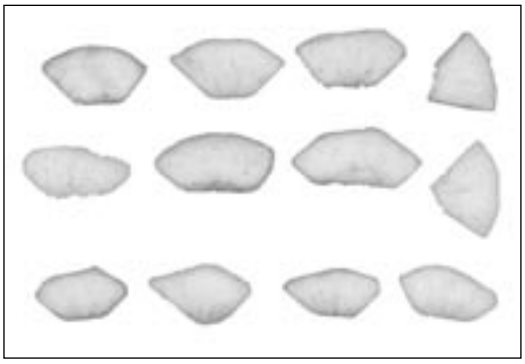


Fig. 1b.

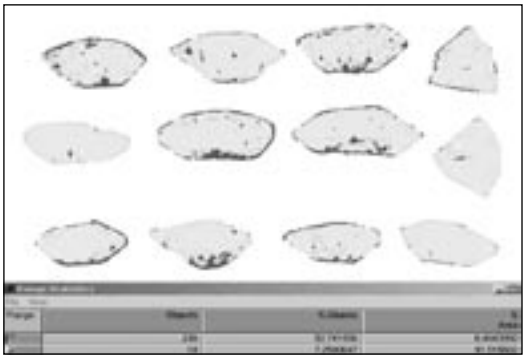


Fig. 1c.

plane scanner (Scan Jet 6300 C) (Fig. 1a). All images were obtained at the same conditions (true colour – 24 bit, resolution of 300 bit pixel) by positioning on the scanner the apple slices of each sample, held in a black box, in order to shut out the surrounding light.

After acquisition, digitalized apple slice images were isolated from the background using the software Photoshop® v. 5.0 (Adobe Systems Incorporated, USA) (Fig. 1b).

With advanced Image Analysis Software (Image Pro-Plus® v. 4.1, Media Cybernetics, USA), using RGB scale, apple images were evaluated in two steps: a) individuation of total sample area and b) evaluation of different level browning areas. On the bases of chromatic characteristics of all samples, a colour model was built up. The same colour model was applied to all apple slice images.

Browning areas were selected in pixel within the images. The software, examining all pixels in the image, calculated browning area percentage in total (Fig. 1c).

The measurements by image analysis were performed on the same slices used for colour determinations made with tristimulus colorimeter.

Statistical analyses

Analysis of variance (ANOVA) and the test of mean comparison according to Fisher least significant difference (LSD) were applied; level of significance was 0.99.

The statistical package STSG Statistica for Windows, version 6.0 (Statsoft Inc., Tulsa, UK) was used.

RESULTS AND CONCLUSIONS

In Table 1 the ANOVA analysis results between colorimetric and physico-chemical data of different samples are reported.

Table 1.

Sample	Physico-chemical and colorimetric determinations*				
	L*	a*	Browning area (%)	Firmness (kg x s)	SS/TA (°Brix/mg malic ac./100 g)
TQ	39.82 [a]	1.95 [b]	79.06 [b]	9.03 [c]	55.19 [b]
A	39.40 [a]	1.72 [b]	81.50 [b]	10.03 [d]	48.11 [ab]
B	42.33 [b]	- 0.21 [a]	39.24 [a]	4.94 [b]	44.35 [a]
C	41.47 [b]	- 0.28 [a]	12.20 [a]	3.91 [a]	41.46 [a]

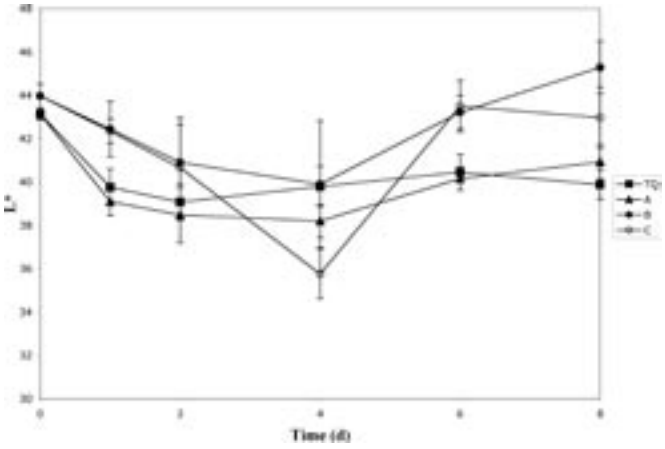


Fig. 2.

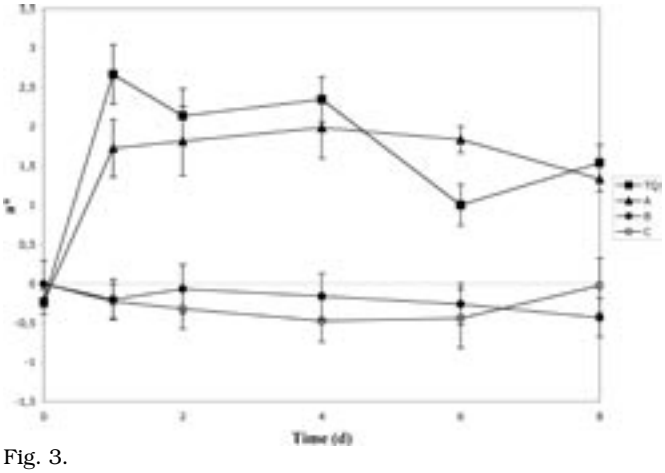


Fig. 3.

On the basis of ANOVA results and showed in Fig. 2 and 3, TQ and A samples were significantly different, in terms of L* and a* values, from dipped samples (B and C). For B and C samples a* parameter data did not show considerable changes during all storage time. To the contrary, just after one day of storage, TQ and A samples reached high a* values, maintained until the end of storage.

Therefore, from tristimulus colorimetric results, in particular red index a*, the dipping treatment was effective in the inhibition of enzymatic browning, according to other experimental works (PIZZOCARO *et al.*, 1993; PONTING and JOSLYN, 1972; SAPERS and DOUGLAS, 1987).

These results were confirmed by those obtained from image analysis (Fig. 4). In fact, TQ and A samples showed the highest percentage (about more than 80%) of browning area, just after the first day of storage. Sample C (dipped, packed in MA) showed the lowest browning level (10% of browning area) after 8 days of storage and seemed to better preserve its original colour.

Therefore, in our experimental conditions, image analysis results, seem to evidence a colour preservative effect of MA on apple slices, but only for samples dipped in the antioxidant solution.

As far as the firmness (energy in penetration during dynamometric test) was concerned, all samples showed significant differences (Table 1).

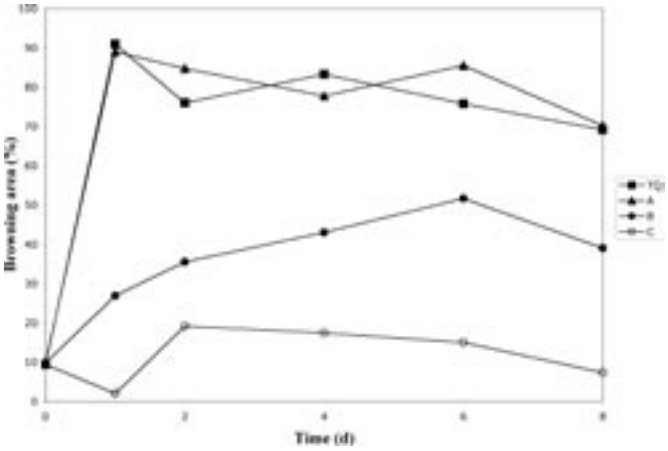


Fig. 4.

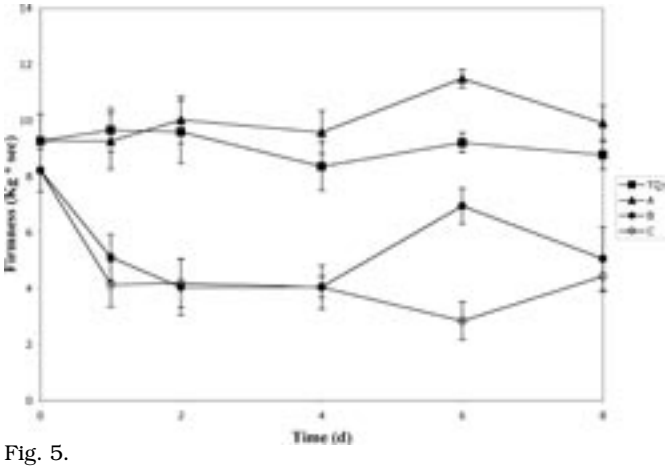


Fig. 5.

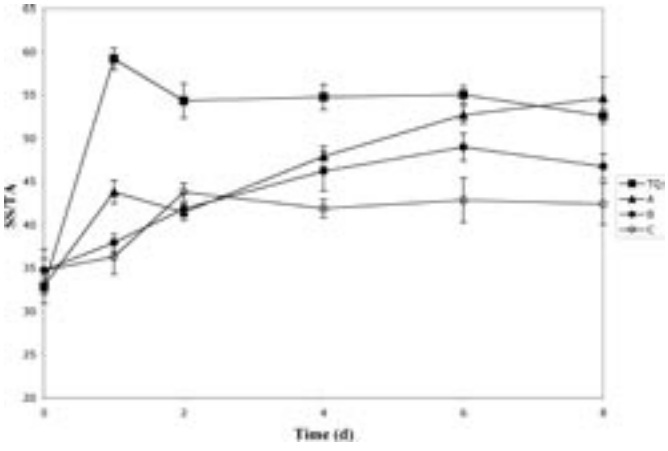


Fig. 6.

In particular dipped samples (B and C) showed a sudden decrease (Fig. 5) of firmness (from 8 to 5 kg s), just after the first day of storage.

Undipped MAP apple (sample A) showed the highest firmness value (more then 10 kg s) after 6 days of storage, while the firmness of undipped in air apples (sample TQ) slightly decreased during storage (from 10 to 8 kg s).

Therefore it would seem that dipping treatment caused a structural decay of fruits.

As shown in other experimental research (Mason, 1976; Johnson, 1979; Sams & Conway, 1984), the use of some salts (like calcium or sodium chloride) in the dipping solution could allow a preservative effect on firmness during refrigerated storage of fruits and have a synergistic effect with AA and CA on colour maintenance.

As show in Fig. 6, TQ sample has the highest ripening index value, just after 1 day of storage; however the ANOVA results of ripening index average values did not evidence significant differences between the other samples (A, B and C).

The lowest ripening index values of dipped samples (B and C) during storage, could be fictitious because of increased titrable acidity due to the effect of the acids used in the dipping solution.

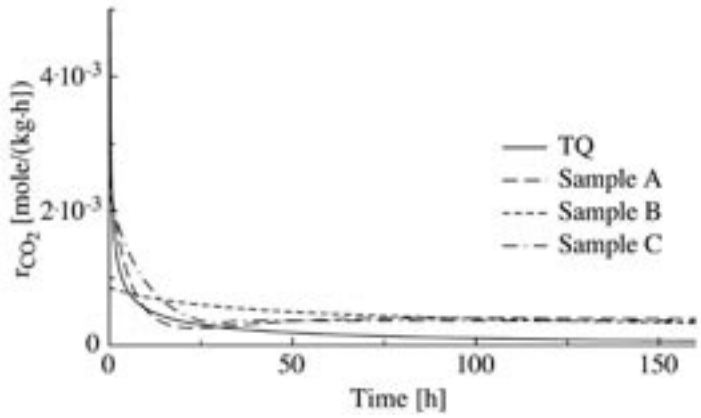


Fig. 7a.

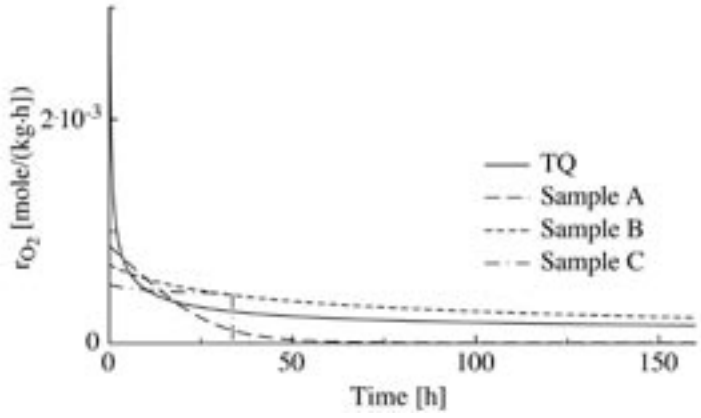


Fig. 7b.

Instead, comparing TQ and A sample ripening index behaviour the effect of MA on retarding ripeness is evident.

As shown in other experimental research on whole fruits, this effect could be due to the action of N_2O gas, that seems have an ethylene antagonist role (Gouble *et al.*, 1995; Qadir and Hashinaga, 2000).

It is worth noting that the model used successfully fits CO_2 and O_2 levels in the case of aerobic and anaerobic respiration in the fresh product.

The developed model successfully fitted our experimental data of CO_2 and O_2 levels inside sample packs, corroborating the hypothesis used to derive it.

In Fig. 7a and 7b, O_2 consumption and CO_2 production rates (r_{O2} and r_{CO2}) of the packed apple slice different samples are reported as a function of time, as well as computed from the respiration models.

As expected, sample TQ, untreated and packed in air, showed the highest respiration rate in terms of O_2 consumption and CO_2 production at the beginning of storage time. For this sample, r_{O2} coefficient remained quite high during all storage period.

Modified atmosphere packaging slowed down the initial r_{O2} in both untreated and dipped samples (samples A and C), while the dipping treatment in combination with air atmosphere (sample B) slowed the r_{O2} only at the beginning of the storage, maintaning this coefficient value during all storage time, as well as TQ sample.

The r_{CO2} level, very high at the beginning for all samples, with the exception of sample B (dipped and air packed apple slices) decreased along the storage time, in particular for the TQ sample that showed the highest r_{CO2} initial value. In the case of other samples (dipped and/or packed in MA), the residual r_{CO2} during all storage period remained slightly higher than in the TQ sample.

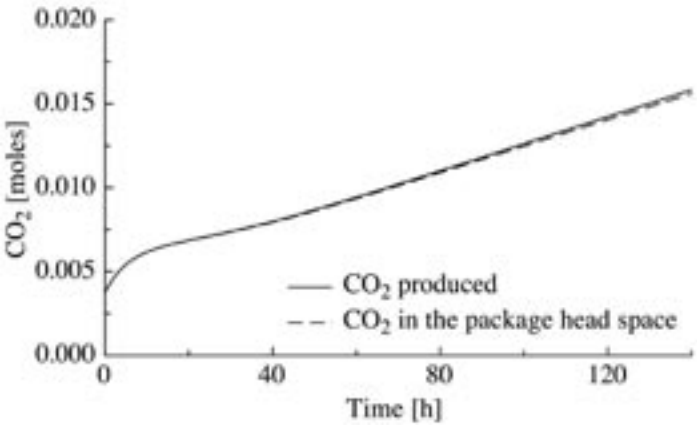


Fig. 8.

In Fig. 8, are showed, as an example, the behaviours of the level of CO_2 present in the package head space and the level produced by sample C apple slices during storage.

In all samples, there was a correspondence of the two CO_2 behaviours (data not reported). This result shows that the permeability of the film used did not influence the CO_2 balance inside the pack.

In conclusion, the dipping treatment was effective in the inhibition of enzymatic browning also if it would seem that it caused a structural decay of fruits. Moreover the effect of MA on retarding ripeness is evident and probably due to the antagonist role of N_2O ethylene.

Image analysis appears to have a potential as an objective assessor of MAP fruit browning, permitting colour evaluation of all product areas.

The model developed successfully fitted our experimental data of CO_2 and O_2, and it has shown an effect of MA used to reduce fruit metabolism.

This effect is probably due to a lower diffusion of O_2 in plant tissues, in the presence of more soluble N_2O gas (Mostardini and Piergiovanni, 2002). Dipping treatment has also been able to reduce the initial oxygen consumption but this effect during all the storage period was shown only in synergism with MA packaging. In all cases, the CO_2 production rate remained slightly higher in treated / MA packed samples than in the TQ sample, showing that the treated / MAP samples were closer than TQ sample to an anaerobic metabolism.

The use of a film with a higher gas permeability could be useful to avoid the incidence of anaerobic metabolism and the quality loss, due to of off flavour production and texture decay.

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INSTRUMENTAL TEXTURE DETERMINATION OF RICOTTA CHEESE DURING STORAGE

VALUTAZIONE STRUMENTALE DELLA TEXTURE DELLA RICOTTA NEL CORSO DELLA CONSERVAZIONE

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ABSTRACT

Textural parameters useful to monitor the shelf-life at 10°C of industrial Ricotta have been evaluated by means of the vane test performed under controlled shear rate operating conditions and by means of lubricated squeezing flow viscosimetry. Results confirm some previous observations describing the decay of the freshness textural attributes according to a transition trend. The Ricotta viscosity, evaluated under elongational deformation resulted to be on the order of 1E6 Pa s and to decrease with storage time. The objective assessment of Ricotta textural characteristics reflects the visual observations of the product spoilage that have been quantified, as color attributes, by means of image analysis techniques.

RIASSUNTO

La shelf-life della Ricotta a 10°C è stata monitorata attraverso parametri di texture ottenuti con il "vane test" operando in condizioni di velocità di deformazione controllata e tramite test di compressione ("squeezing viscosimetry"). I risultati confermano le osservazioni precedentemente effettuate sul decadimento degli attributi di texture tipici dello stato "fresco" secondo un andamento descrivibile da una funzione di transizione. La viscosità della Ricotta, ottenuta in condizioni di deformazione elongazionale, è dell'ordine di 1E6 Pa s e decresce nel corso della conservazione. La valutazione oggettiva delle caratteristiche di texture della Ricotta riflette le osservazioni visive dell'invecchiamento del prodotto che sono state quantificate, come attributi di colore, tramite tecniche di analisi dell'immagine.

- Key words: color, lubricated-squeezing, ricotta, shelf-life, texture, vane-test -

INTRODUCTION

Ricotta is a soft Italian cheese that is becoming popular all over the world thanks to its possible industrial application other than direct consumption. It is a whey protein gel with peculiar structure characteristics that are governed using various processing and/or formulation conditions. Ricotta has high moisture content and its initial pH above 6.0 (Campagnol, 2002) makes it susceptible to microbial spoilage and therefore to textural depletion: even under refrigeration, Ricotta has a limited shelf-life and depletion is perceived in sensory by consumers (Houg *et al.*, 1999). The texture of Ricotta, as well as of other soft dairy products, has been identified as an important property that determines consumers' acceptability. It is mainly affected by factors such as the operative conditions of the thermo-acid protein coagulation process driving the protein aggregation process, as has been be studied by rheology (Clark *et al.*, 2001). By oscillating rheometry, it has been determined that intermolecular disulfide bonds dominate in heat whey protein gels, while hydrophobic interactions in acid-induced milk gels. Covalent disulfide bonds contribute to the gel stabilization: to its elastic attributes, whereas non-covalent interactions give less elastic gels (Keim and Hinrichs, 2003). At the macroscopic scale, textural characteristics of soft solid structures, which necessitate careful sample preparation and loading in the testing apparatus, are often difficult to obtain: this may be the reason why texture testing procedures and indices are not conventionally included in the set of law parameters that are commonly selected to profile Ricotta quality attributes, i.e. microbial spoilage, chemical indexes of acidity, fats, proteins, dried matter, flavor profile, sodium chloride both for fresh product, both during shelf-life (Italian Standard UNI 10978, 2002).

The objective of the present work was to describe the kinetics of industrial Ricotta shelf-life, stored at 10°C, according to simple consistency indices obtained by means of simple mechanical tests. Quality control is an important area of application of the rheological analysis, where a large number of samples could be quickly tested at low unit costs. Results here presented are part of a wider study regarding the modelling of Ricotta shelf-life on the basis of chemical, biochemical, microbiological and rheological data. Biochemical events rapidly take place during storage due to enzymatic activities and they possibly modify the stabilizing bonds between particles and protein-solvent interactions (Campagnol, 2002). Changes of the structure of protein aggregates are thus induced and, hence, rheological properties, which are perceived as macroscopically modification of texture attributes (Philliphs *et al.*, 1994). In particular, as to the texture, we demonstrated from creep -recovery tests and "back-extrusion" analysis that the acidified and coagulated whey protein and casein mass shows a liquid-viscoelastic behavior, and that the protein gel structure is weak and brittle (Piazza *et al.*, submitted for publication), as expected for hydrophobic bonds-stabilized structures (Chardot *et al.*, 2003).

MATERIALS AND METHODS

An industrial fresh Ricotta cheese was studied in the present work. It was processed from whey obtained in Crescenza production and added with whole milk, according to a traditional technological scheme (Fig. 1). Individual packages (1 kg) of Ricotta were stored at 10°C. The product was analyzed after 1, 2, 4, 7, 8, 9 days storage.

Rheological analysis

The "vane test" was performed with the viscosimeter Viscotester VT 500 (Mess Technik Gmb Hu.Co., Germany), connected to a computer. A four-bladed vane rotor (FL 1000) attached to the machine was used for vane analysis. The vane dimensions were 8.8 mm height and 5 mm radius. The vane was gently lowered into the sample and rotated at a constant speed of 2 rpm for 60 seconds. Torque data were collected and vane stress (δ) was calculated. Results presented here are the average of seven replicates. The squeezing test was performed with a dynamometer Instron UTM (mod. 4301, Instron Ltd, High Wycombe, UK), equipped with a 10 kg load cell, operating at various cross-head speed (2,3 and 5 mm/min) and connected to a computer. Data were collected and elaborated by the "Instron SERIE IX" software and Table Curve 2D-v.4 software (Jandel Scientific). Ricotta cylinders (38 mm diameter and 10 mm thickness) were compressed to attain 80% deformation with a plate-plate geometry (35 cm diameter) covered with teflon sheets. The sample/platen interface was lubricated with silicon oil. For each storage time, the test was repeated four times at each compression rate. Viscograms are expressed as elongational viscosity (η_{el}) vs radial extension rate (ε_r). The elongational viscosity (Pa×s) was evaluated from the stress/cross-head displacement data according to Bagley et al. (Bagley *et al.*, 1988):

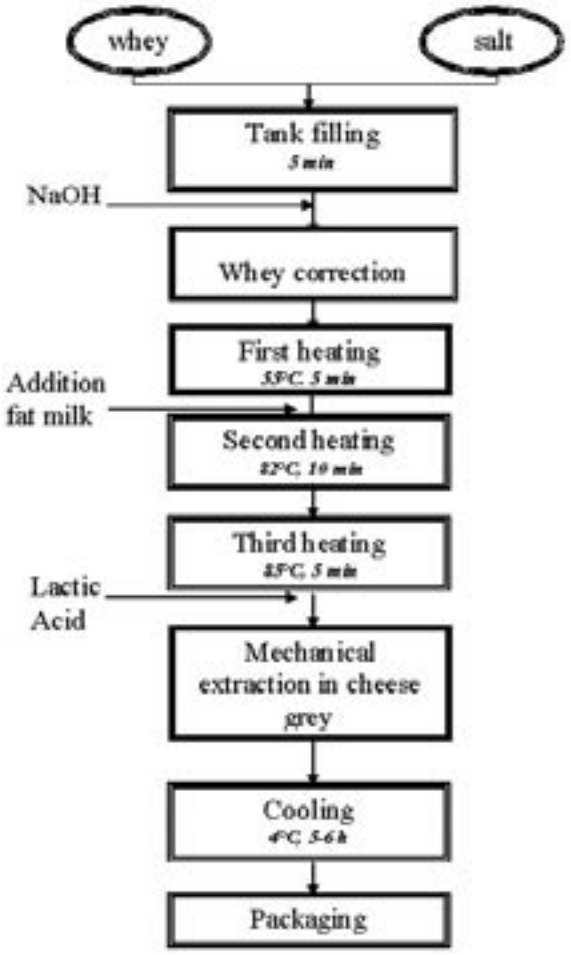


Fig. 1 - Flow sheet for industrial Ricotta production.

$$\eta_{el} = \frac{Fh}{\pi R_0^2 h_0 \varepsilon_r}$$

where F = the applied load; h_0 and h are the staring and the actual height of the sample; R_0 = staring radius; ε_r = radial extension rate =

$$= \frac{h}{2h}$$

Chemical analysis

Ricotta cheese acidity during time was evaluated. 10 g of sample were dissolved in 100 mL of water and homogenized. The solution was filtered and taken to 200 mL with distilled water. 100 mL of the resulting solution were titled by 0.1 N NaOH. Acidity was finally calculated as follows:

$$\text{Acidity} = (V * 0.18 * 10) / P$$

where: V= NaOH mL; 0.18 = conversion factor used for acid lactic; P = sample weight (g).

Image analysis

Digital images of Ricotta were taken by means of a photo-camera "Canon Power Shot Pro 90 Is", transferred to a PC and saved as JPEG images. For image acquisition, samples were lightened with a standard 45°C light angle. A previous calibration of white color was needed before each acquisition set. Color references for the yellow and the gray scales were also included in the digitalization field together with Ricotta samples and their chromatic RGB values were used in the calibration procedure. Digital images were then elaborated by means of the software Adobe Photoshop V 5.5. For each storage time, two images of Ricotta were taken the upper surface and the central slice of the ricotta geometry. A standard circular area of Ricotta was considered for the measure of the chromatic coordinates in the RGB scale. The obtained values were then converted in the L*a*b* (lightness, redness and yellowness) scale (Bonassi, 2002).

RESULTS AND DISCUSSION

A number of important spoilage biochemical events take place during Ricotta storage, resulting in the rapid loss of the freshness attributes of mouthfeel and texture (Hough *et al.*, 1999). Results of the rheological approach followed by our team to study the storage stability of Ricotta, support the hypothesis that the mechanism of Ricotta ageing is due to gel contraction inducing whey exclusion and consequently gel weakening (Piazza et al., submitted for publication). These observations are confirmed by a visual assessment of the structure depletion. In Figs. 2 and 3 the chromatic characteristics of the surface and of a central slice of the product during storage time are shown.

Results of the colorimetric analysis are expressed in terms of L* and b* coordinates. Ricotta surface shows an increase in lightness due to whey expulsion from the protein gel: the solvent layer on the ricotta surface causes light reflection phenomena, easy quantified as higher L* coordinate values. The yellowness parameter also increases with the storage time. On the contrary, the lightness index of the central slice decreased, where the serum macroscopic diffusion towards the outside portions of the mass is responsible for the decrease in reflection phenomena. The tendency to a weaker yellow color may be due to de-coloration of coloring substances (Campagnol, 2002).

The description of Ricotta shelf-life in terms of color attributes is therefore useful to follow the ageing process, but the measures are often affected by the

shadowing effects artificially induced by the sample lightening mode and by the evident non-homogeneity and anisotropy of the structure. The rheological approach seems to be a valid alternative to make fast, routine measurements that may be useful for product development and quality control.

In order to quantify the decay of Ricotta freshness during storage, a routine quality control method was applied. The methodology is called "Texture Profiling with the vane" (TPV). The use of rotating vane geometry has grown in popularity as a simple but effective means of measuring the properties of weak structures. It

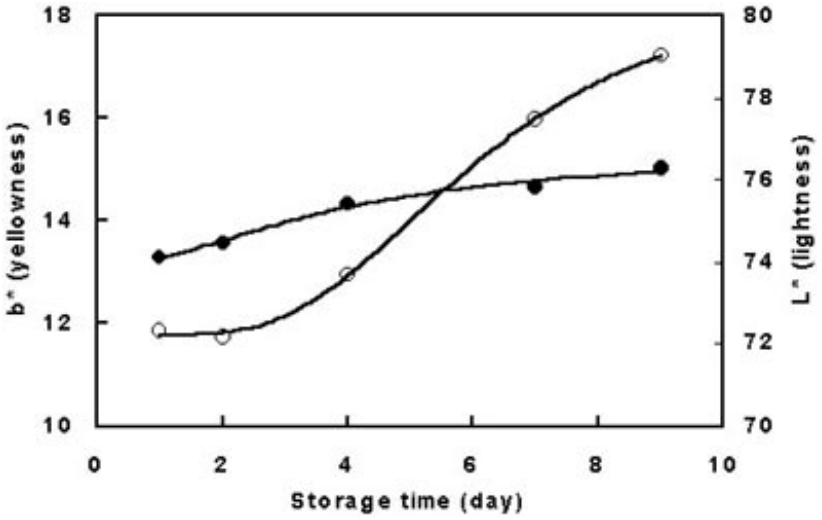


Fig. 2 - Color attributes of lightness L* (•) and yellowness b* (○) for Ricotta surface during storage.

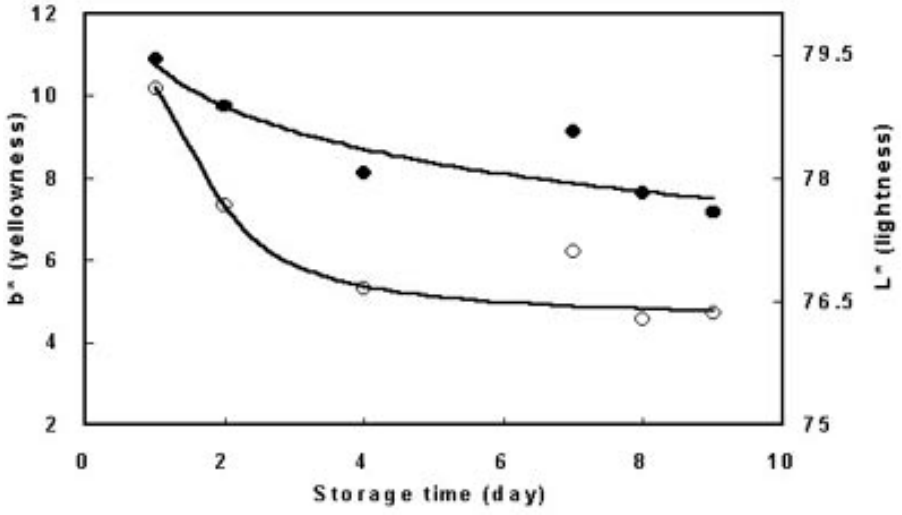


Fig. 3 - Color attributes of lightness L* (•) and yellowness b* (○) for Ricotta central slice during storage.

offers a way of introducing the testing element, a thin-bladed vane, into the sample, even in the original commercial package, with the minimum amount of disturbance to the sample, making it very suitable for materials such as structured liquid or soft solids. The thin-multi-bladed vane is introduced into a structured liquid and then rotated about its axis to make rheological measurements. It has been demonstrated that the product between the vanes is trapped within a circular cylinder and that the shear stress is evenly distributed over the surface of the cylinder, with the cylinder exactly defined by the vane tips. This allows the simplifying assumption that the vane behaves like a solid cylinder that is not slipping (Barnes and Nguyen, 2001). The use of the oscillating vane methods for the determination of the viscoelastic properties using a vane geometry has been validated to characterize soft solid structures in terms of yield stress and viscosity (Yoo and Rao, 1995; Breidinger and Steffe, 2001). Nevertheless, "Texture Profiling with the vane" (TPV) is also widely used to make fast, routine measurements in circumstances where other fundamental techniques are not available (Truong and Daubert, 2001). The vane test method is absolutely simple and timesaving, that being the reason why it is widely used in routine quality control protocols of soft solids. The vane is rotated into the sample at constant rpm for few minutes and peak torque values are plotted as a function of time. Fig. 4 shows a typical experimental curve where the shear stress vs testing time is presented.

The overall shape is characteristic of the sample: the peak may be quite pointed, or may be less rounded; the maximum can be at shorter or longer times; the level of the curve at long times may be higher or lower relative to the peak. These features can be correlated with specific features of the texture. In structured protein materials, the maximum shear value is assumed to be an index of intensity of the tri-dimensional network. In Fig. 5 it is shown that the maximum shear stress (σ_{max}) was modified during storage: the gel weakens and shows a trend that can be described with a transition function: two distinguished levels of σ_{max} are evident, showing a transition from a fresh to a non-fresh structural status.

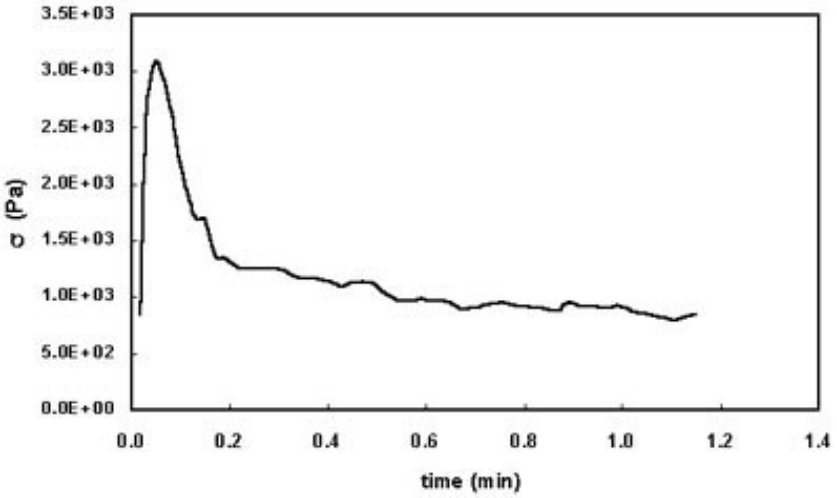


Fig. 4 - Typical shear stress (Pa) vs testing time (min) curve for Ricotta in the "vane" test.

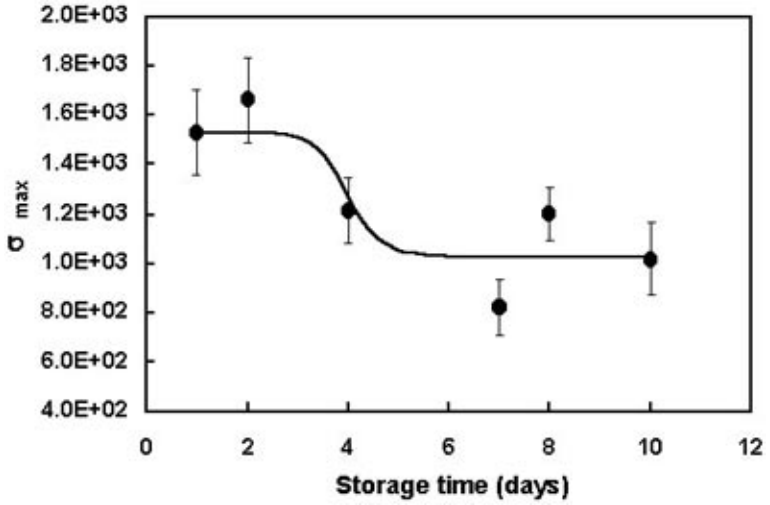


Fig. 5 - Kinetics of Ricotta aging in terms of maximum shear stress σ_{max} (Pa) vs storage time (days).

The presence of a certain degree of textural variability within and among the samples could not be ruled out, because product non-homogeneity exists. The analysis of the function derivatives was performed and the minimum value of the second derivative was identified as the storage time when the maximum acceleration of deterioration phenomena occurs. It was evaluated to 3 days.

Ricotta acidity was also evaluated during storage time (Fig. 6). The analytical index was modified during storage according to the same dependence on the storage time previously observed for the mechanical data: from the mathematical

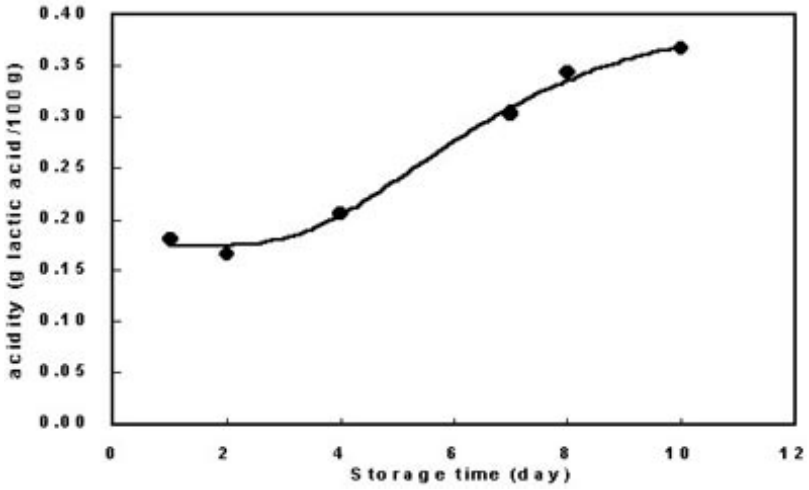


Fig. 6 - Kinetics of Ricotta aging in terms of acidity (g lactic acid/100 g) vs storage time (days).

analysis of the transition function the "stability time" for the fresh product may be quantified to be 3.2 days.

With the purpose of verifying if this analytical method that is currently used for the routine quality control may be suitable for monitoring Ricotta shelf-life, results obtained with the vane method were compared with other consistency indices describing Ricotta textural status. These were obtained with another mechanical test, the "back extrusion" test, designed to measure the thickness and the flowability of the material (Piazza *et al.*, submitted for publication). The term "back extrusion" has been generally used to describe the situation where a solid rod is thrust downward into a cylindrical cup containing the experimental sample. The downward movement of the rod displaces material, causing it to be extruded in the direction opposite that of the rod, i.e., backward; hence the name back extrusion. We assumed the thickness and the flowability parameters from the "back extrusion" test to be useful descriptors for fresh and stored Ricotta texture characteristics. They were modified during storage according to the same dependence on storage time previously observed and discussed for the "vane" test data. A unique straight correlation between a consistency parameter taken from the "back extrusion" test, precisely the maximum extrusion force, and the consistency parameter taken from the "vane " test is not evident all over the range of the parameters studied, but it seems to be effective only for the weaker structure, namely for non-fresh Ricotta cheese (Fig. 7).

Further evaluations are needed because the non-homogeneity of the structure is dramatically enhanced during storage time due to important changes in the solvent-protein relationship. This makes the physical assessment of the texture properties difficult to be reproduced. Nevertheless, for most purposes of the routine control, the comparative measurements are sufficient and, in conclusion, the TPV test seems to be suitable for comparative material characterization of soft-structured foods.

Otherwise, the fundamental rheological behavior of such materials might also be identified and used to optimize fabrication and forming. In these terms, the squeezing flow is an easy to use test and constitutes an interesting alternative to

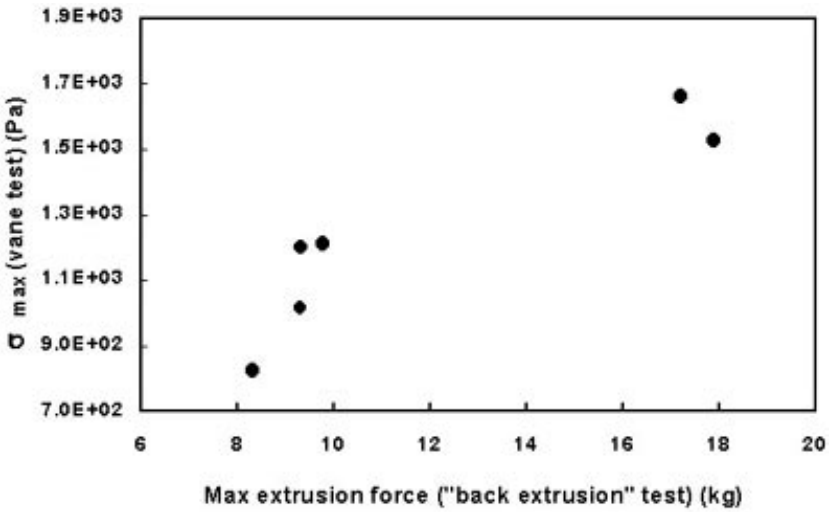


Fig. 7 - Correlation between textural parameters obtained by means of the "vane" test and by means of the "back extrusion" test (Piazza *et al.*, submitted for publication).

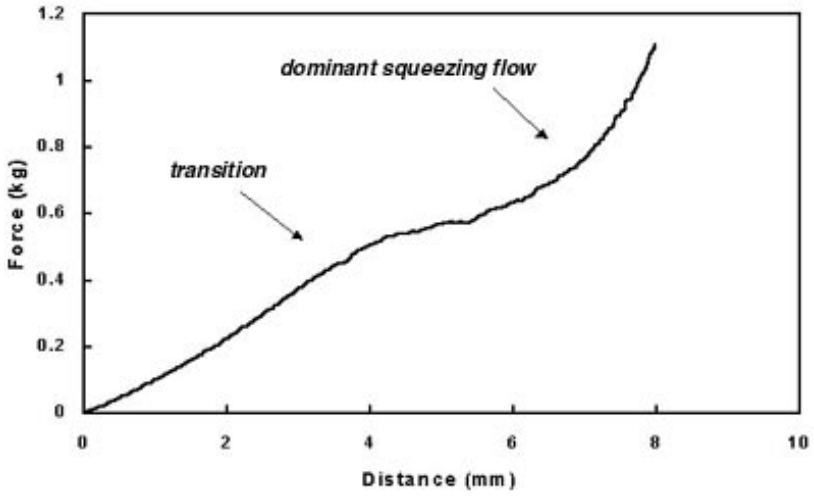


Fig. 8 - Typical Force (kg) vs cross-head desplacement (mm) curve for Ricotta in the "lubricated-squeezing" test.

the traditional rheometry test to quantify flow behavior of high viscosity materials. The test consists in squeezing a fluid-like material sample between two coaxial circular and parallel plates without rotation. If the bottom plate can be replaced with a shallow container what has been called "imperfect squeezing flow viscosimetry" is produced (Suwonsichon and Peleg, 1999). In the case of imperfect squeezing flow the contributions of buoyancy, annular flow, entry and end effects to the compression apparent stress must be accounted in the computation of flow parameters.

The parameters governing the flow are the radial extension rate, the radius of the sample, the starting height of the sample and the compression load. These parameters are imposed or recorded during the test. The plate surface type is also a test parameter. Use of rough plates is supposed to impose a sticking flow. Otherwise, with smooth and lubricated plates the sample can slip along the solid surface. The uniaxial compression in lubricated conditions can then be treated as a biaxial extension in the two directions perpendicular to the axis of compression: the biaxial flow generated makes possible the quantification of the elongational viscosity that is a parameter of interest in the industrial processing of Ricotta cheese, i.e. use of Ricotta as an ingredient in cakes or fresh pasta products. The elongational viscosity (η_{el}) calculated from the experimental force-vs-displacement data is a measure of the resistance against the extensional deformation, defined as the correlation factor between the compression stress and the gradient of the radial deformation (ε_r).

A typical compression curve is plotted in force/distance linear coordinates, as it is shown in Fig. 8 for a compression rate of 5 mm/min. After an initial part reflecting a transient flow regime, probably due to initial packing down of the material, the last part of the curve is considered as representing the region of dominant squeezing flow. The force/distance relationship was remarkably reproducible and enabled characterization of the product in terms elongational viscosity η_{el}.

The viscograms obtained are presented in Fig. 9 in a η_{el} - vs - ε_r plot for Ricotta compressed at a 2 mm/min cross-head speed for the fresh and staled product up to 10 days storage.

The viscogram referred to the oldest product shows that the η_{el} goes to a maximum and then falls off mildly. This qualitative type of behavior has been observed by Casiraghi *et al.* for cream cheese (Casiraghi *et al.*, 1985). Otherwise, for fresh ricotta or for the earlier aging times the curves rise monotonically and then flatten out with the η_{el} becoming independent of strain rate. This type of behavior was previously reported for products with higher solid viscoelastic properties (Bagley *et al.*, 1988). The asymptotic η_{el} values would depend on the deformation rate: they decrease with increasing cross-head speed.

Fig. 10 shows the trend of the extensional viscosity during the storage time at the

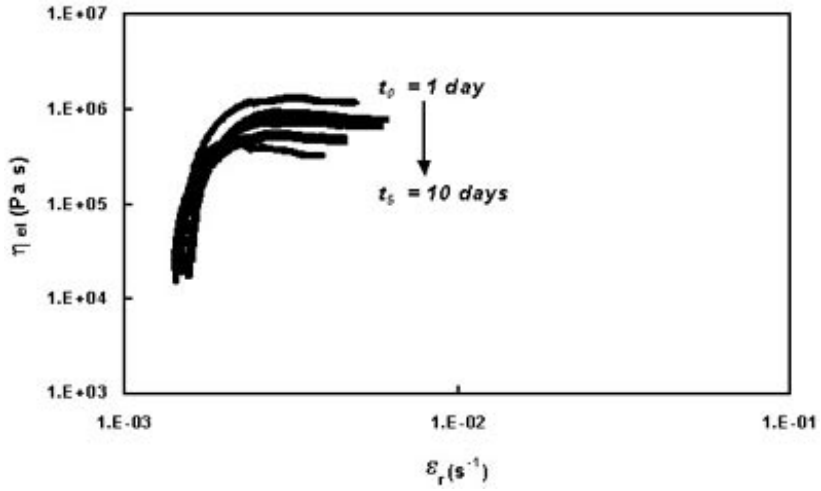


Fig. 9 - Viscograms for Ricotta samples from 1 up to 10 days storage (from the top to the bottom) in terms of elongational viscosity η_{el} (Pa s) vs gradient of the radial deformation ε_r (s^{-1}).

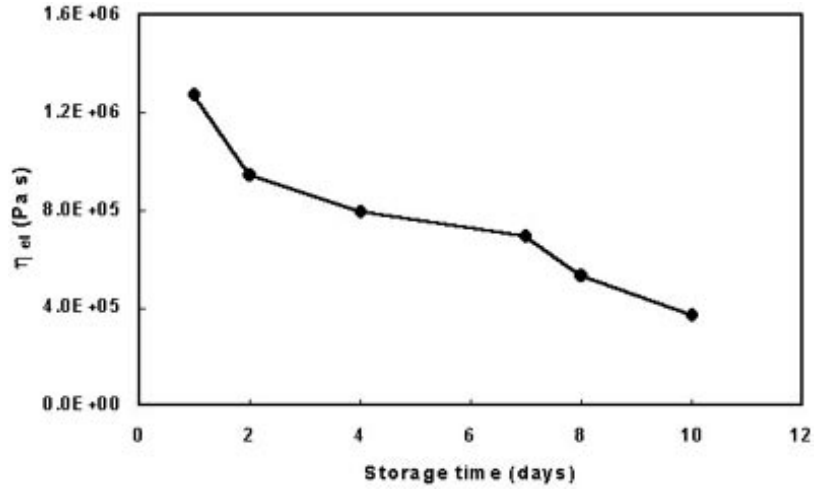


Fig. 10 - Kinetic of Ricotta aging in terms elongational viscosity η_{el} (Pa s) vs storage time (days).

cross-head speed of 2 mm/min, where the values of the parameter of the elongational flow were taken at fixed ε_r equal to 2.86e-3 s^{-1}. Analogous curves have been obtained when the compression has been performed at 3 and 5 mm/min, η_{el} decreasing with increasing cross-head speed for each storage time considered. It remains to verify if this behavior reflects a relaxation time distribution existing in a soft cheese.

In conclusion, a clear transition trend from a fresh to a staled state, as that previously proposed for the monitoring of Ricotta shelf-life by means of the TPV method, has not been obtained again and a threshold of "detectable freshness" is not reliable to propose. Nevertheless, the elongational flow parameter that has been determined by the lubricated squeezing test could still be used to visualize the textural differences between ricotta cheeses of different storage time. The measurements are sensitive enough to distinguish between the different age of Ricotta. Data obtained are a suitable reference for technical problems regarding industrial applications of Ricotta.

CONCLUSIONS

By means of the objective measurement of Ricotta surface and central color, a further way for formulating some hypotheses on the structural modification occurring during the storage period up to ten days at 10°C, has been studied. Two methods have been applied in order to study some textural characteristics of Ricotta cheese. A simple routine "vane" method resulted to be useful in soft-structure food characterization because the testing bob, a multi-bladed vane, can be directly positioned in the ricotta commercial vessel. That represents an advantage because loading of samples in the testing machines is a limitation in the application of physical analysis due to possible structural disruption caused when the sample is inserted into the measuring geometry. This method can be applied only if comparative measurements are sufficient in the product control procedures: by this method, the aim of obtaining a quantitative rheological interpretation of the textural data is actually a difficult task. Reliable data, on the other hand, have been obtained by means of the lubricated squeezing viscosimetry: the extensional viscosity data decrease with the storage time. The absolute values found by this method may be useful for technical purposes. The decrease of viscosity with the aging time confirms the trend we obtained by fundamental rheological analysis of Ricotta cheese (Piazza *et al.*, submitted for publication).

The rheological properties of Ricotta, and more generally of soft cheese, are key parameters required to solve food industry problems in numerous areas: quality control, process design and control, elucidation of food structure, including physicochemical changes which occur during processing and storage, and composition. Concerning Ricotta, further investigations are absolutely necessary to determine the relationships between stabilizing protein-protein interactions and the rheological properties, which allows to optimize storage stability, texture and sensory.

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MODELLING THE BARRIER PROPERTIES OF NYLON FILM DESTINED FOR FOOD PACKAGING APPLICATIONS

MODELLAZIONE DELLE PROPRIETÀ BARRIERA DI FILM DI NYLON UTILIZZATI PER IMBALLAGGIO ALIMENTARE

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ABSTRACT

In this paper a mathematical model able to predict the water and oxygen barrier properties of nylon film as a function of the water activity at the upstream and downstream sides of the film is presented. To validate the model, water sorption and permeation tests and oxygen permeation tests were conducted at 25°C. The fitting and predictive ability of the proposed model was successfully tested by fitting the model to the sorption data and by predicting the nylon water and oxygen permeability coefficient respectively.

RIASSUNTO

In questo lavoro viene presentato un modello matematico capace di prevedere le proprietà barriera di un film di nylon in funzione dell'attività dell'acqua a monte e a valle del film. Per validare tale modello, è stata determinata l'isoterma di assorbimento, le cinetiche di assorbimento e sono state effettuate le prove di permeabilità all'acqua e all'ossigeno, a 25°C. L'abilità predittiva del modello proposto è stata testata con successo interpolando i dati sperimentali con il modello proposto e riuscendo a prevedere il coefficiente di permeabilità all'acqua e all'ossigeno del Nylon. Le implicazioni della strategia adottata sugli aspetti pratici del confezionamento sono stati messi in evidenza confrontando le proprietà barriera all'acqua del nylon valutate con i test di permeabilità e quelle nelle reali condizioni di lavoro.

- Key words: oxygen permeability, sorption, water permeability -

INTRODUCTION

The barrier properties of flexible films play a major role in determining the shelf life of packed foodstuffs (Talasila and Cameron, 1997). In fact, polymeric films controlling the rate at which small molecular weight compounds permeate into or outside the package can slow down the detrimental phenomena responsible for the unacceptability of the packaged product. Generally, the barrier properties of polymeric films are simply determined by evaluating the permeability coefficient of a given polymeric-diffusant system. This approach can be successfully used wherever the permeability coefficient does not depend on the boundary conditions. In fact, in these cases the permeation coefficient is constant since the permeating molecules do not change much the free volume of the polymeric matrix. The above approach fails wherever the permeability depends on the diffusant partial pressure at the upstream and downstream side of the film, like in the case of water vapor transmission through moderately hydrophilic polymers. In fact, water molecules acting as plasticizers increase the macromolecular mobility of the polymer. As a consequence, both the solubility and diffusivity coefficient, and consequently the permeability coefficient, depend on the local water concentration. In these cases, the permeability coefficient can not be determined by a single measurement, instead a more accurate analysis of the permeation process is necessary to properly determine the barrier properties of the film in the real working conditions.

In the present paper a mathematical model able to predict the barrier properties of nylon film as a function of the water activity at the upstream and downstream side of the film is presented. To test the fitting and predictive ability of the proposed model water sorption tests and water and oxygen permeation tests were performed at 25°C.

MATERIALS AND METHODS

A nylon film with an average thickness of 70 μm was used to run both water sorption and permeation tests. It was supplied by SNIA Ricerche, and was used as received.

Water sorption kinetics were determined by means of a Hiden microbalance. After the sample was kept under vacuum (10^{-8} mbar) at 25°C, until it was completely desorbed, water vapor was introduced into the chamber by means of a set of valves connected to a water reservoir. The partial pressure was then increased in a stepwise manner. For the first sorption test, the water activity was increased from zero to 0.05. Afterwards, the water activity was increased as follow: 0.05→0.1, 0.1→0.2, 0.2→0.3, 0.3→0.4, 0.4→0.5, 0.5→0.6, 0.6→0.7, 0.7→0.8, 0.8→0.9. The sample weight, at all the different water activities, was monitored until the attainment of the equilibrium condition.

Water permeability was determined by means of Permatran (Mocon, Model W 3/31). Samples with a surface area of 50 cm^2 were tested at 25°C. The permeation tests were conducted by keeping the relative humidity at the downstream side of the film equal to zero, and changing the relative humidity at the upstream side of the film from 0.35 to 0.90. In particular, the permeation tests were run setting the relative humidity at the upstream side of the film equal to: 0.35, 0.45, 0.55, 0.65, 0.75, and 0.90. A flow rate of 100 mL/min of a nitrogen stream was used.

Oxygen permeability was determined by means of Ox-Tran (Mocon, Model

2/20). Samples with a surface area of 50 cm^2 were tested at 25°C. The oxygen permeability of the investigated film was determined setting the relative humidity at the downstream side of the film equal to 35%, while the relative humidity at the upstream side of the film was varied from 35 to 90%.

MODELLING

Oxygen permeability

According to the definition of the permeability coefficient, one can write:

$$J_{O_2} = P_{O_2}\left(p^1_W, p^2_W\right) \cdot \frac{\Delta p_{O_2}}{\ell} \tag{1}$$

where J_{O2} is the oxygen diffusive mass flux, D_{O2} the oxygen permeability coefficient, p^1_W and p^1_W the water vapor partial pressures at upstream and downstream side of the film, l the tickness of the film and Δp_{O2} the difference between the oxygen partial pressure at the upstream and downstream side of the film. The oxygen mass flux can be related to the oxygen concentration gradient assuming that the oxygen diffusion process can be described by means of the Fick's First Law with an oxygen diffusion coefficient depending only on the local water concentration:

$$J_{O_2} = -D_F^{O_2}\left(C_W\right) \cdot \frac{dC_{O_2}}{dx} \tag{2}$$

D^{O2}_F the oxygen diffusion coefficient, C_{O2} the oxygen concentration and C_W the concentration of total water sorbed at equilibrium into the polymer.

Substituting equation (1) in equation (2) and rearranging, the following expression is obtained:

$$\frac{dC_{O_2}}{dx} = -\frac{P_{O_2}\left(p^1_W, p^2_W\right) \cdot \frac{\Delta p_{O_2}}{\ell}}{D_F^{O_2}\left(C_W\right)} \tag{3}$$

Integrating the left and the right sides of equation (3) over the thickness of the film, and assuming that the solubilization process of oxygen into the polymeric matrix can be described by means of the Henry's law, one obtains:

$$P_{O_2}\left(p^1_W, p^2_W\right) = \frac{\ell}{\int_0^{\ell} \frac{1}{S_{O_2} \cdot D_F^{O_2}\left(C_W\right)} \cdot dx} \tag{4}$$

where S_{O2} is the oxygen solubility coefficient.

As reported in a previous paper (Del Nobile *et al.*, 1997), a simple expression to relate $D^{O2}{}_F(C_W)$ to the local water concentration can be obtained by rearranging the relationship proposed by Fujita (1961):

$$D_F^{O_2}(C_W) = B_1 \cdot \exp\left(\frac{-1}{B_2 + B_3 \cdot C_W}\right) \quad (5)$$

where B_i's are constants to be determined by fitting the proposed model to the experimental data. Substituting equation (5) in equation (4) one obtains:

$$P_{O_2}\left(p^1_W, p^2_W\right) = \frac{\ell}{\int_0^{\ell} \frac{1}{S_{O_2} \cdot B_1 \cdot \exp\left(\frac{-1}{B_2 + B_3 \cdot C_W}\right)} \cdot dx} \quad (6)$$

As can be inferred from equation (6), to determine the oxygen permeability coefficient, the steady state water concentration profile must be evaluated first.

Water permeability

An expression similar to equation (3) can be obtained for

$$\frac{dC_W}{dx}$$

by assuming that the water diffusion process can be described by means of the Fick's First Law with a water diffusion coefficient dependent on the local water concentration:

$$\frac{dC_W}{dx} = -\frac{P_W\left(p^1_W, p^2_W\right) \cdot \frac{\Delta p_W}{\ell}}{D_F^W(C_W)} \quad (7)$$

where $D^W{}_F(C_W)$ is the water diffusion coefficient, $P_W(p^1{}_W, p^2{}_W)$ the water permeability coefficient at the water vapor partial pressures at upstream and downstream side of the film equal to $p^1{}_W$ and $p^2{}_W$ respectively and Δp_W the difference between the water vapor partial pressure at the upstream and downstream side of the film.

To integrate equation (7) (i.e., to calculate the steady state water concentration profile) both $P_W(p^1{}_W, p^2{}_W)$ and $D^W{}_F(C_W)$ must be related to the local water concentration. It can be easily demonstrated that the water permeability coefficient is related to the local water concentration through the following relationship (Del Nobile *et al.*, 2003b):

$$P_W\left(p_W^1, p_W^2\right) = \frac{1}{p_W^1 - p_W^2} \cdot \int_{C_W\left(p_W^2\right)}^{C_W\left(p_W^1\right)} D_F^W\left(C_W\right) \cdot dC_W \tag{8}$$

The relationship proposed by Fujita (1961) was used to describe the dependence of water diffusion coefficient on the local water concentration:

$$D_F^W\left(C_W\right) = A_1 \cdot \exp\left(\frac{-1}{A_2 + A_3 \cdot C_W}\right) \tag{9}$$

where A_i's are constants to be determined by fitting the proposed model to the experimental data.

Substituting equations (8) and (9) in equation (7) one obtains:

$$\frac{dC_W}{dx} = -\frac{\left(\frac{1}{p_W^1 - p_W^2} \cdot \int_{C_W\left(p_W^2\right)}^{C_W\left(p_W^1\right)} A_1 \cdot \exp\left(\frac{-1}{A_2 + A_3 \cdot C_W}\right) \cdot dC_W\right) \cdot \frac{\Delta p_W}{\ell}}{A_1 \cdot \exp\left(\frac{-1}{A_2 + A_3 \cdot C_W}\right)} \tag{10}$$

The set of equations consisting of equation (6) and equation (10) is the model used in this work to predict the oxygen barrier properties of the investigated nylon film. It is worth noting that to numerically integrate equation (10), a quantitative description of both the water solubilization process (necessary to determine the dependence of the equilibrium water concentration at the boundary on the external water vapor partial pressures), and the water diffusion process (necessary to determine the dependence of the water diffusion coefficient on the local water concentration) is necessary. In the following the above two processes are presented.

Water solubilization

Due to the presence of specific interactions between water molecules and hydrophilic sites on the polymer backbone, the sorbed molecules are in part randomly dispersed into the polymeric matrix (dissolved water or free water) and in part physically bonded to the hydrophilic sites (adsorbed water or bound water) (Netti *et al.*, 1996). Therefore, the total amount of sorbed water can be expressed as follow:

$$C_W = C_W^D + C_W^{ad} \tag{11}$$

where C^{ad}_W is the concentration of adsorbed water and C^D_W is the concentration

of dissolved water. The equation proposed by Flory (1953) to describe the mixing process of a linear polymer with a low molecular weight compound was used to relate C^{D}_{W} to the water activity:

$$\ln(a_W) = \ln\left(\frac{C_W^D}{\frac{\rho_W}{\rho_p} + C_W^D}\right) + \left(1 - \frac{C_W^D}{\frac{\rho_W}{\rho_p} + C_W^D}\right) + \chi \cdot \left(1 - \frac{C_W^D}{\frac{\rho_W}{\rho_p} + C_W^D}\right)^2 \qquad (12)$$

ρ_W is the water density and ρ_p the density of the dry polymer.

The dependence of C^{ad}_{W} on water activity was described by means of the Langmuir equation (Netti *et al.*, 1996):

$$C_W^{ad} = \frac{C'_H \cdot b \cdot a_W}{1 + b \cdot a_W} \qquad (13)$$

where C'_H is the adsorption capacity, b is a constant that takes into account the affinity between the adsorption sites and water molecules.

Combining equations (12) and (13), it is possible to relate C_W to the water activity. However, due to the form of equation (12), it is not possible to combine the above equations to obtain a relationship where C_W is an explicit function of a_W. In fact, for a given a_W the corresponding value of C_W was evaluated by first numerically solving equation (12) to determine C^{D}_{W}, then evaluating C^{ad}_{W} through equation (13), and finally summing these two terms.

Water diffusion

Water diffusion related to Brownian motions is generally described by means of the Fick's model with a diffusion coefficient depending on local water concentration. In the specific case of diffusion through a plane sheet, the Fick's model reduces to the following expression:

$$J = -D_F(C_W) \cdot \frac{\partial C_W}{\partial x} \qquad (14)$$

As previously reported, eq. (9) can be used to describe the dependence of the thermodynamic diffusion coefficient on the local penetrant volume fraction.

Several approaches are reported in the literature to describe solvent induced polymer relaxation (Del Nobile and others 1994). Among them, one of the simplest is that proposed by Long and Richman (1960). They assumed that when the external water activity is suddenly changed, the solvent concentration at the polymer surfaces first rapidly increases to a value lower than the equilibrium one. At a later stage, it continues to increase gradually reaching the equilibrium.

The instantaneous response of the system to the increase of the external water activity represents the elastic response of the polymer matrix to the external

perturbation. The value of $C_W^B(0)$ depends on both the initial macromolecular mobility, which in turn depends on the initial water concentration (i.e., $C_W(0)$), and the extent of perturbation (i.e., the difference between the final and initial water concentration at the film boundary, $[C_W(\infty) - C_W(0)]$. Due to the complexity of the phenomena involved, the following empirical relationship is proposed to relate $C_W^B(0)$ to $C_W(0)$ and $[C_W(\infty) - C_W(0)]$:

$$K = \frac{1 - \left[C_W(0)\right]^{K_1} \cdot \exp\left[-K_2 \cdot C_W(0)\right]}{\exp\left\{K_3 \cdot \left[C_W(0) - C_W(\infty)\right]\right\}} \tag{15}$$

K is the normalised initial water concentration at the boundaries of the film, and spans from zero to one.

The rate at which the water concentration at the boundaries gradually increases is directly related to the polymeric matrix relaxation kinetic. In this investigation the following empirical expression is proposed to describe the boundary condition relaxation rate:

$$\frac{d\alpha(t)}{dt} = \left\{\alpha_1 \cdot \exp\left[\alpha_2 \cdot C_W^B(t)\right] \cdot \sqrt{\alpha(t)}\right\} \cdot \left\{1 - \exp\left[-(1 - \alpha(t))\right]\right\} \tag{16}$$

$\alpha(t)$ is the normalised water concentration at the boundaries of the film at time t, it spans from zero to one, and represents the driving force of the polymer relaxation phenomenon.

Water concentration profile

The evolution during hydration of the water concentration profile (or alternatively, the water uptake kinetics) was calculated solving the water mass balance equation. The above differential equation was derived using equations (14) and (9) to describe the stochastic diffusion, and equations (15) and (16) to describe the polymeric matrix relaxation kinetic:

$$\frac{\partial C_W(t)}{\partial t} = \frac{\partial}{\partial x}\left\{\left[A_1 \cdot \exp\left(\frac{-1}{A_2 + A_3 \cdot C_W(t)}\right)\right] \cdot \frac{\partial C_W(t)}{\partial x}\right\} \tag{17}$$

Equation (17) was solved numerically with the following initial and boundary conditions:

$$\begin{cases} C_W = C_W(0) \Rightarrow t = 0;\ 0 < x < \ell \\ C_W = C_W^B(t) \Rightarrow \forall\ t;\ x = 0,\ x = \ell \end{cases} \tag{18}$$

The model proposed to describe water diffusion in the investigated edible film consists of equations (15)-(18), and it will be referred in the following to as the "Anomalous Diffusion Model".

For the sake of comparison, a simplified version of the proposed model (in the following it will be referred to as the "non-Ideal Fickian Model") was also used. The "non-Ideal Fickian Model" was derived neglecting the polymer matrix relaxation phenomenon. Therefore, the evolution of the water concentration profile during hydration was calculated by numerically solving equation (17) with the following initial and boundary conditions:

$$\begin{cases} C_W = C_W(0) \Rightarrow t = 0;\ 0 < x < \ell \\ C_W = C_W(\infty) \Rightarrow \forall\ t;\ x = 0,\ x = \ell \end{cases} \tag{19}$$

RESULTS AND DISCUSSION

Fig. 1 shows the equilibrium water concentration in the investigated nylon film plotted as a function of the water activity at 25°C. Fig. 1 also shows the best fit of equation (1) to the experimental data. The values of parameters appearing in equations (2) and (3), obtained by fitting equation (1) to the experimental data, are:

$$C'_H = 3.23 \cdot 10^{-3} \frac{\text{g Water}}{\text{cm}^3 \text{ Dry Polymer}};$$

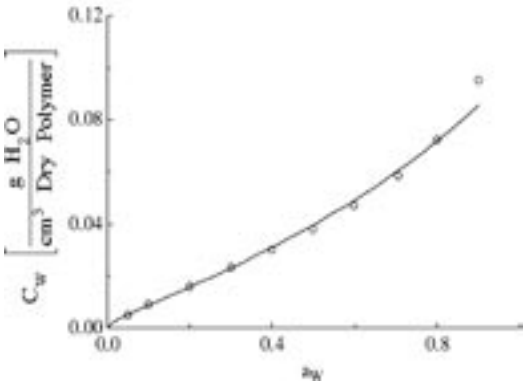


Fig. 1 - Water sorption isotherm at 25°C. (○) experimental data, (——) best fit of equation (1) to the experimental data.

b = 47.5; χ = 1.81. The goodness of fit was evaluated by means of the relative percent difference (Boquet *et al.*, 1978). In the case of the data shown in Fig. 1 a value of $\bar{E}$% equal to 3.22 was obtained. The good agreement between predicted (equation (1)) and experimental data suggests that the sorption process of water into the investigated films can be described in terms of the hypothesized dual sorption mechanism.

Fig. 2 shows the water uptake kinetics of the investigated film at the water activities of 0.4 and 0.5. In the same figure the curves representing the best fit of the proposed models to the experimental data are also shown. It must be highlighted that the proposed models were simultaneously fitted to all the sorption kinetics available. The models' parameters obtained are listed in Table 1, while the calculated values of $\bar{E}$%, obtained for each sorption kinetic curve, are reported in Table 2. As can be inferred from the data shown in Fig. 2 and listed in Table 2, to properly describe the water sorption kinetics, the superposition of the polymer relaxation to the stochastic diffusion must be taken into account.

Fig. 3 shows the diffusion coefficient, as predicted by means of equation (4) using the data listed in Table 1, plotted as a function of the local water concentration. The curves shown in Fig. 3 represent the relationship between D_F and C_W as determined by means of the two proposed models: "non-Ideal Fickian Model" and

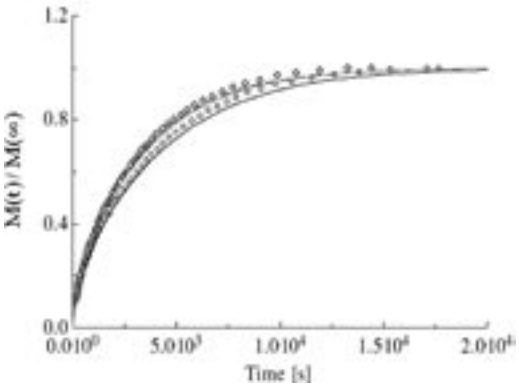


Fig. 2 - Water sorption kinetic at 25 °C. $a_W^{in.}$ = 0.3, $a_W^{fin.}$ = 0.4: (○) experimental data, (——) best fit of the "Anomalous Diffusion Model" to the experimental data, (---) best fit of the "non-Ideal Fickian Model" to the experimental data. $a_W^{in.}$ = 0.4, $a_W^{fin.}$ = 0.5: (◇) experimental data, (——) best fit of the "Anomalous Diffusion Model" to the experimental data, (---) best fit of the "non-Ideal Fickian Model" to the experimental data.

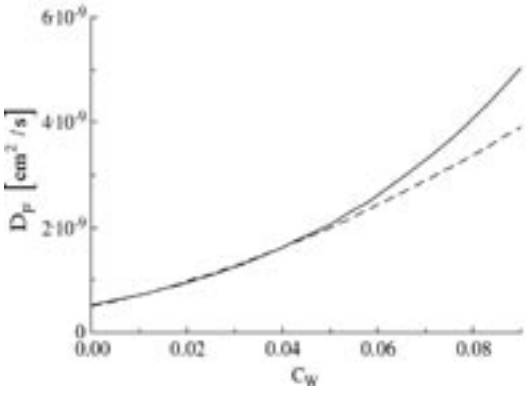


Fig. 3 - Water diffusion coefficient plotted as a function of local water concentration as predicted by the proposed models using the data listed in Table 1. (——) "Anomalous Diffusion Model", (— —) "non-Ideal Fickian Model".

"Anomalous Diffusion Model". The difference between the above curves increases with water activity, demonstrating the necessity to use a more accurate model to determine the above relationship wherever solvent induced polymer relaxation can not be neglected.

To point out the implications of the adopted approach on the practical aspects of packaging, Fig. 4 shows the water permeability of the investigated nylon film, as determined by means of permeation tests, plotted as a function of the water activity at the upstream side of the film. The curves shown in the same figure were obtained by predicting the water permeability coefficient by means of the proposed models using the data listed in Table 1. As it can be inferred from the data shown in Fig. 4, the ability of the "Anomalous Diffusion Model" to predict the experimental data is quite satisfactory ($\bar{E}$% equal to 5.43). The predictive ability of the "non-Ideal Fickian Model" decreases as the water activity increases (i.e., as the influence of the polymer matrix relaxation process on the diffusion becomes increasingly significant), being quite satisfactory at low water activity, and only acceptable at high water activity ($\bar{E}$% equal to 6.79). The above results suggest that at low water activity the water barrier properties of nylon film can be successfully predicted by both models proposed in this paper. While, at high water activity, when the superimposition of solvent induced polymer relaxation to stochastic diffusion

Table 1 - Values of the model's parameters obtained by fitting the model to the experimental data.

	non-Ideal Fickian Model	Anomalous Diffusion Model
$A_1 \left[\frac{cm^2}{s}\right]$	$9.22 \cdot 10^{-8}$	$1.37 \cdot 10^{-4}$
A_2	0.192	$8.03 \cdot 10^{-2}$
$A_3 \left[\frac{g\ Dry\ Polymer}{g\ Water}\right]$	1.38	0.196

Table 2 - Values of $\bar{E}\%$ for each of the water sorption kinetic tests conducted in this study.

$a_w^{in.} \Rightarrow a_w^{fin.}$	$\bar{E}\%$	
	non-Ideal Fickian Model	Anomalous Diffusion Model
$0 \Rightarrow 0.05$	9.46	7.85
$0.05 \Rightarrow 0.1$	9.15	7.75
$0.1 \Rightarrow 0.2$	6.21	3.05
$0.2 \Rightarrow 0.3$	6.73	2.45
$0.3 \Rightarrow 0.4$	4.47	2.56
$0.4 \Rightarrow 0.5$	4.82	2.90
$0.5 \Rightarrow 0.6$	4.40	2.72
$0.6 \Rightarrow 0.7$	5.23	5.31
$0.7 \Rightarrow 0.8$	4.70	2.96
$0.8 \Rightarrow 0.9$	6.36	1.41

Table 3 - Values of the model's parameters obtained by fitting the proposed models to the experimental data.

Model's Parameters	Values
$S_{O_2} \cdot B_1 \left[\frac{cm^3\,(STP) \cdot cm}{cm^2 \cdot s \cdot atm}\right]$	$4.39 \cdot 10^{59}$
B_2	$6.28 \cdot 10^{-3}$
$B_3 \left[\frac{g\ Dry\ Polymer}{g\ Water}\right]$	$1.05 \cdot 10^{-3}$

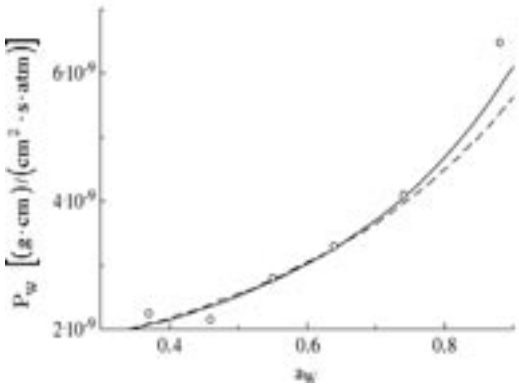


Fig. 4 - Nylon water permeability plotted as a function of water vapor activity at upstream side of the film (the water activity at the downstream side of the film is equal zero). (○) experimental data; (——) "Anomalous Diffusion Model", (— —) "non-Ideal Fickian Model".

cannot be neglected, only the "Anomalous Diffusion Model" is able to satisfactorily predict the water permeability coefficient.

Fig. 6 shows the oxygen permeability coefficient plotted as a function of the water activity (the water vapor partial pressure at the upstream side of the film is equal to that at the downstream side of the film). The data shown in Fig. 6 were obtained setting the oxygen partial pressure at the upstream side of the film equal to 1 atm, while that at the downstream side of the film it was set equal to zero. The curve shown in Fig. 6 was obtained by fitting equation (6) to the experimental data. Equation (6) reduces to the following expression when the water vapor partial pressure at the upstream side of the film is equal to that at the downstream side of the film:

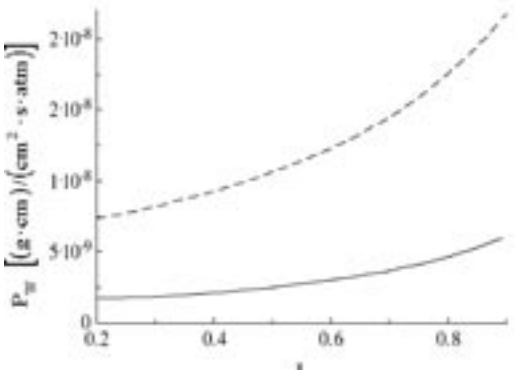


Fig. 5 - Nylon water permeability plotted as a function of water vapor activity on one side of the film; (— —) curve 1, (——) curve 2.

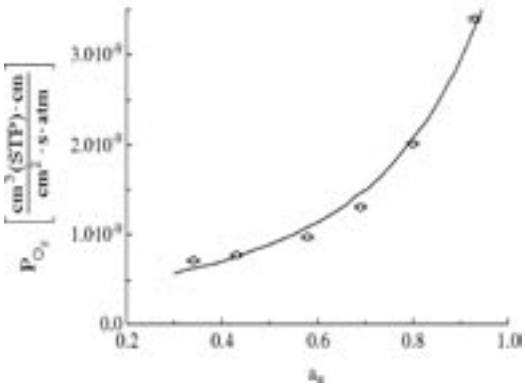


Fig. 6 - The oxygen permeability coefficient plotted as a function of the water activity (the water vapor partial pressure at the upstream side of the film is equal to that at the down stream side of the film). (○) experimental data, (——) best fit of equation (19) to the experimental data.

$$P_{O_2}\left(p'_w\right) = S_{O_2} \cdot B_1 \cdot \exp\left(\frac{-1}{B_2 + B_3 \cdot C^*_{w,f}}\right) \qquad (20)$$

The values obtained for the parameters appearing in equation (20) are listed in Table 3, while the calculated value of $\bar{E}\%$ is 7.04. It is worth noting that C^*_w was evaluated by means of equation (11). As it can be inferred from the data shown in Fig. 6 and from the calculated value of $\bar{E}\%$, the ability of the proposed model to fit the experimental data is quite satisfactory corroborating the assumptions used to derive it.

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A READY-TO-EAT FOOD: STEAMED CARROTS IN PROTECTIVE ATMOSPHERE PACKAGES

STUDIO FINALIZZATO ALLO SVILUPPO DI UN PRODOTTO DI V GAMMA: CAROTE COTTE A VAPORE

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ABSTRACT

In this work an integrated approach methodology is described for developing minimally processed vegetables. As model food steamed carrots were considered. A suitable composition of the protective atmosphere which allowed the appearance of carrots to be preserved for at least two weeks shelf-life, was found to be the one composed of 100% nitrogen. Microbial investigations were made to identify and eventually to eliminate critical points in the preparation technology (HACCP). Carrot samples, packaged in protective atmosphere, were analyzed after different days of storage at 4±1°C. Sensory and instrumental tests to evaluate colour, mechanical properties, weight loss, pH, and °Brix in order to monitor loss quality were performed.

RIASSUNTO

Scopo del presente lavoro è delineare una metodica multidisciplinare che consenta lo sviluppo di un alimento di V gamma. Come prodotto sono state scelte carote cotte a vapore. Indagini preliminari hanno consentito la definizione del range di composizione di un'atmosfera protettiva (100% N_2) tale da garantirne la corretta conservazione per un periodo di tempo pari a due settimane. Indagini microbiologiche hanno garantito la sicurezza d'uso dell'alimento e indicate le problematiche connesse alla tecnologia di preparazione (HACCP). Nelle fasi successive è stato effettuato il rilevamento dei fenomeni di decadimento qualitativo del prodotto, conservato in atmosfera protettiva (PA) per 1, 2, 3, 4, 7 e 14 giorni, mediante analisi colorimetrica, meccanica, gravimetrica, chimico-fisica (pH e grado zuccherino) e sensoriale.

- Key words: carrots, HACCP, PAP, ready-to-use, shelf-life -

INTRODUCTION

Interest in minimally processed foods in general, and in ready-to-use (RTU) fruits and vegetables in, particular, has increased significantly in recent years. Nowadays many ready-to-eat foods are available on the market such as raw and minimally processed (washed, trimmed, sliced) vegetables and cooked vegetables packed under vacuum. Delay quality of food and extending the shelf-life are purposes of protective atmosphere packaging (PAP) technology. Packaging food in an atmosphere different from air and composed of N_2, CO_2, O_2 mixed in different proportions allows chemical and biological phenomenon related to maturation and senescence in fruits and vegetables to be inhibited or slowed down. Different studies on PAP have concerned gas-mixture composition effects on foods: Kader (1985) defined O_2 and CO_2 optimal level, to use in PA in order to preserve quality for fruits and vegetables. For carrots, there are discordant results about CO_2, N_2 and O_2 optimal concentration in PAP. A CO_2 level higher than 4% can reduce the shelf-life of whole carrots, instead a mixture containing CO_2 less than 3% and O_2 higher than 2% can accelerate alterative reactions in stored carrots. Sode and Kuhn (1998) carried out some experiments on shredded carrots, stored in different gas mixtures, in order to study storage optimal conditions and valuate degradation causes. Shredded carrots don't produce ethylene and are not influenced by CO_2 presence in storage atmosphere. A CO_2 increase, however, could boost respiration rate, probably, because of mechanical damage affected during packaging. The aim of the present work was to describe an integrated approach methodology which helps in developing minimally processed vegetables. As model food steamed carrots were considered as a ready-to-use food corresponding to consumer demand and the intrinsic properties of the product.

MATERIALS AND METHODS

Carrots (*Daucus carota* L.) selected by visual inspection, for homogeneity, freshness and integrity, were washed, peeled and then cut in slices of 1 cm thickness and variable diameter. Samples were steamed for 9 minutes and then packaged (50 g each pack) warm in plastic trays (AERPACK mod. B5.37), having 780 cc volume and PO_2 (23°C - 50% UR) of 0.070 $ccatm^{-1}package^{-1}d^{-1}$. Packages were preserved at 4±1°C for 1, 2, 3, 4, 7, 14 days. The total aerobic micro-organisms (TMA) and survey of Enterobacters and Sporigens, in the samples just cooked (t_0), stored for one week (t_1) and two weeks in protective atmosphere (t_2) were performed. Carrots were then analyzed performing sensory and instrumental tests to evaluate colour, mechanical properties, weight loss, pH and °Brix. Eight judges assessed, by means of QDA, sensory properties of just cooked (taken as control) and steamed carrots stored under protective atmosphere. Attributes concerning appearance (colour), odour ("cooked carrot" odour, carrot odour in package), aroma ("cooked carrot" aroma), taste (sweetness, bitterness) and texture (firmness, juiciness) were defined. By means of a colorimeter (MINOLTA mod. CR-300), L* (brightness), a* (+a red; -a green) and b* (+ b yellow; -b blue) defined in CIE (1976) system, and ΔE* (1), valued by Hunter-Judd were measured.

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

To evaluate moisture content as water losses samples were dried in an oven

for one day at 105°C and then weighed. Texture analyses were performed by means of Instron dynamometer (mod. 4467): monoaxial compression with 100 kg loaded cell, 60 mm diameter piston and compression rate 50 mm/min was performed. 80% samples were deformed. pH measurements were done by a pH-meter (Mettler MP20). Sugar content of carrots, defined as °Brix, was performed by ABBE digitally refractmeter (mod. DR-A1, ATAGO). All data were statistically processed by SPSS 11.0 with ANOVA and Duncan test ($p<0,05$) in order to individualize significant differences among samples.

RESULTS

The first step of the design procedure was a preliminary screening among protective atmospheres in order to select those that better preserved appearance for at least two weeks. Steamed carrots were packaged by using 9 different gas-mixtures (Table 1) chosen by taking into account tolerance limits ($2\% < [O_2] < 8\%$ and $1\% < [CO_2] < 10\%$), reported in literature (Myers, 1989). For each gas-mixture two packages, were prepared and stored at 20°C and at 4°C. Additional carrot samples were packaged in air and stored at same temperatures, to study qualitative losses in not protective conditions. Packages were inspected after 7 and 14 days storage. The judgement was expressed by considering sample appearance (colour, mould presence), odour and texture. As one would expect refrigerated conditions preserved the sample better than storage at 20°C. After 1 week samples stored in low CO_2 concentrations (≤5%) became not acceptable anymore because of jelly formation on the surface. After 14 days of storage in atmosphere having low O_2 level (≤4%) carrots resulted not acceptable anymore because of decolouration. In presence of gas-mixtures composed of 70% N_2 - 30% CO_2 carrots showed colour and odour damage. Only the sample stored in 100% N_2 showed good properties after 3 weeks of storage so this atmosphere was choosen for the following analysis.

Microbial analysis

The second step was to verify product safety. Accordingly total aerobic microorganisms (TMA) count and survey of Enterobacters and Sporigens in the samples "just cooked" (R0) and in those stored under PA conditions for 7 and 14 days was performed. In order to avoid microbial risk an HACCP procedure was applied. Microbial data relative to carrots before and after HACCP application are reported in Table 2. During storage, increased microbial populations make the product not safe. However by HACCP procedure it was possible to reduce micro-organism proliferation. Data suggested that the cooking phase assured product microbial safety and that air-cooling of the product is the critical stage from a hygienic-sanitary safety point of view (data not showed). To overcome this risk the cooling phase was carried out in an environmental chamber, however it resulted not effective, hence carrots were packaged warm.

Table 1 - Gas-mixture composition (%).

% N_2	% CO_2	% O_2
100	0	0
90	2	8
90	3	7
90	4	6
90	5	5
90	6	4
90	7	3
90	8	2
70	30	0

Table 2 - Microbial data (CFU/g) of carrots before (No) and after (Yes) HACCP procedure.

Parameters	HACCP	R_0	7 days	14 days
TMA	No	<10	$4.0*10^4$	$2.3*10^6$
	Yes	<10	$2.0*10^3$	$3.3*10^4$
Enterobacters	No	<10	<10	$2.7*10^2$
	Yes	<10	<10	1.7*10
Sporigens	No	<10	<10	<10
	Yes	<10	<10	<10

Sensory analysis

Sensory quality of carrots was investigated in order to define qualitative losses by consumer perception. Data are reported in Table 3. Carrot samples stored in protective atmosphere for 14 days are significantly different to control for juiciness and for aroma attributes (odour of cooked carrot). Data suggested PAP technology allows a ready-to-eat food to be obtained with sensorial properties not different from the control.

Colour

Brightness (L*) of the carrots did not change during storage, by contrast a*(+) slightly decreased (data not showed). However colour difference ΔE* of "just cooked" carrots (R0) with respect to samples stored in protective atmosphere varied significantly (Fig. 1). ΔE* increased linearly during the first week then decreased during the second week.

Moisture content

Weight loss is reported as H_2O/W, in which W is sample weight and H_2O is water loss after 24h at 105°C. Samples stored in PA showed linear time dependence of the moisture content and weight loss increased since the third day of storage (data not showed). Differences among samples were, in particular, due to water respiratory loss. In the first three days of storage higher losses were due to cutting and related to time of scaring tissue. ANOVA data indicated that samples stored

Table 3 - Sensory data of carrots stored in PA until 14 days.

Attributes	Days storage (days)						
	0	1	2	3	4	7	14
At opening odour	7.83b	8.02b	7.71ab	7.63ab	6.95a	7.59ab	8.16b
Colour	6.17ab	6.83b	5.89a	6.38ab	6.13ab	6.18ab	6.77b
Cooked carrot odour	3.81a	4.87b	4.56ab	5.22bc	4.72ab	5.36bc	6.01c
Cooked carrot aroma	4.73a	4.14a	4.77ab	5.83b	4.54a	5.21ab	5.01ab
Sweetness	5.25ab	4.58a	4.27a	5.81b	5.22ab	5.89b	5.27ab
Bitterness	1.56ab	1.76ab	1.97b	1.10ab	1.67ab	1.16ab	0.93a
Firmness	6.33c	6.07bc	5.09ab	4.73a	5.87bc	6.45c	5.01ab
Juiciness	4.93a	6.07ab	5.47ab	5.62ab	5.47ab	6.23b	7.37c

Means within a row with different subscripts are significantly differences ($p \leq 0.05$).

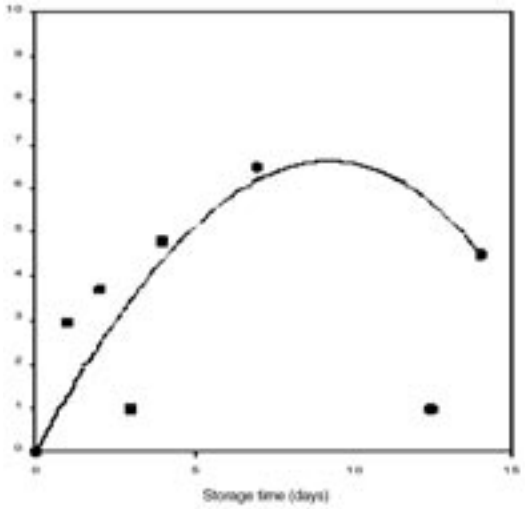


Fig. 1 - Colour difference ΔE* of carrots stored in PA.

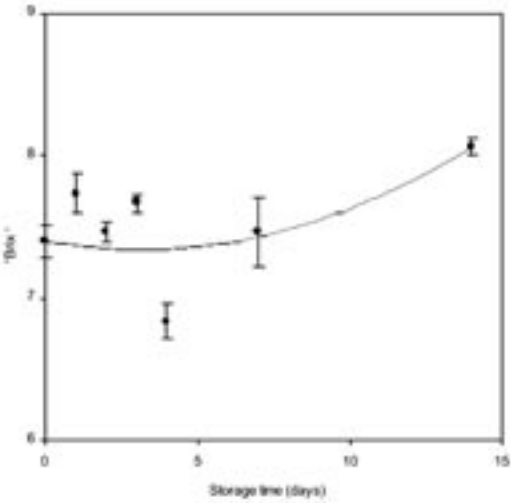


Fig. 2 - Time dependence of °Brix of carrots stored in PA.

for 4 and 7 days in PA were significantly different from control, there weren't differences ($p<0.05$) among other sample (data not showed).

Texture properties

Mechanical data are reported in Table 4. Control and carrots stored for 4 days in PA were different ($p<0.05$) among other samples by strain values. There were not differences about other texture parameters. According to sensory analysis, storage in protective atmosphere did not effect mechanical properties of ready-to-eat carrots.

pH and °Brix analysis

pH analysis performed on carrot samples stored in protective atmosphere suggested storage conditions did not influence the physical-chemical properties of the vegetable (data not showed). °Brix values in samples stored in PA are reported in Fig. 2. Sugar content in carrots stored in PA for 14 days was significantly different

Table 4 - Mechanical properties in carrots.

Days storage	Young modulus	Strain	Deformation	Energy
0	0.039a	0.026a	0.291a	11.44a
1	0.081d	0.036b	0.339ab	17.93b
2	0.04ab	0.036b	0.334ab	22.24ab
3	0.052bc	0.033b	0.323ab	16.00ab
4	0.031a	0.046c	0.345b	17.93ab
7	0.085d	0.037b	0.315ab	16.45ab
14	0.058c	0.034b	0.292a	13.10a

Means within a column with different subscripts are significantly different.

and higher than the control. Probably this phenomenon was due to degradation of starches, naturally present in the roots, in soluble sugars.

CONCLUSIONS

Although instrumental parameters were not significantly different, samples stored in PA exhibited sensory attributes different from the control. In particular sensory analysis demonstrated that, between samples and control, differences in the aroma attributes (odour of cooked carrot) and juiciness exist while the aspect and the gustative characteristics do not change significantly. Results of these analyses show through the application of protective atmosphere packaging it is possible to keep carrot quality at a high level for two weeks.

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TIME-TEMPERATURE EXPOSURE OF FRESH MILK DURING COMMERCIAL LIFE

ESPOSIZIONE TEMPO-TEMPERATURA DEL LATTE FRESCO DURANTE LA COMMERCIALIZZAZIONE

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ABSTRACT

The purpose of our study is to define the reliable time-temperature exposure conditions of traditional pasteurised fresh milk during its marketing and household storage. We tested a new kind of small temperature recording device, directly placed in the middle of the milk package, in order to obtain a number of recordings of overall thermal histories. The package with the device was delivered through the commercial chain. The data obtained were statistically analysed and plotted in time-temperature exposure probability charts (50, 75, 90% probabilities were considered). Our results show that the average exposure temperature in the commercial chain is higher (1°-2°C) than the recommended maximum (6°C). Our findings also show that a joined effort (manufacturer, sale point management, consumer advertising) could reduce the probable exposure temperature by 1°-2°C and justify an extension of the shelf-life of fresh milk from the present 4 days up to 6-7 days.

- Key words: fresh milk, shelf-life, temperature -

RIASSUNTO

Obbiettivo della nostra ricerca è stato il monitoraggio e la modellazione della storia termica del latte fresco pastorizzato durante la sua commercializzazione. Sono stati utilizzati dei micro-registratori in grado di misurare, dopo essere stati programmati e inseriti in una confezione di latte fresco, la temperatura a cui è esposto il latte durante il suo ciclo di vita commerciale. Parallelamente alla verifica delle condizioni di esposizione termica è stato valutato il tempo di residenza del latte fresco attraverso un'indagine diretta sulla rete commerciale rivolta ai clienti e con questionari consegnati a 150 famiglie. Le conclusioni possono essere riassunte in 4 punti: 1) la durabilità del latte fresco pastorizzato, a 6°C effettivi di temperatura, è ben superiore ai 4 giorni imposti dalle norme di legge e raggiunge sicuramente i 6-7 giorni; 2) la temperatura massima di esposizione nella filiera commerciale supera mediamente di 1°-2°C le tolleranze indicate dalle norme e risulta particolarmente "critica" nella fase di conservazione domestica; 3) i tempi medi di commercializzazione risultano più che tranquillizzanti rispetto al mantenimento della garanzia di freschezza del prodotto; 4) uno sforzo congiunto potrebbe ridurre di 1°-2°C le temperature più probabili di esposizione ed, a questo punto, giustificare, anche a livello normativo, un allungamento della durabilità del prodotto "tradizionale" da 4 a 6-7 giorni.

INTRODUCTION

Fresh pasteurised milk is a safe but delicate commodity, and its shelf-life is affected by psychrotrophic bacterial growth. Shelf-life temperature plays a fundamental role. In the commercial chain, temperature exposure and fluctuations are often unpredictable (Riva and Sardi 1998a, Riva and Sardi 1998b) and higher then recommended regulation tolerances (6°C) (Italian DLg n. 169, 03-05-1989).

The classical temperature measurements and recording (i.e thermocouples) are not efficient methods to monitor the commercial chain, because they reveal the environmental temperature instead of product conditions. Another issue is the extension of the shelf-life of traditional fresh milk, which is nowadays under discussion as a consequence of the introduction on the market of new products, assuring 8-10 days shelf-life.

The purpose of this study is to define the reliable time-temperature exposure conditions of traditional pasteurized fresh milk during its marketing and household storage.

We tested a new kind of small temperature recording device, directly placed in the middle of the milk package, in order to obtain a number of recordings of overall thermal histories. The final aim of our research is the monitoring and prediction of milk shelf-life.

MATERIALS AND METHODS

A Time Temperature Recording device (TB Econorma SAS), water proof, ±0.5°C temperature tolerance, was employed. Fig. 1 shows the features and the size of

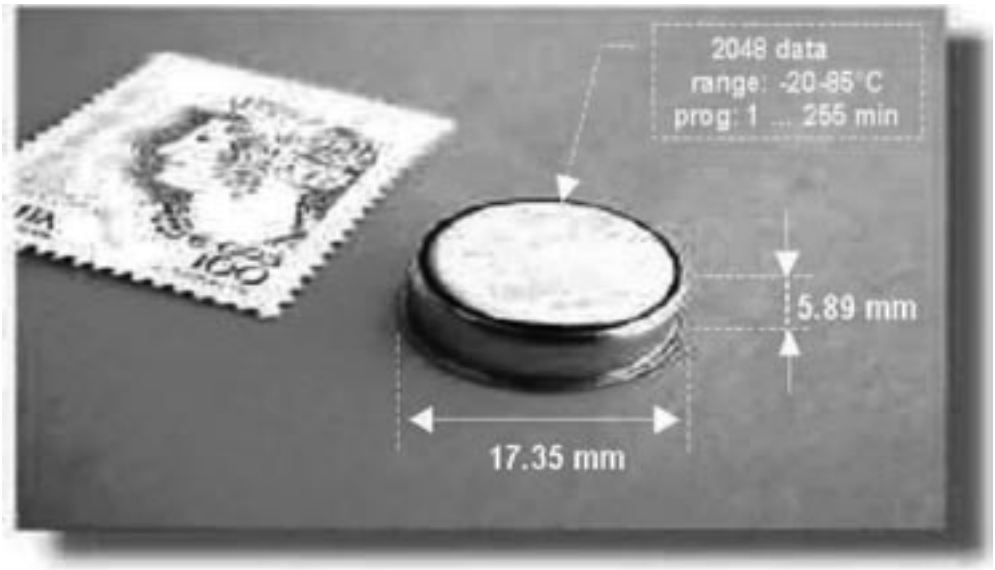


Fig. 1 - Picture of feature and size of time temperature recording devices.

a device. Each device was set and read by a PC. The devices were suspended at the verycentre of a fresh milk package at the beginning of its commercial life (Fig. 2). The package with the device was then delivered through the commercial chain: selected buyers were asked to take the product from the store and simulate the usual household storage. At the end of conventional shelf-life (4 days after packaging), the device was brought back by the consumers and data were downloaded into a PC. Thirty thermal histories, relating to twenty sales points, were so collected at the end of summer of 2002. Time data relating to the transfer to the consumers home and to the household storage (obviously affected by the methodology) were replaced with the results of a sample survey. In the survey two hundred consumers were asked to record accurately: time (day, hour and minute) of the purchase, time when the pack was placed in the domestic refrigerator, and time when the pack was first opened and consumed. Collected data



Fig. 2 - Placement of a time temperature recording device in a package of milk.

were segmented into five stages: 1) transport, 2) delivery, 3) sale points storage, 4) purchasing and transferring home and (5) home storage. For each stage and for each time temperature history the average time and the average temperature were computed.

Fig. 3 shows a complete record of a thermal history.

RESULTS AND DISCUSSION

The data obtained were statistically analysed and plotted in time-temperature exposure probability charts (50, 75, 90% probabilities were considered). The 50% probability (or cumulative frequency) refers to an average exposure if the 75% probability is used to "really" describe the thermal history of the product (July 1984).

Fig. 4 presents the probability chart of temperature exposure.

The transport and the sales point storage show the less critical thermal conditions (T°< 8°C in 75% of cases). Delivery, transport to consumers home and household storage are more critical because they depend on several factors, such as the climatic conditions. Household storage stage is obviously affected by intrinsic variability (in 1 out of 4 times, the refrigeration temperature was more than 10°C, and in 1 out of 10 times more than 12°C).

As our results show, it's difficult to keep the temperature at 4°C, usually used as a reference for fresh product storage, and at 6°C, recommended as maximum

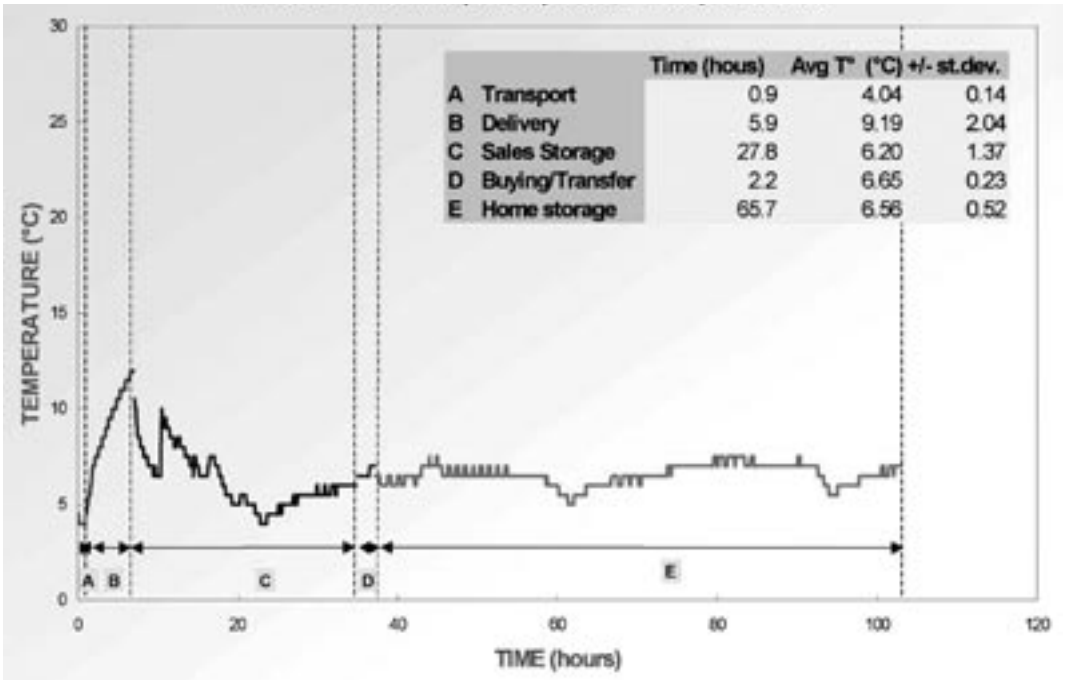


Fig. 3 - An example of complete recording of a thermal history: the insert shows the average data for each commercialization stage.

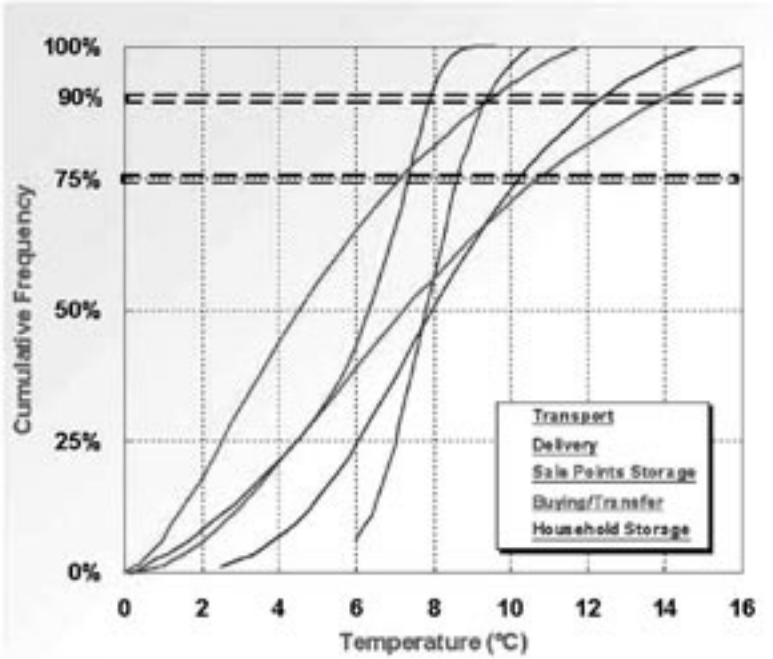


Fig. 4 - Probability chart of temperature exposition in different stages of fresh milk commercialization.

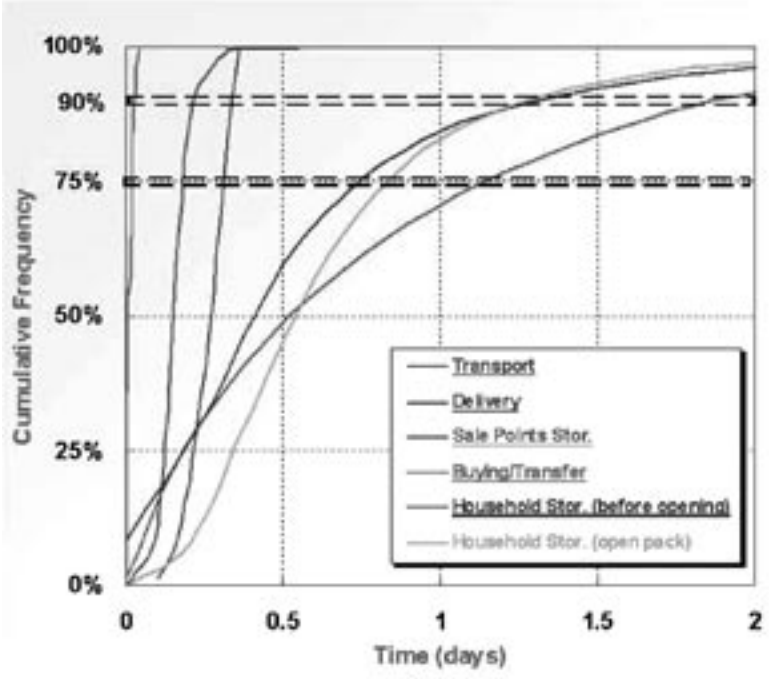


Fig. 5 - Probability chart of time exposition in different stages of fresh milk commercialization.

exposure temperature. In the time exposure probability chart (Fig. 5) the household storage period was segmented into two sub-stages: storage before and after package opening. These two sub-stages are the critical points for milk shelf-life: together they are longer than 1 day and, in 1 out of 4 times, more then 1.5 days. Our results show that the consumer generally buys fresh milk every 1 or 2 days, as a consequence a continual delivery of new stock.

In Fig. 6 our results are compared with a previous (five years ago, same local area, spring season) survey. In that study the temperature data were acquired by an indirect and less accurate device (TTI labels) (Riva and Sardi 1998a, Riva and Sardi 1998b). New data are slightly better than the older ones regarding the sales point and household storage. In the first case this is due to the logistics' and, probably, in the second one to the consumers' improved knowledge. From the obtained data, fresh milk shelf-life was measured based on time-temperature exposure in the commercial chain.

According to scientific literature (Labuza 1982, Singh and Heldman 1993) the following equation was applied:

$$RSL\ (Remaining\ Shelf\text{-}Life) = 1 - \sum_{i=0}^{n} \left(\frac{t_n}{t_s}\right)_{Tn}$$

Where t_n is the time lapse of the n-stage and t_s is the corresponding stability time at the temperature Tn.

The stability time (t_s) depends on temperature by the subsequent classical relation:

$$t_s = to \exp [Tn - (\ln Q10/10)]$$

with Q10 = increase of a degradative reaction rate for a 10°C temperature increase.

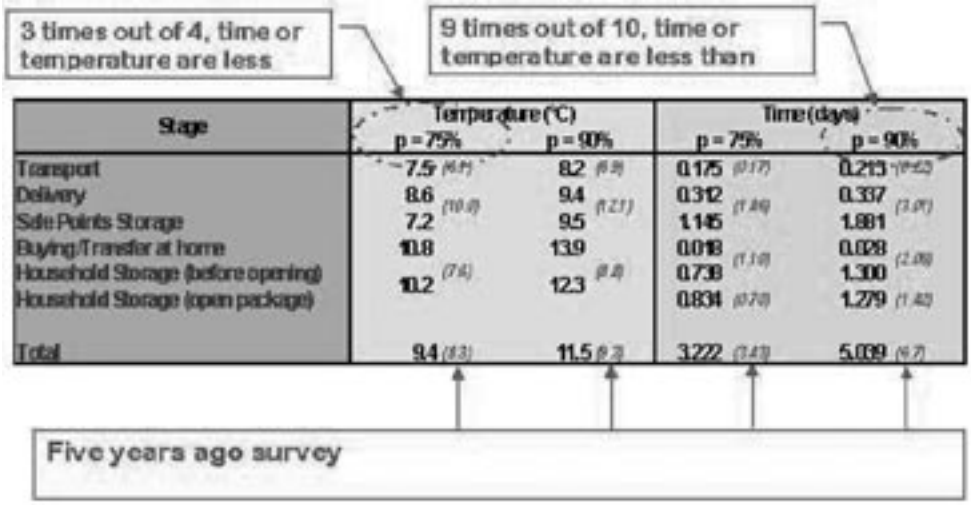
Q10 values were obtained in a parallel study (Lorenzini 2003) based on accelerated shelf-life tests on the same fresh milk. Results of microorganism growth were modeled with a Gompertz function and Psychrotophyc Bacteria Counts, Total Bacteria Counts and acidity modifications lag phases were evaluated and related to applied storage temperatures.

As Fig. 7 shows, Psychrotophyc bacteria lag phase is the most sensitive index (Chandler and McMeekin 1989), showing a 8.7 days time-tolerance at 4°C and a Q10 of 5.82. A "prudent" estimation of shelf-life can be consequently drawn with this reference.

The most probable (75%) time-temperature exposure data registered in our survey were integrated with an appropriate routine calculation based on the previous relationships, in order to define the remaining shelf-life (RSL) (Riva and Piergiovanni 2002). Results of this approach are shown in Fig. 8.

When fresh milk had been consumed, the remaining shelf-life is more than 10% 3 times out of 4. This condition is related to an integrated average temperature of 9.4°C and to a 3.22 days overall commercialization time. On purchasing fresh milk (see arrow in the plot of Fig. 8), less than 30% of the total shelf-life had been consumed when the more reliable exposure conditions (75% probability) were considered.

A post-simulation shows that a 2°C reduction of refrigeration temperature at home (with an appropriate advertising campaign) could increase the shelf-life to



Stage	Temperature (°C) p = 75%	Temperature (°C) p = 90%	Time (days) p = 75%	Time (days) p = 90%
Transport	7.5 (6.4)	8.2 (6.9)	0.175 (0.17)	0.213 (0.42)
Delivery	8.6 (10.0)	9.4 (12.1)	0.312 (1.86)	0.337 (3.01)
Sale Points Storage	7.2	9.5	1.145	1.881
Buying/Transfer at home	10.8	13.9	0.018	0.028
Household Storage (before opening)	10.2 (7.4)	12.3 (8.8)	0.738 (1.10)	1.300 (2.06)
Household Storage (open package)			0.834 (0.70)	1.279 (1.42)
Total	9.4 (8.3)	11.5 (9.3)	3.222 (3.43)	5.039 (6.7)

Fig. 6 - 75 and 90% probability time and temperature exposure in different stages of fresh milk commercialization (data in brackets are referred to a previous study).

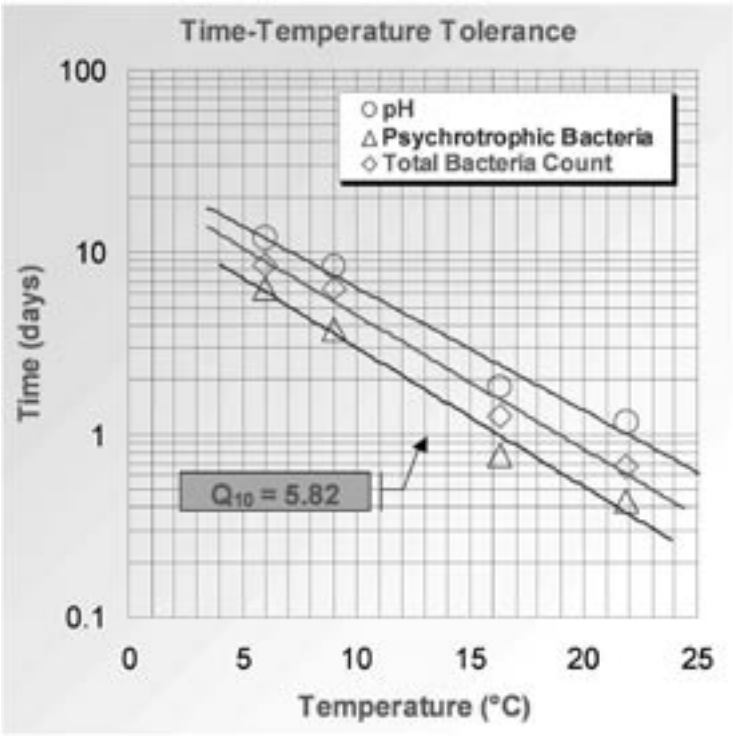


Fig. 7 - Time-Temperature Tolerance chart of fresh pasteurized milk based on different shelf-life indices.

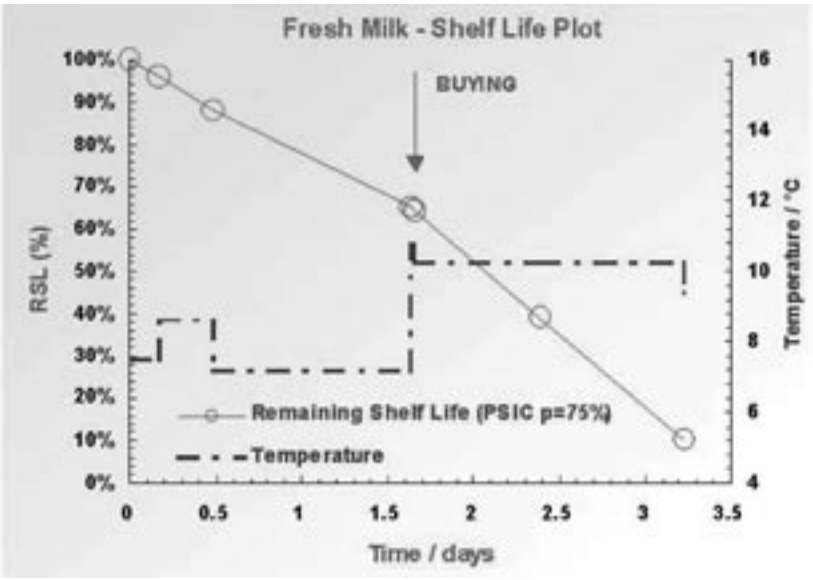


Fig. 8 - Remaining shelf-life parameterization of fresh milk shelf-life.

5-6 days and another day could be gained with a better handling at sales point and delivery.

CONCLUSIONS

In the shelf-life surveys, a very accurate time temperature history can be obtained by an "in situ" recording with micro-devices. The fresh pasteurized milk shelf-life at 6°C is more than the 4 days established by the law. The average exposure temperature in commercial chain is higher (1°-2°C) than the recommended maximum (6°C). Delivery at the sales point and household storage are the critical stages. The average time or the most probable commercialization time can clearly assure the freshness of milk. A joint effort (manufacturer, sale point management, consumer advertising) could reduce the probable exposure temperature by 1°-2°C and justify an extension of fresh milk shelf-life from the present 4 days to 6-7 days. Obviously, this last point should be investigated further: our study suggests only a methodology.

At the time of reviewing this short communication, a recommendation of Italian Ministery of Agriculture (decree 24/07/2003 - GU Italian Republic n. 179, 4-8-2003) increased the commercialization time of fresh milk from previous 4 days to 6 days. This choice is in agreement with the findings of our study.

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ACCURATE DETERMINATION OF PRESSURE, COMPOSITION AND AMOUNT OF UNFILLED VOLUME (UFV) IN PACKAGED FOODS. SET UP OF A NEW QUASI-AUTOMATIC DEVICE

DETERMINAZIONE ACCURATA DI PRESSIONE, COMPOSIZIONE E VOLUME DELL'«UNFILLED VOLUME» (UFV) IN ALIMENTI COMFEZIONATI. SVILUPPO DI UN DISPOSITIVO SEMI-AUTOMATICO

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ABSTRACT

Head space, free volume and unfilled volume (UFV) are expressions widely used to indicate the volume in the package which doesn't belong to the mass of packed product. This volume is mainly an air volume but, very often, it is represented by a gas mixture used for protective purposes. Therefore UFV is a quite important parameter of the packaged food system, both for packaging optimisation and for shelf-life regulation. In this paper a modified version of a method for UFV determination, based on Boyle's Law, is presented. The original apparatus proposed has been modified in a quasi-automatic device, obtaining a faster determination of all the relevant parameters of UFV, i.e. amount, pressure and composition. The comparison between expected and observed values of volume, pressure and gas composition of several and different unfilled volumes always demonstrated good accuracy and precision of the new device.

RIASSUNTO

Head space, free volume e unfilled volume (UFV) sono espressioni largamente utilizzate per indicare il volume libero di atmosfera all'interno di una confezione. Questo volume, generalmente di aria ma molto spesso costituito da un'atmosfera protettiva, è un dato importante per l'ottimizzazione delle dimensioni di un imballaggio e nella modulazione della shelf-life. In questo lavoro viene presentata una versione modificata di un metodo noto, basato sulla legge di Boyle, per determinare l'UFV. L'apparato originale è stato modificato in un dispositivo quasi automatico, in grado di determinare rapidamente sia il volume che la pressione e la composizione dello spazio di testa. Il confronto statistico tra valori osservati ed attesi, in un ampio intervallo di volumi e pressioni, ha dimostrato soddisfacente accuratezza e precisione del dispositivo messo a punto.

- Key words: head space, rigid packages, unfilled volume -

INTRODUCTION

Beverages and liquid foods, as well as completely compacted and sized solid foods, have well defined "head space" in the packages where they are sealed. On the contrary, for particulate or porous foods the free volume in their package includes a volume which is inside or between the product. Thus, for those kinds of food the definition of "Unfilled Volume" appears more pertinent and, in general, UFV seems a more precise expression for whatever food packaging situation. This volume is mainly an air volume but, very often, it is represented by a gas mixture used for protective purposes and, in any case, it contains and dilutes the volatile substances produced or released from the food and the gases permeated. In other words, UFV is a quite important parameter of a packaged food system and its accurate knowledge might be crucial in packaging optimisation (e.g. saving of materials, designing of effective shape and dimension) and in shelf-life regulation (1,2). Actually the "Unfilled Volume" is strictly related to the packaging capacity and food volume and may represent both the amount of protective atmosphere present in the package around the food and the volume in which the oxygen possibly permeated from external, becomes diluted. From this point of view, its importance is quite evident and, for modified atmosphere packaging, the larger is the UFV, the longer may be the shelf-life. In the past several authors studied or proposed systems for measuring the free volume inside a flexible or rigid package (3-6) and therefore many different methods are now available. Some of them may be considered destructive because they require breakage of the packages (e.g. in opening the pouch under water level for collecting the air escaped) or its perforation, like the method which involves the addition of a known amount of tracer gas, before an analysis of gas composition is performed. Some others are defined as non destructive methods, and, generally they are based on Archimedes's principle ("neutral buoyancy method") or on Boyle's Law.

In this paper a modified version of a method, described in an internal procedure of Nestec Ltd (7), is presented. The original apparatus proposed has been widely modified in order to have the possibility of faster and more accurate determination of all the relevant parameters of UFV (amount, pressure and composition) and to make the construction and the operation of a real quasi-automatic instrument easier.

MATERIALS AND METHODS

The quasi-automatic device

The prototype instrument developed in this project for the complete evaluation of UFV characteristics is presented in Fig. 1. The unfilled volume (UFV) and its pressure (P_{pack}) are measured by two algorithms, based on Boyle's Law:

$$UFV = \frac{P_3(V_{ref} + V_a) - (P_2V_a) - (P_{ref}V_{ref})}{(P_2 - P_3)} \quad \text{(i)}$$

$$P_{pack} = \frac{P_2(UFV + V_a)}{UFV} \quad \text{(ii)}$$

where:

P_2 = pressure (mbar) after piercing the package;

P_3 = pressure (mbar) after connection with the reference volume;

V_a = volume of the device (mL);

V_{rel} = reference volume (mL).

The quali-quantitative composition of UFV is determined, after the volume and pressure estimations, by HW-GC determination (8) of a withdrawn gas sample after that the total pressure inside the apparatus is balanced with external atmosphere, loading an appropriate amount of Helium.

After an accurate determination of V_a and V_{ref} by means of an external calibrated reference volume, the accuracy and precision of the UVF estimates (i) provided by the new device, were evaluated comparing expected (theoretical) and measured (experimental) volumes of glass vials (22, 60, 120, 310, 580 mL) closed with Teflon/silicon septum and an aluminium crimp top, filled with small glass balls in order to reduce the volume to a perfectly measurable value. Three determinations for each volume were performed and the values averaged.

The accuracy and precision of the total pressure estimates of UVFs in vials closed at different values of residual pressure were evaluated comparing the calculated (ii) pressure (experimental) with the pressure measured (theoretical) by an external calibrated electronic manometer. Three determinations for each value of residual pressure were performed and the data averaged.

The accuracy and precision of the UFV gas composition were evaluated comparing the HW-GC analyses performed on glass vials (experimental), closed under established atmospheres (Table 1), with analyses performed on gas samples (theoretical) withdrawn from the device after the UFV determination were completed and the pressure inside the apparatus balanced.

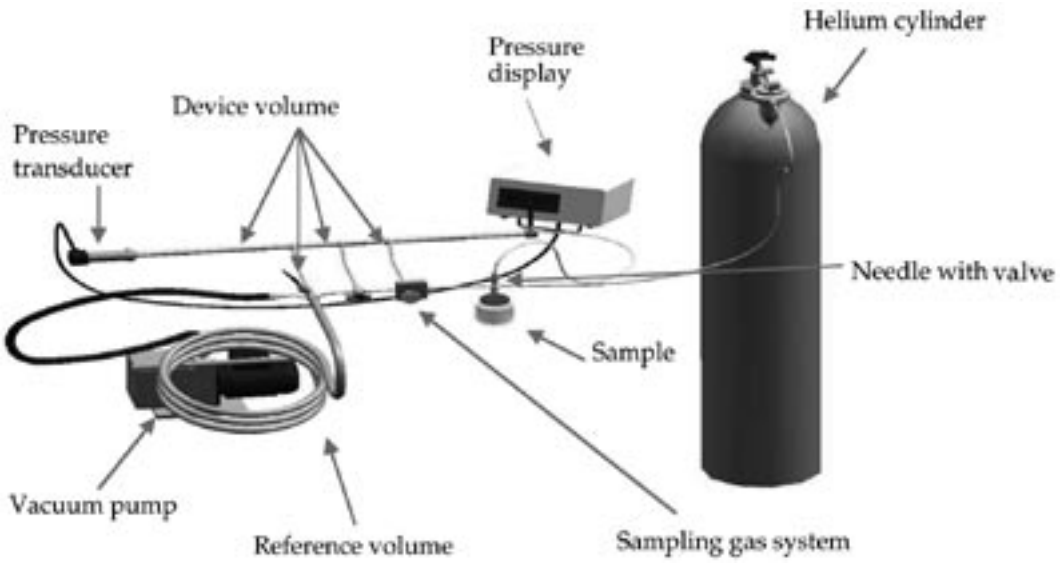


Fig. 1 - The quasi-automatic device.

RESULTS AND DISCUSSION

Accuracy and precision of UFV amount

The linear regression between theoretical and experimental data of all the average values is presented in Fig. 2. The equation of the regression line is reported in the table within the figure, together with the coefficient of determination (R^2) and the uncertainty of slope (I_m) and intercept (I_q) of the regression line. Since slope = 1 and intercept = 0 are included in the confidence interval at 95% of probability around the calculated slope and intercept, it is possible to conclude that there is no significant difference among theoretical values and the measured data (i).

Accuracy and precision of UFV pressure

The linear regression between theoretical (measured) and experimental values of residual pressure inside the glass vials is presented in Fig. 3. The equation of the regression line is reported in the table within the figure, together with the coefficient of determination (R^2) and the uncertainty of slope (I_m) and intercept (I_q) of the regression line. Since slope = 1 and intercept = 0 are included in the confidence interval at 95% of probability around the calculated slope and intercept, it is possible to conclude that there is not significant difference among the pressure values measured with the external manometer and the values calculated by the algorithm (ii).

Accuracy and precision of UFV composition

The two linear regressions between oxygen and carbon dioxide percentages measured sampling the atmosphere directly from the vials (theoretical) and from

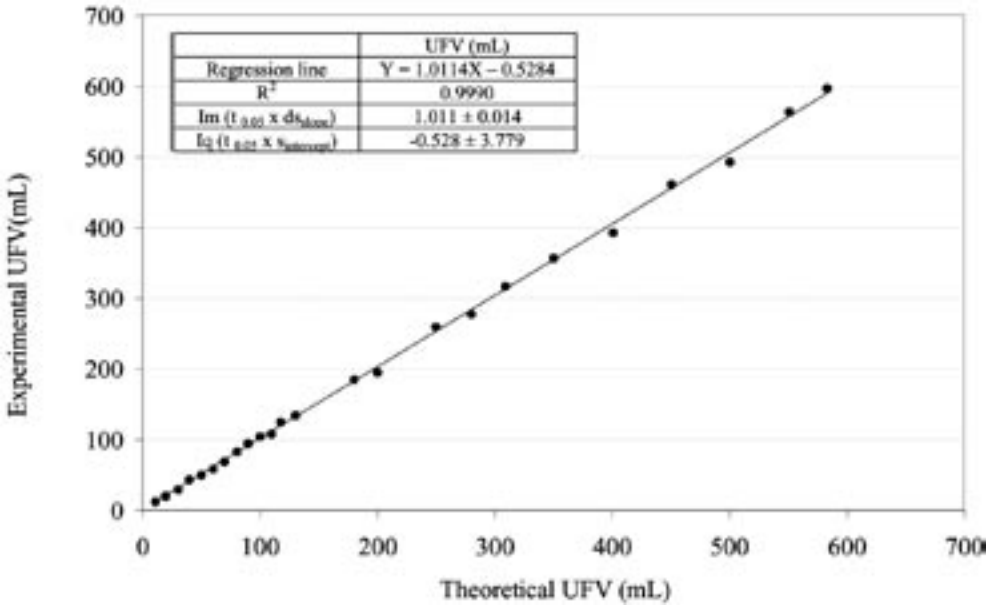


Fig. 2 - Linear regression between theoretical and experimental data of UFV.

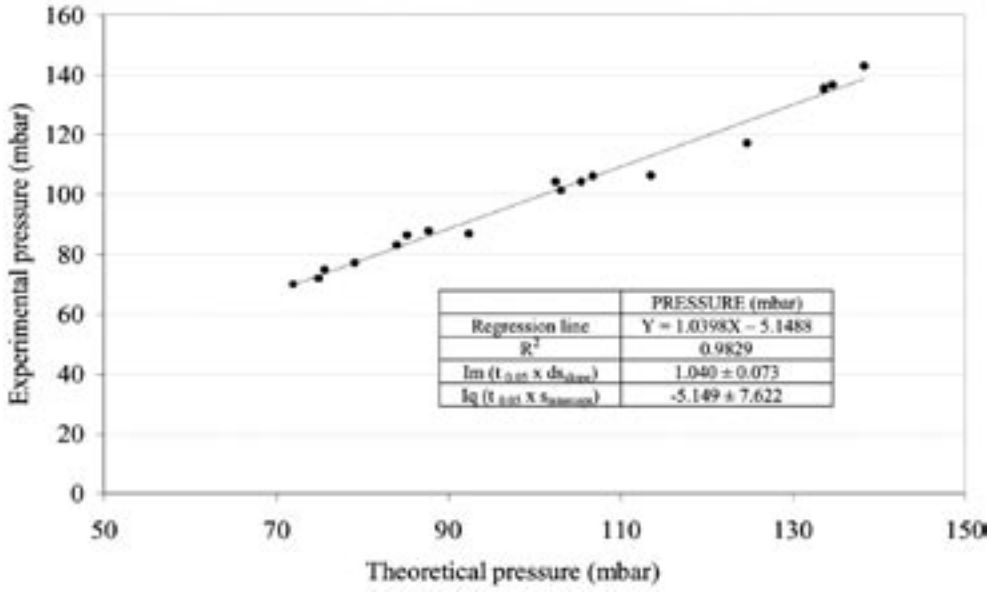


Fig. 3 - Linear regression between theoretical and experimental data of UFV pressure.

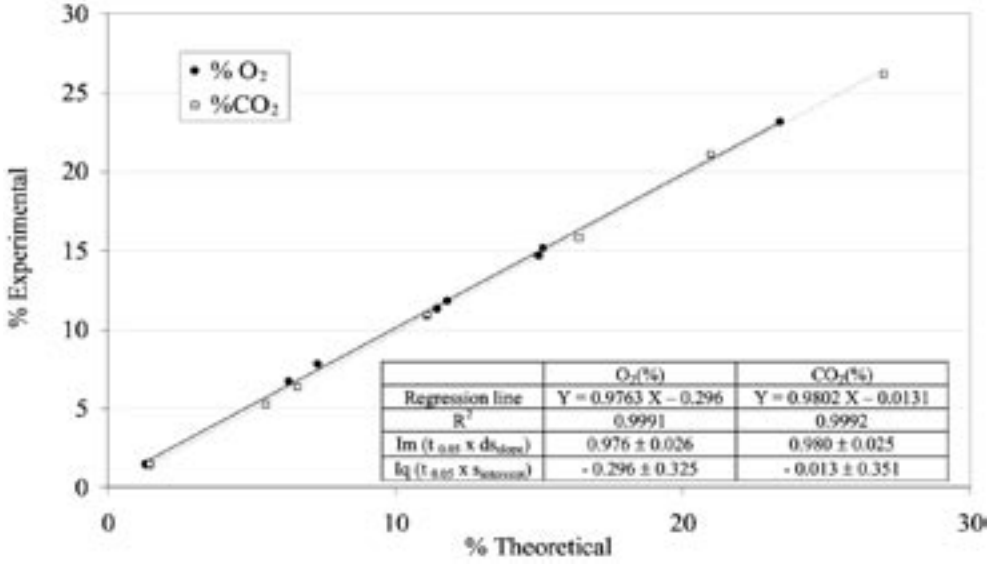


Fig. 4 - Linear regression between theoretical and experimental data of UFV composition.

the apparatus after that the UVF amount and residual pressure were calculated (experimental) are presented in Fig. 4. The equations of the regression lines are reported in the table within the figure, together with the coefficients of determination (R^2) and the uncertainties of slope (I_m) and intercept (I_q) of the regression lines. Since

slope = 1 and intercept = 0 are included in the confidence interval at 95% of probability around the calculated slopes and intercepts, it is possible to conclude that there is not significant difference among the UFV composition measured in the atmosphere withdrawn from the apparatus and the original packed atmosphere.

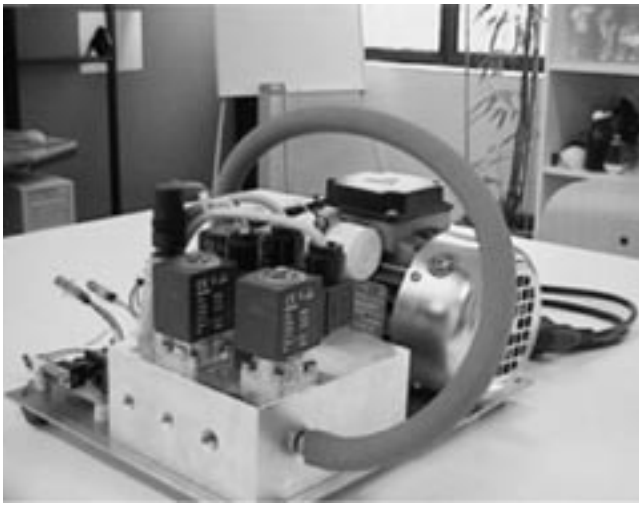


Fig. 5 - The industrial prototype of the quasi-automatic device.

CONCLUSIONS

The results obtained demonstrated satisfactory accuracy and precision of the measurements of UFV amount, residual pressure and composition, provided by the device developed. The errors in determining the UFV amount were lower than 3% for all the measurements except for free volumes smaller than 15 mL, for which a 5% error was estimated. The same error (5%) was the maximum recorded for residual pressure determinations, whereas the oxygen and carbon dioxide analyses gave errors around 5-10% only when the gas concentration was lower than 5%. Poor accuracy was evident when moisture was present in the vials; in these cases, the water vapour pressure developed affected deeply the pressure readings. This factor must be controlled (through a theoretical correction or using a water vapour absorber) for the widest applicability of the instrument. The overall results, however, justify the continuity of development and, actually, an industrial prototype of the new device (Fig. 5) is under construction.

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ACCELERATED SHELF-LIFE TESTING: POSSIBLE APPLICATIONS OF A NEW INSTRUMENT (OXITEST) TO OXYGEN SENSITIVE FOOD PRODUCTS

POSSIBILI APPLICAZIONI AD ALIMENTI SENSIBILI ALL'OSSIGENO DI UN NUOVO DISPOSITIVO PER TEST DI SHELF LIFE ACCELERATA (OXITEST)

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ABSTRACT

Chemical reactions occurring between atmospheric oxygen and food sensitive components are some of the most important causes of food product degradation, thus the possibility to foresee their effects in time is always of great interest in shelf life studies. The aim of this study was to evaluate the applicability, in autoxidation reaction studies as well as colour and flavour loss investigations, of an empirical method based on the absolute pressure change in a closed and thermostatted room, monitoring the oxygen up-take by reactive substances. The instrumental prototype Oxitest is presented as an apparatus able to measure, using accelerated conditions, the oxidative stability of fats, oils or foods, in order to determine their quality or to evaluate their state of preservation. The information potentially obtainable from instrument response regard the induction time of autoxidation process, the amount of oxygen potentially consumable by the product, rate and acceleration of the autoxidation process itself. Different food products were considered, each of them chosen in order to underline specific problems pertinent to the use of the instrument in accelerated ageing tests. Following the obtained results the instrumental prototype Oxitest can be defined as a useful previsional instrument, suitable for studies concerning food products degrading reactions involving oxygen consumption.

- Key words: accelerated shelf life test, automatic device, oxygen sensitive products -

RIASSUNTO

Le reazioni che intervengono tra l'ossigeno atmosferico ed alcuni componenti sensibili degli alimenti rappresentano una delle principali cause di alterazione qualitative durante la vita commerciale dei prodotti a lunga conservazione; conseguentemente, la possibilità di poterne prevedere per tempo gli effetti è di grande importanza negli studi di shelf life. Lo scopo di questo lavoro è stato quello di valutare l'applicabilità di un metodo empirico basato sulle variazioni di pressione assoluta in un sistema chiuso e termostatato, per valutare il consumo di ossigeno legato a reazioni di auto-ossidazione e a variazioni di colore o aroma di alcuni alimenti. Il prototipo strumentale, denominato Oxitest, è proposto come un dispositivo in grado di valutare la stabilità ossidativa di oli e grassi in condizioni accelerate. Le informazioni ottenibili da questo strumento riguardano la quantità di ossigeno potenzialmente consumabile da un prodotto, la velocità e l'accelerazione del processo auto-ossidativo. Sono stati testati diversi alimenti, ognuno dei quali rappresentativo di più generali forme di deterioramento. I risultati ottenuti suggeriscono che lo strumento è un utile strumento previsionale della conservabilità di prodotti sensibili all'ossigeno.

INTRODUCTION

Very often, food shelf-life is shortened by oxygen, which can cause both chemical and biological degradation: rancidity, colour changes, flavour loss, enzymatic activity, aerobic micro-organisms growth are the main phenomena which are correlated to oxygen concentration (1,2). For medium and long shelf lives, however, food stability depends mainly on fat and flavour or colour preservation. For such kind of foods an Accelerated Shelf-Life Test (ASTL) may be very useful as may be saved time and money in designing new formulations. The aim of this work was to evaluate the performance and applicability of a new instrument, named Oxitest, which, using accelerated ageing conditions, permit food stability to be investigated. The instrument was tested evaluating both fats, oils and heterogeneous whole foods.

MATERIALS AND METHODS

A prototype instrument supplied by the manufacturer (VELP Scientifica, Usmate, Milano) was used for all the trials (Fig. 1). The instrument permits absolute pressure changes to be measured inside two closed and independent rooms, where food samples are submitted to high temperature and high oxygen concentration in the dark. A suitable device, connected to the outlet valve, was also developed for the evaluation of the residual atmosphere gas composition (Fig. 2) which permitted the gas recovery into a glass vial and its subsequent GC analysis (3).

Several different products were submitted to tests: olive oil, extravirgin olive oil, sunflower oil, pork lard and shelled walnuts, for oxidative rancidity investigation; ground arabica coffee, for flavour loss investigation; crushed chilly (lycopene oxidation), powdered spinach and crushed parsley (chlorophyll oxidation), for colour loss investigation. For the sake of brevity, only the most representative data will be presented.

Solid foods were milled and then homogeneously distributed in the sample holder; liquid foods were directly distributed in the sample holder using an automatic pipette. An adequate pressure of pure oxygen was then established inside the two rooms, the temperature was raised to a prefixed value and changes of the internal pressure were recorded by a specific software each minute (Table 1). In order to reduce the instrument signal noise, the pressure values recorded during tests were processed by a smoothing procedure provided by the mathematical software Table Curve 2D version 4. The same software was used to calculate several different parameters from the "pressure versus time" curves that were obtained.

The tests were interrupted after 12-24 hours at 100°-120°C and after more than 100 hours at 90°C.

Colour evaluation of food products (chilly and powdered spinach) was carried out using a reflective colorimeter (CHROMA METER CR-210 MINOLTA ver. 3.0), performing 4 replicate measurements



Fig. 1 - Oxitest prototype.

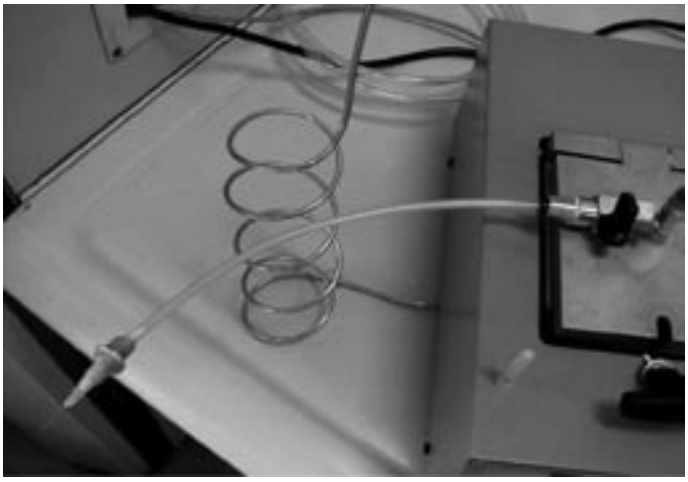


Fig. 2 - Device for the recovery of the residual atmosphere.

Table 1 - The experimental conditions used to perform the tests.

Temperature °C	Absolute pressure bar	Gas	Sample amount g	Sample holder	Sampling rate min
120, 100, 90	3	oxygen	10	Glass	1

Table 2 - Artificial ageing conditions.

Aged products	Ageing temperature	Light irradiation	Ageing time
olive oil, pork lard, shelled ground walnuts	40°C	absent	10 days
chilly, parsley	60°C	150 W	10 days

on each sample, using CIE Lab parameters. To evaluate the instruments' ability to discriminate between fresh and aged products, some food portions were submitted to the accelerated ageing conditions presented in Table 2.

RESULTS AND DISCUSSION

Information from instrument response

For food sensitive to rancidity, the plot obtained from the instrument response (Fig. 3) describes completely the product behaviour. The first portion of the curve shows the oxidative stability of the product under high temperature and high oxygen concentration and the complete curve makes its overall oxygen consumption measurable.

However, the information obtained by processing the instrument response is many: rancidity induction time, amount of oxygen consumed by the product (in specific accelerated conditions), velocity and acceleration of autoxidative degradation, cumulative area under the curve "pressure versus time"; this area is defined as "oxidation work" and measured in bar x min units.

Rancidity

Fig. 4 shows the instrument response for olive oil conditioned at 120°C, both in oxygen atmosphere and inert gas (argon). The oxidation tests were performed

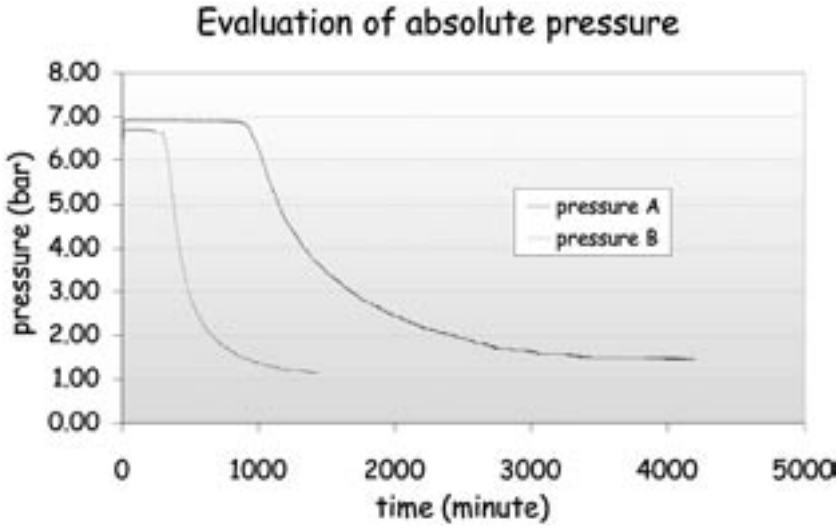


Fig. 3 - An example of the instrument response.

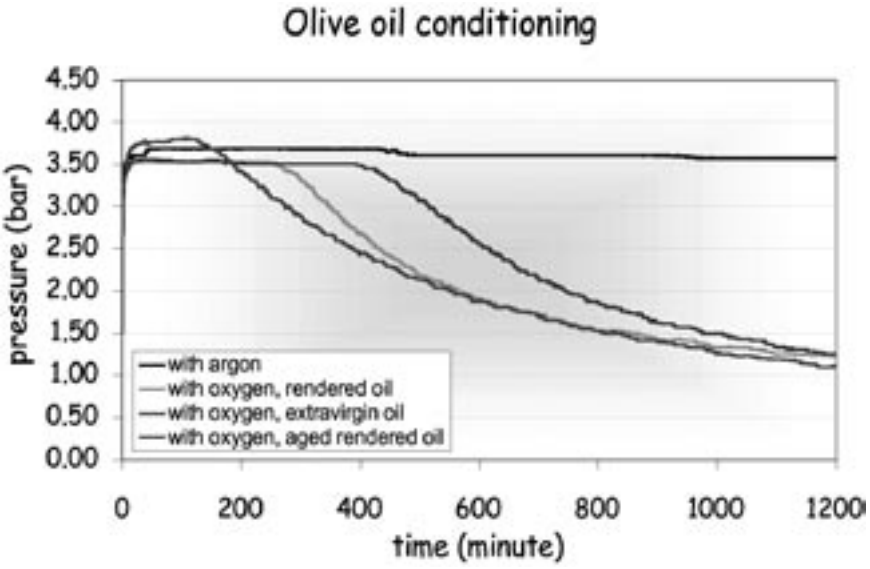


Fig. 4 - Instrument response for olive oil oxidation.

on fresh products, both olive oil and extravirgin olive oil, and on a sample of olive oil artificially aged as previously described.

In Table 3 and in Fig. 5, the induction times and the other parameters obtained by processing experimental data are detailed.

As can be seen from the previous figures and tables, the information obtained from the instrument response permits the different samples to be discriminated easily, both for different state of preservation and different market category. The amount of oxygen consumed by the product is higher for the aged sample: this happens because in the degraded products, the oxidation phenomenon occurs in a shorter time, leading to the production of volatile compounds able to react with oxygen as well (4). The longer induction time recorded for extravirgin olive oil is very likely due to the content of antioxidants (vitamin E) in this special product.

Interaction between NEB and autoxidation

Testing whole and heterogeneous products, also non enzymatic browning reactions may occur. Their volatile products have antioxidant properties, so interfering in the evaluation of sample oxidative stability (5). To eliminate such a problem, a

Table 3 - Parameters obtained from the oxidation test performed on olive oil.

Product	Average induction time (min)	Δ%	Oxidation work (bar*min)	Oxygen consumption (mmol/g)
olive oil	249.46±10.41	-	635.16	1.11
aged olive oil	124.82± 9.24	**-49,96**	573.98	1.34
extravirgin olive oil	404.31±13.41	**+62.07**	802.73	1.19

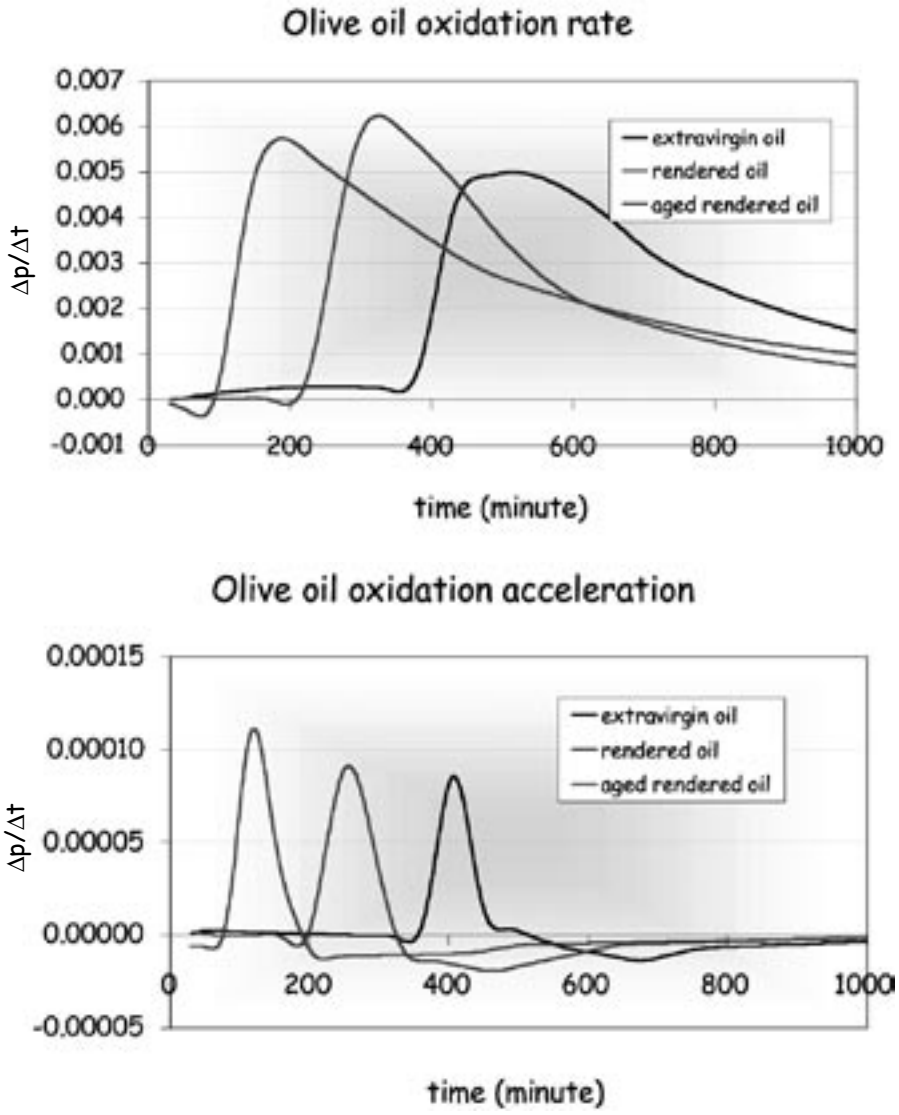


Fig. 5 - Olive oil oxidation reaction rate and acceleration.

procedure was developed in order to have, at first, browning reaction in inert gas, after that the inert atmosphere is eliminated and the oxidation reaction is monitored in a new oxygen atmosphere. Figure 6 shows the instrument response for walnuts (without shell and ground); the oxidation tests were performed at 90°C, both on fresh and artificially aged product. To avoid sample burning, it was necessary to perform the tests at a lower temperature than olive oil.

In Table 4 and in Fig. 7, the results obtained by processing the experimental data are shown. In the computing of induction times, the initial 4 hours of inert gas conditioning were not taken into account.

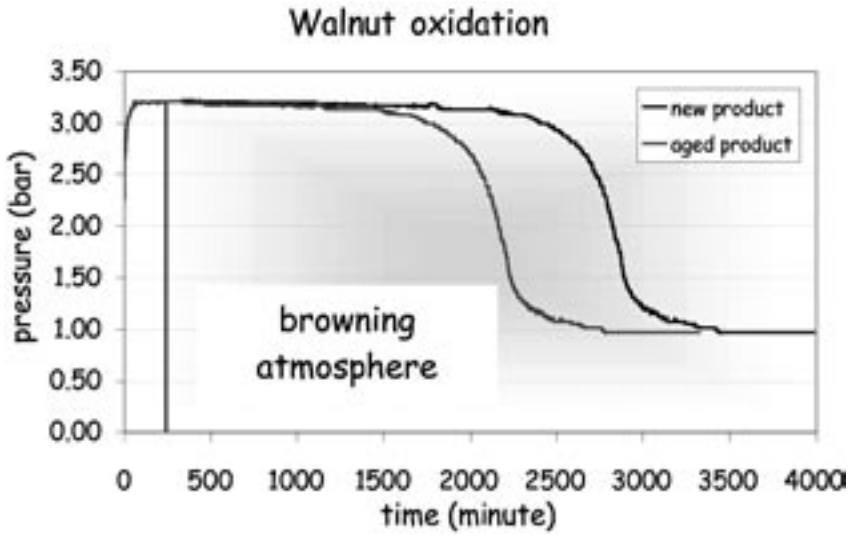


Fig. 6 - Instrument response for walnut oxidation after browning in inert gas.

Table 4 - Parameters obtained from the oxidation test performed on walnuts.

Product	Average induction time (min)	% difference	Oxidation work (bar*min)	Oxygen consumption (mmol/g)
new walnuts	2,208.54±79.86	**100.00**	2,805.36	1.13
aged walnuts	1,352.29±42.71	**61.23**	2,067.62	1.15

As can be noticed, the information from the instrument response permits the two samples to be discriminated. It is reasonable to consider this information as pertinent only to the characteristics of lipids in the product, because it was previously conditioned in argon, coming in contact with oxygen only after discharge of browning reaction volatile products. It is worth noticing that, even if the amounts of oxygen consumption are very similar, the induction time and the "oxidation work" are decidedly different between fresh and aged products.

Colour loss

Fig. 8 and 9 show, respectively, the instrument response for crushed chilly and powdered spinach, conditioned both in oxygen and in argon. The oxidation tests were performed both on fresh product and artificially aged product. The amount of carbon dioxide generated by the sample, conditioned at 100°C and in oxygen atmosphere, was evaluated by GC analyses and taken into account in the determination of pressure drop out.

Table 5 reports detailed information obtained in relation to carbon dioxide percentage measured after 24 hours of reaction time.

In Table 6, the colour parameters for new, artificially aged and exhausted

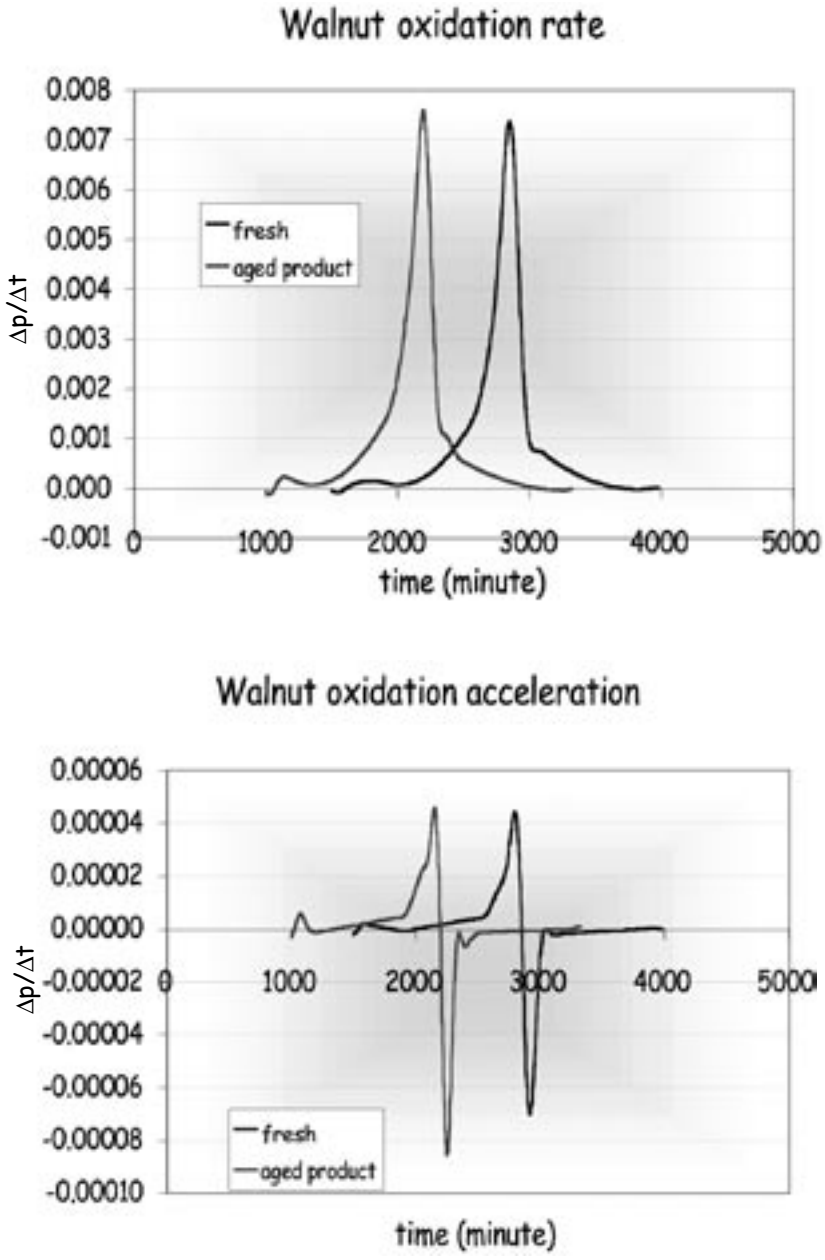


Fig. 7 - Walnut oxidation reaction: rate and acceleration.

products are shown. In these two samples, the oxygen consumption is mainly due to reactions causing product colour changes. For powdered spinach only a variation of greenness (CIE Lab "a" parameter) occurs, indicating reduction in the chlorophyll content. For chilly powder, a variation of both redness (CIE Lab "a" parameter)

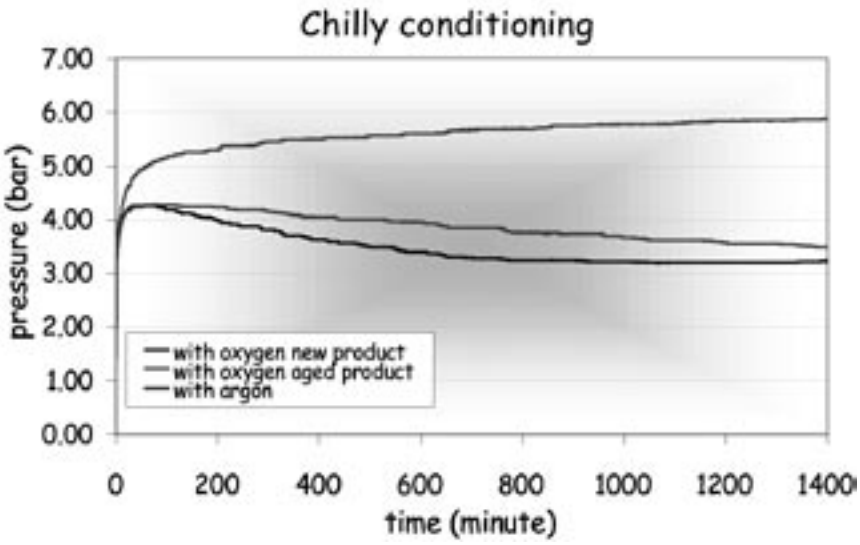


Fig. 8 - Instrument response for chilly in inert gas and oxygen.

Table 5 - Oxidation data of Chilly and powdered spinach samples.

	Chilly		Powdered spinach	
	new	aged	new	aged
Apparent oxygen consumption (mmol/g)	0.24	0.19	0.09	0.04
% CO_2	60.20	32.34	16.27	17.97
Real oxygen consumption (mmol/g)	**0.71**	**0.46**	**0.22**	**0.21**

Table 6 - Colour variation of samples.

Product		L	a	b
Chilly	new	**43.42**±0.22	**18.48**±0.04	**26.29**±0.12
	aged	**47.27**±0.24	**14.20**±0.08	**27.98**±0.14
	new exhausted	**25.28**±0.03	**5.20**±0.03	**7.12**±0.02
	aged exhausted	**25.69**±0.05	**5.34**±0.04	**7.06**±0.01
Powdered spinach	new	**41.87**±0.49	**-14.74**±0.20	**24.92**±0.40
	aged	**43.74**±0.04	**-11.02**±0.01	**22.59**±0.02
	new exhausted	**44.70**±0.03	**-0.52**±0.01	**21.56**±0.01
	aged exhausted	**44.52**±0.07	**-0.53**±0.02	**21.60**±0.03

and lightness (CIE Lab "L" parameter) were evident, indicating destruction of the original pigments and production of new brown compounds. When a product is submitted to Oxtest analysis, the test is performed in complete darkness, thus it is possible to conclude that the oxygen involving reactions are not due to a pho-

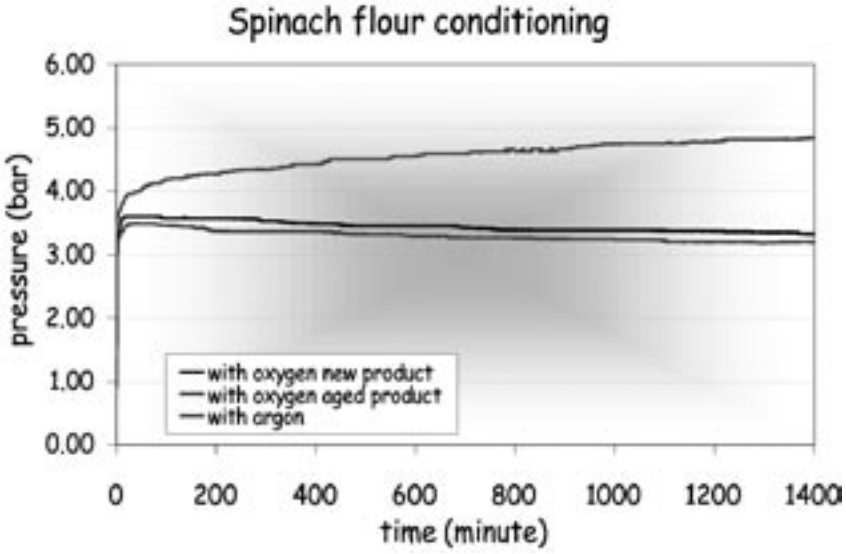


Fig. 9 - Instrument response for powdered spinach in inert gas and oxygen.

tochemical event but to events, related to the production of reactive radicals from sample liposoluble components (2).

CONCLUSIONS

The instrumental prototype Oxitest demonstrated to be a useful device for evaluating lipid stability, under accelerated conditions, of fats, oils and whole products. The Oxitest also makes possible, indirectly, to measure the efficacy of preservation conditions and to evaluate the product shelf-life because it is able to discriminate among different aged products. The instrument seems to be potentially useful, also, for other fields of research, where the oxygen consumption produces colour or flavour losses. A possible very useful improvement, however, could be to provide the instrument with an oxygen partial pressure monitoring system, not affected by gaseous/volatile substances produced or consumed during testing.

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SESSION II

NEW TECHNOLOGIES FOR EXTENDING SHELF-LIFE

Main lecture: Prof. Joseph H. Hotchkiss
Cornell University, NY - USA

CURRENT AND FUTURE PACKAGING APPROACHES TO EXTENDED SHELF LIFE OF FOODS

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ABSTRACT

There are several reasons for wanting to extend the shelf life of foods. Extended shelf life foods, by definition, have higher quality at anytime compared to conventional products of the same age. Longer shelf life can be the basis for new products or allow products to be distributed through new channels. Extending shelf life is often one method of reducing distribution costs or using new or unconventional distribution methods. Shelf life also allows companies to enter into new markets and reduces restocking costs.

Shelf life can be defined as the time for a product's quality to deteriorate to an unacceptable degree under specific conditions of processing, packaging, and storage. This time is also defined by several factors including product composition, storage temperature and atmosphere, processing conditions, packaging, and initial quality.

One of the most difficult tasks is to define at what point a product becomes unacceptable. That is a limit of deterioration and finding a minimum acceptable quality (MAQ) must be defined. These are difficult questions for which the answers are not apparent. The goal of shelf life extension is not to achieve an indefinite shelf life but to have an optimum shelf life given the nature of the product and the desired distribution system.

Several conventional strategies have been used to extend shelf life including additives and ingredients (e.g. antioxidants, antimicrobials), modified atmospheres, vacuum packaging, optimal barrier films (e.g. high barrier to perforated films), oxygen absorbers, ethylene absorbers, direct CO_2 injection, irradiation, pasteurization, and others. Recently several newer technologies have emerged to extend shelf life including ultra high pressure processing, dense-phase CO_2 processing, UV treatment, direct O_2 absorbers and interceptors, odor absorbers, ethanol vapor release systems, and active packaging. Even newer processes such as antimicrobial polymers (films), selective barriers, temperature compensating films, low pressure processing, low impact thermal processes (e.g., hmic heating), low impact non-thermal processes (e.g. pulsed light) are being developed.

This presentation will focus on several examples of newer technologies including permselective barriers for respiring produce, direct addition of CO_2 to dairy products, processing with dense phase carbon dioxide, and antimicrobial polymers and films. Each of these new technologies is designed to extend the shelf life of perishable foods while providing high sensory quality.

LECTURE

PRODUCT INNOVATION IN FOOD SCIENCE: READY TO EAT PRODUCTS

L'INNOVAZIONE DI PRODOTTO IN CAMPO ALIMENTARE:
I PRODOTTI DELLA V GAMMA

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ABSTRACT

Importance and role of ready-to-eat products is due not only to the high convenience level that they offer, but also to their high sensory and nutritional standards. From a market research, steamed carrots and potatoes, with a shelf-life of two weeks, resulted to be a suitable product of interest for consumers and catering operators. Once the process conditions have been defined (composition of protective atmospheres to introduce into the package, assurance of safety product through microbial analysis and HACCP), the quality decay of product during storage period was investigated by means of colorimetric, mechanical, gravimetric and sensory analysis.

RIASSUNTO

I prodotti della V gamma, presentando un elevato livello di preparazione, garantiscono al consumatore un risparmio di tempo, di lavoro e di sprechi. Il confezionamento di tali prodotti avviene, generalmente, in atmosfera protettiva, tecnologia

- Key words: carrots, HACCP, potatoes, protective atmosphere packaging, ready-to-eat -

che, accoppiata alla refrigerazione, consente di ottenere prodotti con caratteristiche non molto dissimili da quelle degli alimenti preparati a livello domestico. Considerando i bisogni dei consumatori come punto focale dell'intero processo, scopo di questo lavoro è stato quello di illustrare una metodica multidisciplinare che consenta di sviluppare un prodotto di V gamma dotato della capacità di coniugare la corrispondenza tra le attese espresse dai consumatori e le caratteristiche intrinseche del prodotto stesso. Individuato il range di composizione di due atmosfere protettive che consentivano di ottenere un prodotto (patate e carote cotte a vapore), di aspetto accettabile e con una shelf-life medio-breve di due settimane, indagini microbiologiche hanno garantito la sicurezza d'uso dell'alimento confezionato ed hanno consentito di individuare e superare, mediante l'applicazione di un adeguato piano HACCP, i punti critici del processo produttivo. I fenomeni di decadimento qualitativo dei prodotti sono stati, infine, rilevati mediante analisi colorimetrica, meccanica, gravimetrica e sensoriale.

INTRODUCTION

As defined in France at the end 70s, ready-to-eat products are foods with a higher level of preparation, in comparison to minimally processed ones, being cooked before packaging in protective atmosphere. Extending shelf-life and obtaining products with characteristics comparable to fresh-made ones are the purposes of this technology. By means of Concurrent Design (CD) and Quality Function Deployment (QFD) methodologies it is possible to obtain these products. Even if both of them are based on simultaneity of all industrial functions, QFD differs from CD because all the project is oriented to the consumers' needs, understanding and fulfillment. In the food industry the QFD methodology is usually adopted, consisting in the following steps:

1. screening of the market segment to which the product is destined (ready-to-eat foods were chosen by those don't have time to dedicate to meal preparation and carry out food commodities supply daily);
2. screening of consumers' needs: through interviews (focus groups);
3. converting consumers' needs to technical data, obtaining measurable data. (Sensory analyses were performed by means of a panel, after assuring hygienic sanitary safety of products);
4. definition of production process and development of new technologies, if necessary;
5. set optimum process variables.

Packaging in protective atmosphere allows the qualitative characteristics of the product to be preserved, limiting the development of degradative phenomena activated from atmospheric agents (Piergiovanni and Fava, 1987). Packaging vegetables in PA could surely give good results. In fact, an opportune choice of the gaseous atmosphere, could allow degradative processes to decrease and their shelf-life to be extended. For "minimally processed" foods, usually a concentration of CO_2 in the range between 5 and 10%, and an oxygen level between 2% and 5% (Kader *et al.*, 1989) is appropriate. Carlin *et al.* (1990) showed that O_2 level (2-10%), and CO_2 level (10-40%), reduce sugar consumption of carrots with respect to those preserved in air, even though lactic bacteria proliferation caused spoilage. It is observed that some quality losses of "minimally processed" carrots are due to off-flavour development, to acidification, tissue softening and decolouration (Bolin and Huxsoll, 1991; Howard and Griffin, 1994).

Kato-Noguchi and Watada (1996 and 1997), stated that, glycolysis is accelerated and ethanol and acethaldeyde levels increase when O_2 level is less than 2% in the package. After 8-12 days at 8°C the quality of carrots stored in 50% O_2 and 30% CO_2, resulted better than samples stored in 1% O_2 and 10% CO_2 (Amanatidou *et al.*, 2000). Massignan *et al.* (1992) worked with carrots stored at low temperatures (0°C and U.R. 90-95%) for 120 days, in a climatic room: weight loss, soluble solid, titratable acidity, ascorbic acid and β-carotene were determined. Data relative to carotenoids are probably results of biosynthesis and degradative oxidative processes, that also caused off-flavour synthesis. Processing also could have an effect on quality, in fact cutting increases oxidation and produces bitter taste sensation, due to isocumarine and cumarine synthesis (Bruemmer, 1988). For ready-to-eat potatoes, the most important degradation process is enzymatic browning which manifests itself after few hours from cutting. To extend shelf-life and to reduce browning effect, Mencarelli *et al.* (1993) performed some experiments on potato slices, dipping them in solutions having different composition. 1% ascorbic acid and citric acid solution was unable to control browning, while calcium chloride (1M) and sodium chloride (0.2M) solution were more effective. However with respect to browning, low temperature preservation is more effective. According to Sapers *et al.* (1995) browning, in raw slices of potatoes, depends on superficial damage during peeling: surface cellular structures were broken, phenols and the polyphenoloxydase (POD) released by cells reacts with O_2 leading to enzymatic oxidation (Huxsoll *et al.*, 1989). Sapers and Miller (1993) prevented browning by means of ascorbic acid dipping, NaOH superficial digestion and vacuum packaging. P.A.P. technology alone can't prevent browning of "minimally processed" potatoes, instead, under vacuum packaging coupled with anti-browning treatments, could extend the shelf-life of raw and cut potatoes up to 2 weeks (Langdon, 1987). Gunes and Lee (1997) evaluated colour changes in slices of potatoes, dipped in anti-browning solutions and stored in protective atmospheres composed of: 100% N_2, 9% CO_2 and 91% N_2 and 9% CO_2 - 3% O_2 - 88% N_2. Atmosphere effect on product was indifferent if anti-browning treatments were carried out in comparison to the control in air, nevertheless, it advantage of such procedure of packaging was shown.

MATERIALS AND METHODS

Carrots (*Daucus carota* L.) and potatoes (*Solanum tuberosum* L.) where purchased in a local store. Packages were made by using film (PET 12+COEX/EVOH/PE 95) with high gas-barrier properties (PO_2 at 23°C - 0% U.R. <5 $cm^3m^{-2}d^{-1}atm^{-1}$ and PH_2O at 38°C - 100% U.R. <5 $gm^{-2}d^{-1}$). The film was cleaned with ethyl alcohol (70% v/v), and then used for preparing the packages. N_2, O_2 and CO_2 were mixed in different percentages under controlled conditions.

Market investigation

Distribution of minimally processed products on local markets was analyzed by means of a market research with the application of the Conjoint Analysis. In this method different types of metric and non-metric scales are used to measure consumer preferences for various concept profiles that are the basis for estimating their utility functions for the different concept attributes. Consumers' opinion on such products, was obtained, instead, performing focus groups.

Sample preparation

Carrots, were peeled, washed and cut in cylindrical samples (1 cm thickness). Samples were steamed for 9 min, and then air-cooled in an environmental chamber. Carrots, then, were packaged in protective atmosphere (50 g each pack). Same operations were performed for potatoes: raw samples were cut in parallelepiped shape (9 mm x 4.5 cm).

Data obtained from all the analyses were elaborated by means of One-way Anova and Tuckey' Test (α = 95%), to determine possible differences among samples.

Microbial analysis

Microbial analysis, concerning the evaluation of TMA (Total Aerobic Microflora) and the survey of Enterobacters and Sporigens on samples "just cooked" (t_0), and stored one (t_1), two (t_2) and three (t_3) weeks in PA, were analyzed. The analyses were performed on the following samples:

a. whole sample, peeled and washed;
b. sample, washed and cut;
c. sample steamed and packaged warm;
d. sample steamed and air cooled;
e. sample steamed and cooled in environmental chamber;
f. sample "just cooked" (control);
g. sample stored for 24 h;
h. sample stored for 48 h;
i. film used for packages.

Instrumental analysis

Colour evaluation

Colour evaluation was performed by means of a colorimeter MINOLTA Chroma Meter, mod. CR-300.

According to Hunter, a*, b* and L* (CIE 1976) coordinates, white index (WI*):

$$WI^* = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2} \quad (1)$$

and colour difference (ΔE^*) with respect to control, were calculated:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (2)$$

Moisture content

Carrots and potatoes were weighed, set in oven (Continental Equipment) for about 24h at 105°C, then were cooled in a drier and weighed.

Sensory analysis

To monitor vegetable sensory quality, Quantitative Descriptive Analysis (QDA) was performed. Analyses were effected on steamed carrots and potatoes stored in different atmospheres, for 7 and 14 days at 4±1°C. Data were analyzed by

One-way Anova and Tukey' Test ($p<0.05$), the "just cooked" vegetable was taken as control.

RESULTS AND DISCUSSION

Consumers' opinion

Conjoint analysis is a very powerful tool for obtaining information about the effect of different product attributes on liking and/or purchase intent of food products (Green & Srinisvan, 1978). The attributes studied are usually varied according to a factorial design plan and each consumer gives scores, either liking or purchase intent, for a number of combinations of the attributes. In most cases, different consumer groups respond differently to the attribute combinations. In such cases, it is of great importance for the purpose of generating marketing strategies to identify the segments and then to interpret them in terms of demographic or other external information (consumer variables).

By means of focus groups principal characteristics were individualized, that a new product has to possess to satisfy consumers' needs, and attributes (Table 1) that, required in ready-to-eat products, drove consumers' purchase choice. Consumers usually prefer to buy a brand name product, with a high quality level, without additives, even if the price is high. There are no preferences about cultivation methods, even if we expected more interest in organic agriculture crops. The packaging material was very important. In fact consumer' purchase choice depends, first of all, on the possibility of seeing the inside of the packaging, so the film has to be transparent.

Table 1 - Attributes and levels obtained from focus groups and elaborated by means of Conjoint Analysis.

Attributes	Levels
Firmness	- Hard
	- Soft
Agriculture	- Biologic
	- Integrated
	- Conventional
Additives	- With
	- Without
Origin	- North countries
	- South countries
	- Central countries
Time to prepare meal	- +10 minutes
	- -10 minutes
Brand	- Ortobell
	- Ghisetti
	- Cielo e Campo
	- brand of distribution chain
	- others
Sizing	- 1-2 portions
	- 3 portions
	- 4-5 portions
Prize/portion	- € 0.60
	- € 0.80
	- € 0.30
Packaging	- Coloured
	- Transparent

Preliminary research of gaseous mixtures

As a first step carrots and potatoes were packaged in different mixtures of N_2, O_2 and CO_2 in order to make a first screening. According to Myers (1989) the tolerance level for O_2 (2-8%) and CO_2 (1-10%), lower oxygen level and higher CO_2 could increase microrganism proliferation and create organoleptical damage to the product (jelly presence, exudate losses, tissue acidification, lipid oxidation) consequently gas mixtures explored are shown in Table 2. For each gas mixture, and for both vegetables, two packages were pre-

Table 2 - Gaseous mixtures analyzed in the preliminary phase.

	Analysed mixtures
A	100% N_2
B	70% N_2, 30% CO_2
C	90% N_2, 2% CO_2, 8% O_2
D	90% N_2, 3% CO_2, 7% O_2
E	90% N_2, 4% CO_2, 6% O_2
F	90% N_2, 5% CO_2, 5% O_2
G	90% N_2, 6% CO_2, 4% O_2
H	90% N_2, 7% CO_2, 3% O_2
I	90% N_2, 8% CO_2, 2% O_2

pared: one of them was stored at 4°C and the other at 20°C. In addition some samples were stored in air, as control. Gas-mixture screening was performed by visual by inspecting the vegetables every week. As discriminative indexes, packages, looking at the colour variation, presence of moulds and presence of alterative diseases, were monitored. At the end of the preliminary screening it was shown that it was not possible to preserve vegetables in air for 3 weeks, because of colour alteration: in particular carrots become orange-red and potatoes white, the same for samples stored at high temperature (20°C). Only samples packaged in 100% N_2 (atm. A) and in 70% N_2 and 30% CO_2 (atm. B), after visual inspection, still appeared acceptable after three weeks of storage.

Microbial analysis

Table 3 lists microbial results for both carrots and potatoes, relative to different processing steps. The microbial investigation revealed that the cooking phase had a stabilizing effect, while air-cooling (CP) could lead to a risk of microrganism spoilage. To reduce the risk, a cooling stage, in environmental chamber was introduced. The results of this are reported in Table 4.

Instrumental analysis

Colour analysis

ΔE^* values for carrots and potatoes are reported in Fig. 1 (a and b). Both PA

Table 3 - Screening of critical points during the processing of carrots and potatoes (CFU g^{-1}).

Carrots	TMA	Enterobacters	Sporigens
Peeled, washed	$4.8x10^4$	$4.0x10^3$	$6.0x10^3$
Cut	$6.5x10^3$	$1.4x10^3$	<10
Steamed	<10	<10	<10
Air-cooling	2.0x10	4.0x10	5.0x10
Potatoes	**TMA**	**Enterobacters**	**Sporigens**
Peeled, washed	2.0x10	7.0x10	<10
Cut	$5.2x10^2$	$2.4x10^2$	2.0x10
Steamed	<10	<10	<10
Air-cooling	2.0x10	<10	1.0x10

TMA = total aerobic microflora.

Table 4 - Microbial data (CFU g^{-1}) after the application of HACCP on carrots and potatoes.

Carrots	TMA	Enterobacters	Sporigens
Peeled, washed, cut	$1.5x10^5$	$3.0x10^3$	<10
Steamed	<10	<10	<10
Cooling in environmental chamber	<10	<10	<10
Packaged in air	<10	<10	<10
Potatoes	**TMA**	**Enterobacters**	**Sporigens**
Peeled, washed, cut	$1.3x10^5$	$1.1x10^3$	<10
Steamed	<10	<10	<10
Cooling in environmental chamber	<10	<10	<10
Packaged in air	<10	<10	<10

TMA = total aerobic microflora.

atmospheres lead to colour variation. For carrot, the 70% N_2-30% CO_2 atmosphere give rise to some variation, hence 100% N_2 appears to be a better solution. In potatoes, instead, atmosphere A caused a higher colour difference than the control. It is worth noting that in carrots the a* value (red index) increased during storage, and

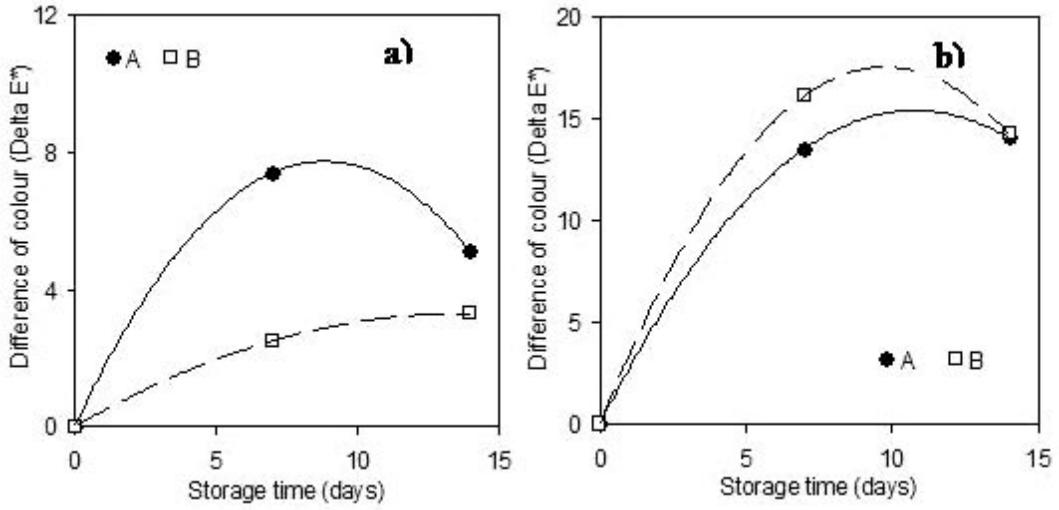


Fig. 1 - Difference of colour of carrots (a) and of potatoes (b) during storage time.

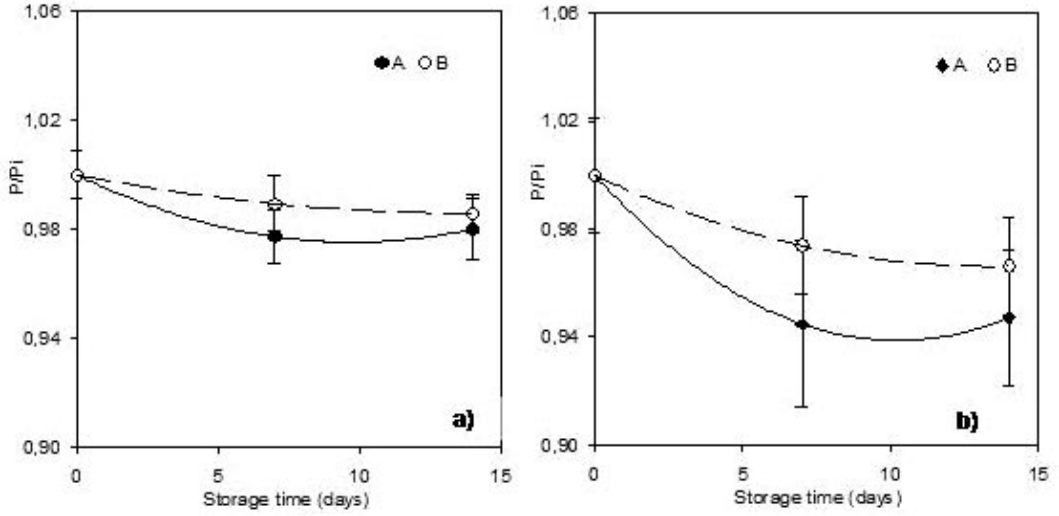


Fig. 2 - Weight loss of carrots (a) and of potatoes (b) during storage time.

therefore the samples stored 14 days in both atmospheres showed a more intense red colour. In potatoes, brightness (L*) and white index (WI*), since the first week of storage, increased for both atmospheres considered, and their value remained constant up to 14th day.

Moisture content

Figs. 2a and 2b report the moisture content variation of carrots and potatoes, stored in protective atmospheres A and B, after 7 and 14 days. Although the weight losses for both vegetables were large after one day of storage, aimed to reduce and keep constant until 14th day, atm. B guaranteed more contained losses than samples stored in atm. A.

Sensory analysis

In order to explore the effect of PA on the quality of ready-to-eat foods, with respect to "just cooked" ones, QDA profiles were developed, for carrots and potatoes (Fig. 3 and 4). During storage, in both the atmospheres, steamed carrots presented colour variation and differ from the control after 14 days. In potatoes, sensorial attributes decrement it's faster than carrots. Colour variation is already perceivable after 7 days of storage, in both tested mixtures. In 100%N_2 time dependence of the other attributes (Fig. 4) remained constant. In samples stored under 70%N_2-30%CO_2 atmosphere all attributes, and taste in particular, depend on storage under protective atmosphere: odour and aroma losses are already recorded after 7 days of storage, so it happens for sweetness, while acidity increases during storage. In a group discussion, it was possible to estimate relative importance (expressed in %) that each judge gave to every attribute: taste attributes influence final quality of both vegetables, colour has great importance in potatoes. Considering the relative importance for two vegetables a global quality score was calculated (pQDA), at different storage times.

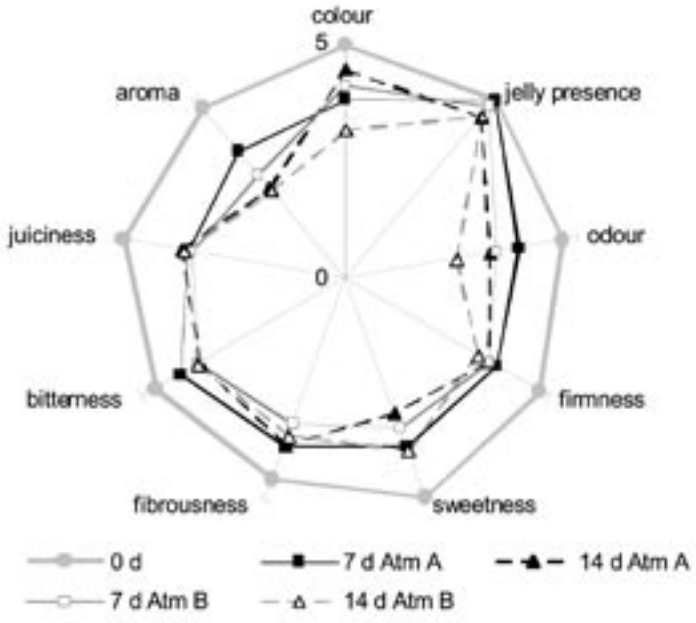


Fig. 3 - Qualitative profile of carrots packaged in 100% N_2 (atm. A) and in 70% N_2-30% CO_2 (atm. B).

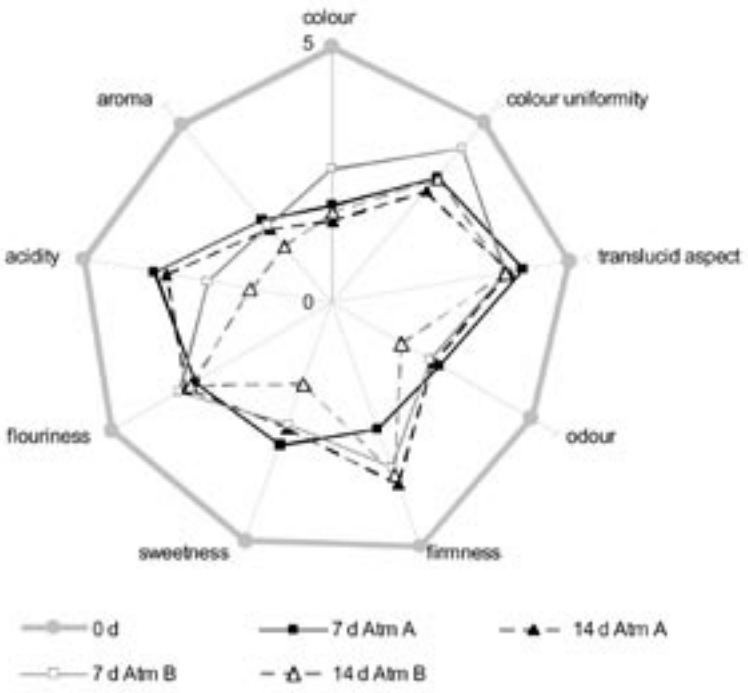


Fig. 4 - Qualitative profile of potatoes stored in 100% N_2 (atm. A) and in 70% N_2-30% CO_2 (atm. B).

By means of the following equation (Pompei and Lucisano, 1991), it was possible to estimate sensorial quality losses:

$$pQDA = \sum_{i=1}^{9} \left[I_i \cdot \frac{(V^1_i - l_i) \cdot 10}{V^0_i - l_i} \right] \quad (3)$$

where $V^1_i \leq V^0_i$, and:

l_i = relative importance of i descriptor;
V^0_i = optimal value of i descriptor (control);
V^1_i = average value of i descriptor;
I_i = 0 (left extreme of the scale).

In Fig. 5a and 5b QDA scores are reported, respectively, for carrots and potatoes packaged in the presence of two gas mixtures. Carrot quality, in both atmospheres, decreased in the first week of storage, and then kept constant in the following 7 days. Potato quality (Fig. 5b), during the first 7 days, decreased, both for samples packaged in A and B atmosphere, but in the second week, a quality decrement is higher for samples stored in atm. B, than for samples stored in atmosphere A, whom total quality, remaining constant, revealed a better effect on ready-to-eat vegetable quality.

CONCLUSIONS

Considering consumers' needs as focal point of the whole innovative process, results that the role of the technologists in developing a new product, is to find technological solutions able to realize a product satisfying a segment of the market to which it is destined.

Market research underlining consumers' interest to ready-to-eat foods, put basis for future works, while by means of focus groups it was possible to individualize characteristics that a new product has to possess for satisfying consumers' needs.

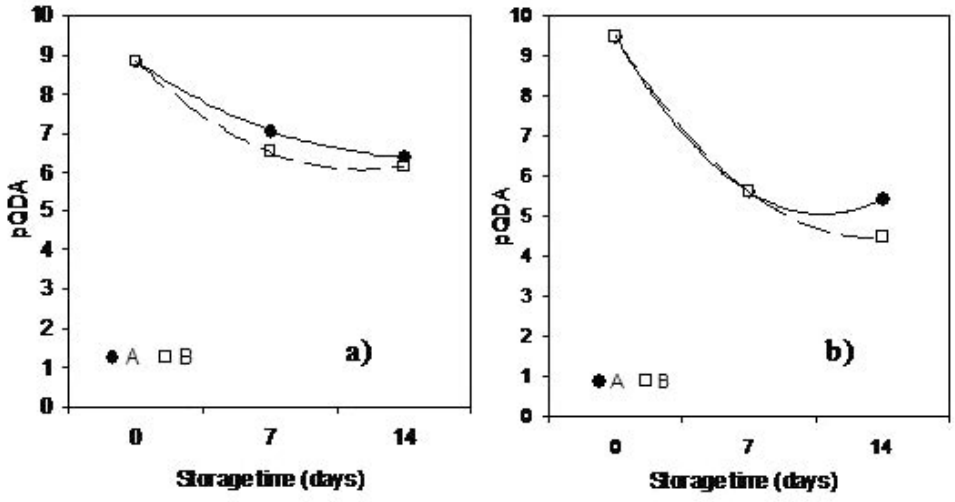


Fig. 5 - Carrots (a) and potatoes (b) pQDA during the storage time.

Relative to processing, PA could extend the shelf-life of steamed carrots and potatoes at least for two weeks with an acceptable quality, even if HACCP application results to be necessary.

Relative to product, carrot quality decay, during the storage period, is, principally, due to odour attribute losses, while appearance, texture and taste, also decaying, remain at acceptable levels. Potato quality loss, instead, is due to decreasing of all the attributes, in particular colour, aroma and odour of "cooked potato". Sensory analysis was found to be more relevant than instrumental in order to estimate product shelf-life, so in developing of a new product, by means of QFD, this method is necessary to evaluate quality.

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BEHAVIOUR OF FILM WRAPPED PONKAN MANDARINS TREATED WITH IMAZALIL AND SODIUM CARBONATE

CONSERVABILITÀ DEL MANDARINO "PONKAN" CONFEZIONATO INDIVIDUALMENTE CON UN FILM PLASTICO E TRATTATO CON IMAZALIL O CARBONATO DI SODIO

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ABSTRACT

"Ponkan" mandarins were dipped in a solution of imazalil (1.000 mg L^{-1}) or sodium carbonate (3%) at room temperature for 2 min. Fruit was then individually wrapped with a 19 µm thick heat shrinkable polyolefinic film and stored for 10, 20 or 30 days at 20°C and 65% RH. Wrapping completely inhibited weight loss, reduced dramatically ageing and even after 30 days of storage all healthy fruit were still saleable. In contrast, un-wrapped fruit due to excessive weight loss aged very rapidly and no fruit was saleable after 30 days of storage. Sodium carbonate increased the rate of transpiration and hastened ageing in un-wrapped fruit, but reduced significantly the incidence of decay, especially in wrapped treatment, even if its efficacy was at a lower level than imazalil.

RIASSUNTO

Frutti di mandarino "Ponkan" trattati con imazalil (1.000 mg L^{-1}) o con carbonato di sodio (3%) per immersione (2 min) in soluzione a temperatura ambiente e confezionati singolarmente con film plastico termoretraibile sono stati conservati a 20°C e 65% UR per 10, 20 e 30 giorni. I frutti non confezionati sono andati incontro ad un rapido invecchiamento, ad una accentuato scadimento commerciale e a gravi alterazioni fisiologiche, anche se non sono state rilevate sostanziali variazioni

- Key words: Citrus fruit, decay, imazalil, individually film wrapping, quality change, sodium carbonate, storage -

delle componenti chimico-nutrizionali (pH, acidità titolabile, solidi solubili totali, vitamina C). In modo particolare il trattamento con carbonato di sodio ha incrementato significativamente le perdite di peso e l'invecchiamento dei frutti nella tesi non confezionata. Il confezionamento con il film plastico ha contenuto le perdite di peso a meno del 3% dopo 30 giorni di conservazione, rallentato drasticamente l'invecchiamento dei frutti per tutta la durata della prova. I frutti confezionati hanno manifestato una più intensa attività respiratoria, una più elevata pressione parziale della CO_2 endogena e una più rapida degradazione dell'acidità titolabile, rispetto ai frutti non confezionati. Il carbonato di sodio e soprattutto l'imazalil, hanno contenuto a livelli accettabili le perdite dovute ai marciumi, specie nei frutti confezionati, in cui gli agenti dei marciumi per le più elevate condizioni igrometriche hanno trovato condizioni più favorevoli al loro sviluppo.

INTRODUCTION

Shelf-life of tangerines and mandarins can be considerably extended by the application of film wrapping even at stringent conditions of temperature and humidity. Sealing "Minneola" tangelos in trays with a semi permeable polyolefinic film allowed freshness to be maintained for 30 days at 20°C and 60% relative humidity without important changes in sensory quality (D'Aquino *et al.*, 1998). Inhibition of weight loss and prolonging shelf-life in similar experimental conditions were also obtained with "Miho" satsumas (D'aquino *et al.*, 1996), "Fairchild" mandarins (D'Aquino *et al.*, 1999) and "Salustiana" oranges (D'Aquino *et al.*, 2001). However packaging citrus fruit in trays can promote decay due to moist saturated in-package atmosphere, especially if the product is stored in non-controlled temperature conditions. Consequently, even if one single fruit decays the whole tray in most cases must be thrown away. For this reason a very effective fungicide is needed to reduce the risk of decay. Imazalil as well as thiabendazole are very effective fungicides against green and blue mould and have been successfully applied even at low concentrations in packaged citrus fruit. However, their use is not allowed for certified organic production. Since products approved for organic produce are less effective than synthetic fungicides, sealing different fruits in films bags, trays or cartons is very risky. Individual seal packaging offers the same beneficial effect of packaging the fruit in trays in prolonging shelf-life and improving maintenance of quality in retail conditions, reducing at the same time the incidence of decay by providing a barrier against the spreading of microorganisms spores and allowing an easy removal of infected fruit avoiding the downgrade of the healthy ones.

Objective of this experiment was to study physiological, qualitative and spoilage decay of individually film wrapped "Ponkan" mandarins treated with imazalil or sodium carbonate, a product which has been used extensively to preserve decay in the California citrus fruit industry before the introduction of synthetic fungicides and which is generally recognized as a safe compound (GRAS) by the US Food and Drug Administration.

MATERIALS AND METHODS

The "Ponkan" mandarins used in this study were harvested from the experimental station of the Institute located in Oristano (central-west Sardinia).

The day following harvest, fruit was sorted for uniform size and absence of visual alterations and divided into three lots. Lot one (control) was dipped in tap water for 2 min; lot two was dipped in a 3% solution of sodium carbonate; lot three was dipped in a solution containing 1.000 mg L^{-1} of imazalil. The temperature of water (control) or fungicides solutions (imazalil and sodium carbonate) was of about 20°-22°C. The day after half the fruit of each treatment was individually wrapped with a 19 mm thick polyolefinic heat shrinkable film (water transmission rate = 16 g/24 h m^2 at 35°C and 95% delta RH; O_2 = permeance 1.000 cm^3/24 h m^2 bar at 23°C and 0% RH) named BX (Bolphane, Bollorè, France) using a wrapping machine (Minipack-R.A.S. – Costruzioni Meccaniche Torre, Dalmine, Italy).

Storage conditions

Fruit was stored at 20°C and 65% relative humidity for 10, 20 or 30 days. Inspections and analysis were carried out at harvest and at the end of each storage period, with the exception of respiration activity and ethylene production rate, which were measured at harvest and at five-day intervals.

Respiration activity, ethylene production rate

Fifteen fruits for each treatment chosen at the beginning of the experiment were used over the storage period to measure respiration activity and ethylene production rate. Since, at the end of the trial, only eight fruit were free of decay in control-wrapped treatment, only the first eight fruit of each treatment were used for statistical analysis. Fruits were individually closed in 1 L jars and after 1 hour respiration activity was measured. The lid of each jar was fitted with two rubber septa. Through the two septa two needles connected with a combined CO_2/O_2 analyser (Combi Check 9800-1, PBI-Dansensor A/S, Denmarck) were inserted. A pump continually inspired air through the first needle from the head-space. The sample, after being analysed was re-injected through the second needle into the jar. The analysis lasted until the values of CO_2 and O_2 were constant, and this took about 1 min. After one more hour of sealing a 2 mL sample from each jar was withdrawn with a syringe for ethylene measurement, which was determined by gas chromatography (D'Aquino *et al.*, 1998).

Endogenous gas composition

Analysis was carried out on ten fruits for treatment. Each fruit was submerged in water and a 2 mL sample was withdrawn. One mL of air was used for CO_2 and O_2, the other half for C_2H_4. Determinations were performed by gas chromatography, using a Helett Packard gas-chromatograph equipped with a thermal conductivity detector for CO_2 and O_2 and a Varian 3300 gas chromatograph equipped with a flame ionization detector for C_2H_4. Experimental conditions were the same as previously reported by D'Aquino *et al.* (1998).

Firmness and weight loss

Firmness was measured as the resistance (g) opposed to puncture onto two opposite sides of the equatorial region of a 2 mm-diameter needle applied to an Ef-

fegì penetrometer (TR Turoni & C., Forlì, Italy) or as deformation. Deformation was the reduction of fruit in diameter (mm) after compression on the equatorial axis for 5 sec with a 1 kg load using the devise described by D'Aquino *et al.* (1998).

Thirty fruits per treatment were used to determine weight loss.

Visual quality evaluation

Fifty fruits per treatment were used for visual quality evaluation. Fruits were first checked for decay. The remaining healthy fruit was evaluated for visual appearance on a subjective scale of 1-9, ranging from very aged to fresh. A value of 6 expressed the limit of saleability. The percentage of saleable fruit was determined as

$$\left(1 - \frac{N_{rej}}{N_{tot}}\right) \cdot 100.$$

Where N_{tot} was the total number of fruit and N_{rej} was determined by the number of rotten fruits and fruits with a rating value less than 6.

Chemical analysis

Thirty fruits for treatment divided into three replications were used for chemical analysis of the juice, which comprised pH, titratable acidity (expressed as g citric acid in 100 mL of juice), total soluble solids (TSS), expressed as °Brix and vitamin C, as mg ascorbic acid in 100 mL of juice. The methods used were the same as reported by D'Aquino *et al.* (1998).

Statistical analysis

The experiment was a completely randomised 4 (7) x3x3 block design, with factor A being the storage period with 4 levels (7 for respiration activity and ethylene production rate) (harvest, 10, 20 and 30 days), B the fungicidal treatment (control, imazalil, sodium carbonate) and C the wrapping (no wrapping, wrapping). All data were subjected to analysis of variance and separation of the means was obtained by the LSD test. The Statgraphics software (plus 5 version, Manugistics, Rockville, Maryland, U.S.A.) was used.

RESULTS

Respiration activity, ethylene production rate and endogenous gas composition

Both storage period and wrapping significantly affected respiration activity, whilst the fungicidal treatment had no influence. The interaction between the storage period and wrapping was also significant (Table 1). During storage respiration activity decreased progressively in both wrapped and un-wrapped fruit, however in wrapped fruit, apart from the last inspection period, the intensity of the respiration activity was always significantly higher than in un-wrapped ones (Fig. 1).

Ethylene production rate increased during storage but was not affected by the other factors (Table 1). In particular, ethylene production was very low and

Table 1 - Analysis of variance of respiration rate, ethylene production, internal carbon dioxide, internal oxygen, internal ethylene, deformation and resistance to puncture of individually film wrapped "Ponkan" mandarins stored at 20°C and 65% RH for 10, 20 or 30 days.

Source	Significance of experimental factors						
	Respiration activity	Ethylene production rate	Internal Carbon dioxide	Internal Oxygen	Internal Ethylene	Deformation	Resistance to puncture
Storage period (P)	***	***	***	***	ns	***	***
Wrapping (W)	***	ns	***	***	ns	***	***
Treatments (T)	ns	ns	ns	ns	ns	ns	ns
PxW	***	ns	***	***	ns	***	***
PxT	ns	ns	ns	ns	ns	ns	ns
WxT	ns	ns	ns	ns	ns	ns	ns
PxWxT	ns	ns	ns	ns	ns	ns	ns

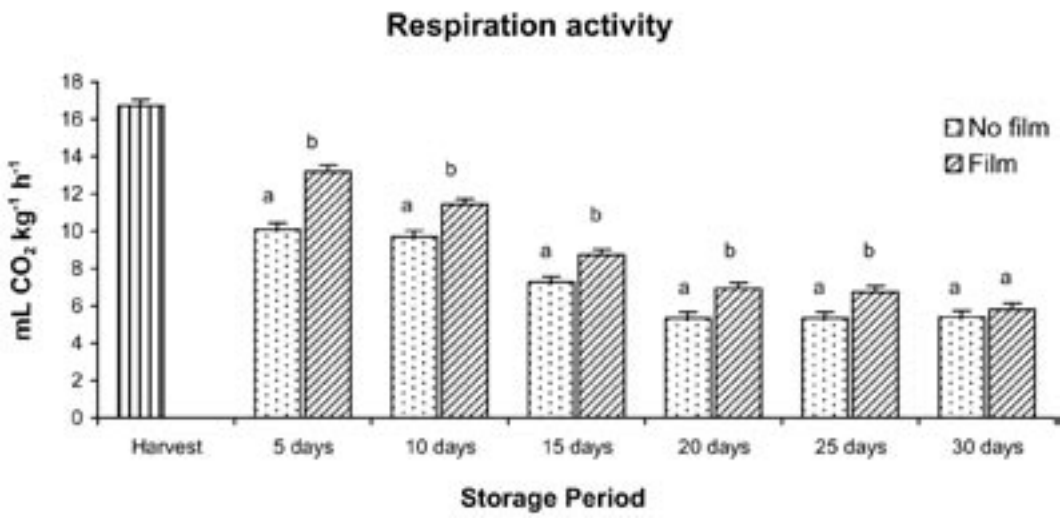


Fig. 1 - Respiration activity of "Ponkan" mandarins over the storage period as affected by film wrapping. For each storage period histograms with different letters are significantly different at P≤0.05. Vertical bars indicate the standard error (n=8).

detectable only from the tenth day of storage, with a peak of 0.038 μL kg^{-1} h^{-1} (data not shown).

Storage period and wrapping as well their interaction affected endogenous CO_2 and O_2 (Table 1). Endogenous CO_2 increased considerably over storage both in un-wrapped and wrapped fruit. However, the rate of increase of endogenous CO_2 in wrapped fruit was more rapid than in un-wrapped fruit, with final values twice higher in the first than in the latter (Fig. 2). However, differences between wrapped fruit and un-wrapped fruit were significant only at the last inspection time (Fig. 2). Conversely, endogenous O_2 decreased progressively in treatments, but at a higher

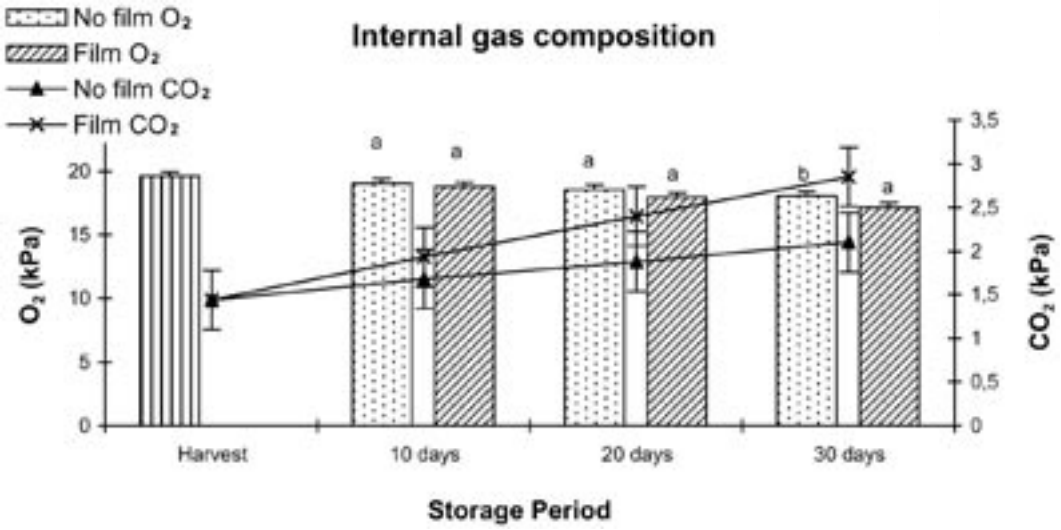


Fig. 2 - Evolution of internal carbon dioxide and internal oxygen in "Ponkan" mandarins as affected by the storage period and wrapping. For each storage period histograms (O_2) with different letters or overlapping vertical bars (LSD) crossing the lines (CO_2), are significantly different at P≤0.05.

rate in wrapped than un-wrapped fruit, with differences being significant at the third inspection (Fig. 2).

Firmness and weight loss

The storage period and wrapping affected significantly deformation (Table 1). Specifically, an irrelevant increase was observed in wrapped fruit, whereas the rapid reduction of turgor which affected un-wrapped fruit led deformation of un-wrapped ones after three weeks of storage to a three time increase with respect to harvest time (Fig. 3). The resistance to puncture of the rind changed with storage period and wrapping, while it was not influenced by fungicides; a significant interaction was also observed between the storage period and wrapping (Table 1). The change of the resistance to puncture of the rind had an opposite trend in wrapped and in un-wrapped fruit, with slightly decreasing values in the first and significant increases in the latter (Fig. 3).

Visual quality evaluation

Decay rose progressively in all treatments over the storage period (Table 2) and was always significantly higher in wrapped fruit than in un-wrapped ones (Table 2). Fungicidal treatments had a significant effect on decay (Table 2). Imazail completely inhibited microorganism growth during the first ten days of storage and reduced decayed fruit to only 2-2.8% after 20 days of storage. However, after 30 days of storage decay in imazalil-treated fruit ranged between 10.1 (un-wrapped) and 15.6% (wrapped) (Table 2). Sodium carbonate was less effective than imazalil but however reduced the incidence of decay during the first twenty days of stor-

age compared to untreated fruit (Table 2). At the last inspection decay incidence increased considerably (Table 2).

The interaction between wrapping and fungicidal treatments was highly significant (Table 2). In fact, while in imazalil and sodium carbonate wrapped fruit the incidence of decay was 52 and 43% higher than the respective un-wrapped treatments, in control wrapped fruit decay incidence was more than 200% higher than in control un-wrapped fruit. Decay caused by *Penicillium digitatum* accounted for more than 90% of total loss, about 6% was caused by *Penicillium italicum*, whereas others pathogens represented only 4% of total decay (data not shown). Although no significant differences were detected among treatments in relation the incidence of the pathogens, in general in fruita treated with imazalil and sodium carbonate the loss due to presence of botrytis and phythophtora was higer than control fruita (data not shown).

Weight loss increased progressively in all treatments going on with storage. However in wrapped fruit after 30 days of storage weight loss was of about 2.4% against 20% of un-wrapped one (Table 2).

The fungicide treatment affected significantly weight loss (Table 2); at each storage period, fruit treated with sodium carbonate always reported higher loss than control or imazalil treated fruit. However, wrapping inhibited transpiration in sodium carbonate-wrapped fruit and no statistical differences were observed with control or imazalil-wrapped fruit (Table 2).

Wrapping also had a dramatic effect in slowing down ageing. (Table 2). Over all the storage period the aesthetical aspect of wrapped fruit did not change whereas the loss of freshness was negligible even after 30 days of storage. In fact, wrapped fruit were never rated less than 8. In contrast, un-wrapped fruit since the first inspection showed severe signs of aging even if most of them were still marketable. However at the second inspection time only 20 and 16.7% of unwrapped control

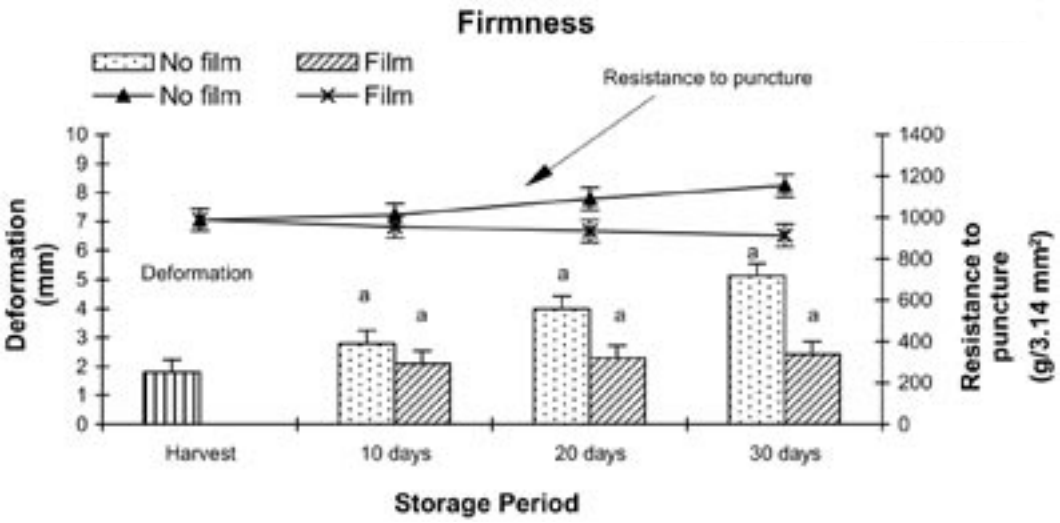


Fig. 3 - Changes in deformation and in the resistance to puncture of "Ponkan" mandarins as affected by storage period and film wrapping. For each storage period histograms (deformation) with different letters or overlapping vertical bars (LSD) crossing the lines (resistance to puncture), are significantly different at P≤0.05.

Table 2 - Changes in weight loss, appearance, decay and marketability of "Ponkan" mandarins as affected by the storage period, film wrapping and fungicidal treatment.

Storage Period	Weight loss (%)	Appearance[1] (index number)	Fruit rated more than 5 (%)	Rotten fruit (%)	Saleable fruit[2] (%)
Harvest	0	9	100	0	100
10 days					
Cnt	9.9 b[3]	6.7 b	100 b	12.7 c	87.3 b
Imazalil	10.4 b	6.6 b	100 b	0 a	100 d
Sod. Carb.	12.7 c	5.9 a	76.7 a	3.5 b	73.8 a
Cnt-film	1.3 a	9 c	100 b	22.7 d	77.3 a
Imazalil-film	1.4 a	9 c	100 b	0 a	100 d
Sod. Carb.-film	1.4 a	9 c	100 b	4.6 b	95.4 c
20 days					
Cnt	14.6 b	5.2 b	20 c	15.6 d	16.9 b
Imazalil	15.4 b	5.2 b	16.7 b	2 a	16.4 b
Sod. Carb.	19.4 d	4.7 a	0 a	4.8 b	0 a
Cnt-film	1.8 a	8.8 c	100 d	29.3 e	70.7 c
Imazalil-film	2.0 a	8.7 c	100 d	2.8 a	97.2 d
Sod. Carb.-film	2.1 a	8.5 c	100 d	6.8 c	93.2 d
30 days					
Cnt	18.2 b	3.7 b	0 a	20.1 e	0 a
Imazalil	19.3 c	3.8 b	0 a	10.1 a	0 a
Sod. Carb.	24.6 d	3.1 a	0 a	12.2 b	0 a
Cnt-film	2.1 a	8.3 d	100 b	45.5 f	54.5 b
Imazalil-film	2.5 a	8.4 d	100 b	15.6 c	84.4 c
Sod. Carb.-film	2.6 a	8 c	100 b	18 d	82 c
Source	Significance of experimental factors				
Storage period (P)	***	***	***	***	***
Wrapping (W)	***	***	***	***	***
Treatments (T)	***	***	***	***	***
PxW	***	***	***	ns	***
PxT	***	***	***	ns	***
WxT	***	***	***	***	***
PxWxT	***	***	***	ns	***

[1] Visual appearance was evaluated according to a subjective scale, where 9 was the fruit as fresh as at harvest, 5 the limit of commerciability and values less than 5 indicated aged and unmarketable fruit.

[2] Saleable fruit were determined by subtracting decayed fruit and fruit rated less than 5 from the total number of fruits.

[3] For each storage period means in a column followed by different letters are statistically significant for $P \leq 0.05$. Means separation by the Duncan's multiple range test.

and un-wrapped imazalil fruit, respectively, were rated more than 5, whereas at the same inspection time no un-wrapped fruit treated with sodium carbonate was rated more than 5 (Table 2). The combined positive effect of wrapping and fungicide treatment is better shown by the percentage of saleable fruit, which after 30 days of storage was still higher than 82%. On the contrary, at the same inspection only 54,5% of wrapped un-treated fruit, due to decay, was saleable, whilst no un-wrapped fruit was saleable (Table 2).

Chemical analysis

All chemical parameters were affected significantly by the storage period and wrapping (Table 3). The interaction between the storage period and wrapping was also significant (Table 3). In particular, over the storage period pH increased both in wrapped and un-wrapped fruit, but at a higher rate in wrapped fruit than in un-wrapped one. In contrast, titratable acidity as well as vitamin C content decreased slightly in un-wrapped fruit and markedly in wrapped one (Table 3). Total soluble solids were stable in wrapped fruit whilst un-wrapped fruit showed a slight increase. The fungicide treatment had no influence on chemical parameters (Table 3). From the gustative point of view, the above described changes did not negatively affect

Table 3 - Changes in chemical parameters in "Ponkan" mandarins over the storage period*.

Storage period	pH	Tiratable acidity g/100 mL citric acid	Total soluble solids (°Brix)	Vitamin C mg/100 mL ascorbic acid
Harvest	3.78	1.05	10.2	33.27
10 days				
No wrapped	3.87 a	0.98 a	10.85 a	34.63 a
Wrapped	3.92 a	0.92 a	10.57 a	32.94 a
20 days				
No wrapped	4.10 a	0.93 b	11.44 b	31.00 b
Wrapped	4.35 b	0.81 a	10.60 a	26.51 a
30 days				
No wrapped	4.18 a	0.90 b	11.90 b	28.84 b
Wrapped	4.44 b	0.79 a	10.42 a	22.02 a
Source	Significance of experimental factors			
Storage period (P)	***	***	***	***
Wrapping (W)	***	***	***	***
Treatments (T)	ns	ns	ns	ns
PxW	***	***	***	***
PxT	ns	ns	ns	ns
WxT	ns	ns	ns	ns
PxWxT	ns	ns	ns	ns

*For each storage period means in a column followed by different letters are statistically significant for P≤0.05. Means separation by the Duncan's multiple range test.

taste, but lowered the nutritional value by lowering vitamin C content, which at the end of storage accused a reduction of about 13% in un-wrapped fruit and of about 34% in wrapped one (Table 3).

DISCUSSION

Plastic film has been used extensively in retail conditions to protect horticultural commodities in the last two decades. Wrapping with plastic films can reduce respiration activity, transpiration, ageing, and spoilage microrganisms (Miller and Risse, 1988). However, differently than other commodities, citrus do not receive beneficial effect from modifications of the atmosphere surrounding the fruit (Purvis, 1983a). In contrast, physiological activity of the juice sacs, the edible portion of citrus, can be enhanced, even if this is not clearly detectable when respiration of the intact fruit is measured. In fact, wrapping may reduce respiration activity of flavedo and albedo (peel), but at the endocarp level, where aerobic and anaerobic metabolism normally coexist, the increase of internal CO_2 and the concomitant decline of O_2, will increment the fraction of anaerobic respiration. Consequently, the rate of degradation of fruit metabolic substrates and vitamins enhances (sugars, organic acids, ascorbic acid) whereas anaerobic by-products, such as aldehydes, alcohols, lactate, which produces off-flavours and alters the eating quality, accelerate (Bruemmer and Roe, 1970). When fruit is stored at room temperature anaerobic respiration at the endocarp level may increase at different times and fruit may become inedible within a few days. In contrast with the results of Ben-Yehoshua *et al.* (1987) and Eaks (1991) who found respiration activity of individually wrapped "Salustiana" oranges and "Eureka" lemons stored at 20°C significantly lower than un-wrapped fruit, in our experiment respiration activity as well as internal CO_2 partial pressure were constantly higher in wrapped fruit than in un-wrapped fruit. Thus wrapping stimulated anaerobic metabolism. As a result wrapped fruit showed a higher rate of degradation of TSS, titratable acidity and vitamin C content, whereas pH was always higher in wrapped fruit. However, not all these changes altered the eating quality of the fruit, which, due to the relatively initial high level of acidity received an overall beneficial effect on taste, without any presence of off flavour.

Wrapping confirmed all the beneficial effect previously reported in other experiments in preserving appearance and freshness (Ben-Yehoshua *et al.*, 1985; Ben-Yehoshua, *et al.*, 1987; D'Aquino *et al.*, 1998; D'Aquino *et al.*, 2001; Hale *et al.*, 1986; Miller and McDonald, 1989; Purvis, 1983b): softening as well as weight loss and ageing were almost completely inhibited. Therefore, after the 30 days of storage all wrapped fruit free of decay were totally marketable.

Even if film wrapping prevented spoilage by containing decay spores and release of juice as reported by other authors, (Albrigo and Ismail, 1983; Ben-Yehoshua, 1985), wrapping increased decay. Probably the high in-package humidity favoured microorganism growth. Imazalil confirmed its efficacy in reducing decay caused by green and blue mould (Eckert and Ogawa, 1985), and its activity was particularly evident in wrapped fruit. Sodium carbonate, which was extensively used as a standard procedure in California packinghouses to preserve lemons from decay (Fawcett, 1936), was also effective, even if at a lower level than imazalil. In different studies sodium carbonate has shown its effectiveness in reducing the

incidence of green and blue mould in inoculated citrus fruit when applied as heat dipping (Houck, 1965; Smilanick, 1995; Palou *et al.*, 2002), although at temperature of 20°-26°C it has demonstrated no beneficial effect (Houck, 1965). The mode of action of sodium carbonate is still unknown, but in vitro it has only shown a weak activity on spore germination (Marloth, 1931). As different compounds or physical treatments (Barkai-Golan, 2002) sodium carbonate may induce phytoalexin production in host tissue. However, their synthesis and accumulation in treated tissue may reach protective levels some days after the treatment (Rodov *et al.*, 1992). In the experiments in which sodium carbonate was used as heated solution there might be a direct and instantaneous effect on pathogen growth due to the hot temperature followed by a possible induced resistance of the host by sodium carbonate.

CONCLUSIONS

Wrapping citrus fruit with plastic films can have a beneficial effect in delaying ageing, in maintaining freshness and in prolonging marketability. However, when different fruits are packaged together microorganism growth represents the major problem to face: one rotten fruit in short time will contaminate other fruits in the package. In addition, liquid and volatiles coming out from decomposed tissue (ethylene and other compounds responsible for off-flavour) will alter the quality of healthy fruit. Sealing citrus fruit individually prevents the spreading of spores from decayed fruit, does not allow liquid and volatiles produced by decayed fruit to contaminate healthy ones and makes the removal of decayed fruit easy. Moreover, according to the characteristics of the film, metabolic activity of fruit and storage temperature, the prediction of respiration activity as well as of the potential postharvest life of individually wrapped fruit will result easier than when different fruits are packaged together in cartons or plastic bags. In this last case, in fact, any model must also take account of the volume of the space between the fruit and plastic film, of the amount of packaged fruit as well as of the ratio of the surface of the film/weight of fruit. Yet, in the case of organic fruit that can be protected from decay only by the use of residue-free non-toxic compounds, which, such as sodium carbonate, are less effective than conventional fungicides, individual wrapping of fruit can be the only means that can make the use of film wrapping economically feasible. In fact, if different fruit was packaged together in cartons, plastic bags or trays, the incidence of decay might be excessively high to nullify all the possible advantages deriving from wrapping. Unlikely, the cost per single fruit can be so high to cause individual wrapping to be not convenient on a large scale. Nevertheless, the availability of very efficient machines which can wrap fruit very fast and the possibility that in the near future the cost of plastic film will decrease, could make this technology economically viable.

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- Authors contributed equally to this study.

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LECTURE

BIOPRESERVATION OF FRESH VEGETABLES: MICROBIOLOGY AND ECOLOGY

BIOCONSERVAZIONE DI VEGETALI DI QUARTA GAMMA: ASPETTI MICROBIOLOGICI ED ECOLOGICI

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ABSTRACT

The ecological aspects of Scarola salad leaves, mechanically damaged and singly inoculated with *Lactobacillus casei*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Escherichia coli* and *Listeria monocytogenes*, were studied. SEM observations and bacterial counts of damaged tissues or leaf surface demonstrated that the added cells of each species mainly colonized the cut-edges, trichomes and stomata of salad leaves. Challenge experiments performed on salads inoculated simultaneously with *Lactobacillus casei* and each single pathogen, showed a remarkable inhibitory effect by the lactic culture towards all the pathogenic strains. The protective effect of antimicrobical-producing lactic acid bacteria in relation to bacterial cell location is discussed.

RIASSUNTO

Sono stati studiati gli aspetti ecologici delle foglie di insalata Scarola, meccanicamente danneggiate e singolarmente inoculate con *Lactobacillus casei*, *Staphylo-*

- Key words: biopreservation, *Lactobacillus casei*, ready-to-eat, vegetables -

coccus aureus, Aeromonas hydrophila, Escherichia coli e Listeria monocytogenes. Le osservazioni in microscopia SEM e le conte microbiche sulla superficie fogliare o lungo le sezioni di taglio, hanno dimostrato che le cellule di ciascuna specie aggiunta colonizzano prevalentemente le parti danneggiate, i tricomi e gli stomi.

Gli esperimenti di competizione condotti su foglie di insalata Scarola, inoculata simultaneamente con *Lactobacillus casei* ed ogni singolo patogeno, hanno evidenziato un notevole effetto inibitorio da parte della coltura lattica nei confronti di tutti i patogeni esaminati.

La tendenza delle cellule di tutte le specie batteriche aggiunte ad accumularsi nelle stesse nicchie ecologiche può fornire una spiegazione del notevole effetto protettivo dei batteri lattici produttori di sostanze antimicrobiche.

INTRODUCTION

In the recent years, the increasing consumer demand for more convenience food items led to a wide diffusion of new categories of vegetable products in the ready-to-eat form on the market. Freshness, economic handling and attractive presentation are essential characteristics for their progressive success, as well as the absence of a further washing, slicing or chopping (Nguyen-the and Carlin, 1994; Vescovo *et al.*, 1997).

Pathogens can readily contaminate fresh produce via contact with the decaying vegetation, soil, polluted water, manure or sewage sludge (Beuchat, 1998). In addition, contamination with pathogens such as *Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli* may also occur during post-harvest handling, processing, distribution and marketing, as demonstrated by several researchers (Berrang *et al.*, 1989; Beuchat and Brackett, 1990; Odumeru *et al.*, 1997). To preserve the typical fresh character only mild stabilizing treatments are reserved to such products, as the combination of chemical and physical barriers (chlorine, low temperatures, MAP) (Vescovo *et al.*, 1997). However, controversial opinions exist among researchers on the effectiveness of classical sanitizers like chlorine, as the current procedures cannot eliminate the contaminating microorganisms (Seo and Frank, 1999; Takeuchi and Frank, 1999; Takeuchi *et al.*, 2000). In fact, it is well known that the difference of total microbial counts between vegetables treated with 200 ppm chlorine or only water washed, is usually not more than 1 log CFU/g (Beuchat, 2002). Furthermore, chemical treatments can reduce the freshness image. These reasons stimulated food microbiologists to try new biological approaches in the preservation of minimally processed fruits and vegetables (Vescovo *et al.*, 1995; Vescovo *et al.*, 1996, Cay *et al.*, 1997).

Lactic acid bacteria (LAB) completely fulfill this purpose, due to their protective effects widely demonstrated in a large variety of foods: the ability to produce organic acids, H_2O_2 and particularly antimicrobial compounds as bacteriocins (Stiles, 1996; O'Sullivan *et al.*, 2002) and bacteriocins-like (Scolari *et al.*, 1999) explain their competitive action towards undesirable microorganisms.

A better understanding of the microbial ecosystem surface of the surface of raw vegetables, would be extremely useful when developing interventions to minimize contamination, to prevent the growth of pathogens and to kill or remove pathogens at various stages of processing, marketing and preparation for consumption.

The objective of this research was to study the microbial ecosystems of Scarola

salad leaves, contaminated with single pathogenic strains of four species or inoculated with an antimicrobial-producing *Lactobacillus casei* strain. Moreover challenge experiments were carried out between the lactic culture and each pathogen to evaluate the inhibition potential of *L. casei* in fresh vegetables during storage at 8°C.

MATERIALS AND METHODS

Salad

Scarola salad was purchased at a local supermarket and immediately utilized after transport to the lab. Outer leaves were discarded, while the inner pale-coloured leaves were subjected to a extensive washing at 25°C for 15 min in a mild ultrasound water-bath; the washing solution was sterile milli-Q water containing 200 ppm chlorine. Treated samples were rinsed repeatedly in sterile milli-Q water up to the complete removal of the sanitizer, then cut in 3x3 cm pieces by a sterile surgical knife. Cut-edges were made on the leaf surface to simulate post-harvest mechanical damage.

Strain and culture conditions

L. casei LC34GF strain was selected among various mesophilic LAB strains isolated from fresh vegetables and characterized for their peculiar inhibitory activity against pathogenic and spoilage microorganisms (Vescovo *et al.*, 1996). Further characteristic traits of this strain were: absence of pectinolytic activity and growth at refrigeration temperature.

E. coli Ct1 and Ct2 were isolated from raw milk, while *E. coli* EcV8 was from fresh mixed salads; *S. aureus* Sa4 and Sa5 strains were isolated from lettuce and basil, respectively. *L. monocytogenes* VR13560 was from the collection of Dipartimento Scientifico e Tecnologico, Università degli Studi Verona, Italy. *L. monocytogenes* LGB and Sp2 were isolated from cauliflower and smoked salmon, respectively. *Areomonas hydrophila* 2 was kindly provided by Prof. A. Galli (Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università di Milano, Italy). All the strains were maintained at -80°C until use.

L. casei LC34GF was cultured 15 h at 30°C in MRS broth (Oxoid). Each pathogenic strain was activated by three successive 24 h transfer in Tryptic Soy Broth (Difco) at 37°C.

Inoculum procedure

Bacterial cells were collected from active cultures by centrifugation at 3,000 x g, 4°C, 15 min; pellets were twice washed and resuspended in sterile milli-Q water to obtain an inoculum level of 6-7 log CFU/g for *L. casei* strain and 4-5 log CFU/g for each pathogen on salad leaves. The inoculum of each strain was sprinkled singly on leaves which were left 18 h at 4°C in sterile bags to allow the complete absorption of the adjuncted cells. Following two gentle rinses in sterile water, samples were incubated 6 days at 8°C in sealed polyethylene bags. A control was prepared using sterile water as inoculum.

Challenge experiments

Challenge experiments were carried out in two phases using the inocula of LAB and pathogens above described. At first, salad leaves were sprinkled with the

mixed strains of each pathogenic species and left 2 h for absorption in a sterile cabinet; then, LAB inoculum was added and leaves were maintained 18 h at 4°C for the complete absorption. Samples were twice gently rinsed before incubation at 8°C for 6 days.

Labeling of *L. casei* LC34GF strain

To evaluate the ability of the adjuncted LAB cells to colonize the Scarola salad leaves, *L. casei* LC34GF strain was transformed by electroporation with pPSC22 plasmid vector (Cocconcelli *et al.*, 1996) carrying the resistance to erythromycin and chloramphenicol. The transformation procedure was performed employing the apparatus and the protocol previously described (Vescovo *et al.,* 1991) with some modifications: *L. casei* was grown up to 0.630 OD560; pulse time was 3.8 ms and 1 h was required for gene expression. Transformed cells were selected on MRS containing 50 μg mL^{-1} erythromycin.

Bacterial counts

Ten samples of the outermost tissue (0.3 cm from cut-edge) were removed from salad leaves with a sterile surgical knife and homogenized in 10 mL of sterile Ringer solution. Samples were taken also from leaf surface. Decimal dilutions were plated in MRS agar added of 50 μg/mL erythromycin for *L. casei* LC34GF strain and in Tryptic Soy Agar for pathogenic strains counts. Plates were incubated 48 h at 30°C under anaerobiosis for *L. casei*, 24 h at 37°C for *E. coli* and 48 h at 37°C for the remaining pathogens, under aerobiosis.

SEM observations

Salad samples were dehydrated by increasing ethanol concentrations up to the critical point, then coated with gold under vacuum (Scolari and Vescovo, 1996). Observations were made by electron microscope Philips XL30 ESEM.

RESULTS AND DISCUSSION

The results from challenge experiments carried out on salad leaves between *L. casei* LC34GF strain and single mixed cultures of *S. aureus* Sa4 and Sa5, or *E. coli* Ct1, Ct2 and EcV8, or *L. monocytogenes* VR13560, LGB and Sp2, or *A. hydrophila* 2, are summarized in Table 1. It is possible to observe that during the incubation period, the inoculated LAB progressively increased by about 2 log CFU/g. *S. aureus* appears to be the most sensitive to the antagonistic effect of *L. casei* cells, as it was not detectable after 6 days at 8°C; the inhibition was already evident since the second day (2 log reduction).

A. hydrophila and *E. coli* followed a similar kinetics of count decrease, ranging from 0.7 log CFU/g on the second day to more than 5 log CFU/g at the end of leaf incubation. *L. monocytogenes* was the most resistant among the examined species, although 1 log CFU/g reduction was observed since the second day, and 2.5 log CFU/g at the end of incubation time.

As the potential of *L. casei* LC34GF strain was more evident when employed in fresh vegetables compared to other food matrix, the study of ecological interactions between microflora and the abiotic components of this ecosystem, is of great interest and offers new perspectives to explain the observed phenomena.

Some indications on these aspects are provided by the attachment of the dif-

Table 1 - Antagonistic effect of *Lactobacillus casei* LC34GF against pathogens added to Scarola leaves incubated at 8°C.

Strains	Incubation time (days)			
	0	2	4	6
Staphylococcus aureus Sa4-Sa5	5.0±0.2[a]	2.3±0.2	<1	n.d.[b]
Lactobacillus casei LC34GF	6.2±0.2	6.5±0.3	7.2±0.6	8.5±0.5
Aeromonas hydrophila 2	5.0±0.3	4.3±0.2	2.2±0.2	<1
Lactobacillus casei LC34GF	7.2±0.3	8.4±0.3	8.6±0.1	9.1±0.3
Escherichia coli Ct1-Ct2-EcV8	4.8±0.3	4.2±0.2	2.5±0.2	<1
Lactobacillus casei LC34GF	6.9±0.2	7.5±0.2	7.9±0.4	8.1±0.4
Listeria monocytogenes VR13560-LGB-Sp2	4.9±0.3	4.0±0.2	3.2±0.1	2.4±0.2
Lactobacillus casei LC34GF	6.6±0.3	7.1±0.2	8.0±0.5	8.0±0.7

[a]: data are the means of three separate experiments and are expressed as log CFU/g ± SD;
[b]: not detectable.

ferent bacterial species to Scarola salad leaves after the artificial contamination. Bacterial counts related to leaf surface and cut-edges respectively, are shown in Table 2. The counts detected on the cut-edges were higher than those present on the surface of about 1.5; 0.9; 1.1 and 0.5 log CFU/g for *L. casei, S. aureus, A. hydrophila* and *E. coli,* respectively. Similar results were obtained by Seo and Frank (1999) and by Takeuchi *et al.* (2000) for *E. coli* O157:H7 on iceberg lettuce leaves. The results from bacterial counts were also confirmed by SEM observations, as shown in some significant microphotos.

Microcolonies of *S. aureus* Sa5 are visible as biofilm structure adhering to the cut-edge tissues of a salad leaf (Fig. 1) as well as for *S. aureus* Sa4 (Fig. 2). Cells of this species are usually concentrated near the damaged lymphatic vessels in cut-edges of leaf (Fig. 3) probably for a greater availability of nutrients. The same reason explains the higher concentration of *E. coli* cells in cut-edges, as illustrated in Fig. 4, in which the difference in cellular density between surface and cut-edges is evident; frequently cells of *E. coli* strains accumulate deep within the leaf stomata

Table 2 - Bacterial counts in different sites of Scarola leaf, after single strain inoculation and incubation at 8°C for 6 days.

Bacterial strains	Inoculum size[a]	Surface[a]	Cut-edge[a]
Lactobacillus casei LC34	6.1±0.2	6.6±0.3	8.2±0.2
	7.0±0.4	6.9±0.3	8.5±1.0
Staphylococcus aureus Sa3-Sa4	4.1±0.1	4.0±0.1	5.0±0.3
	5.2±0.2	4.8±0.3	5.6±0.4
Aeromonas hydrophila A2	4.3±0.1	4.5±0.2	5.8±0.4
	5.1±0.2	5.2±0.2	6.1±0.3
Escherichia coli Ct1-Ct2-EcV8	4.2±0.1	4.0±0.1	4.5±0.1
	5.0±0.3	5.2±0.3	5.8±0.3

[a]: data are the means of four separate experiments and are expressed as log CFU/g ± SD.

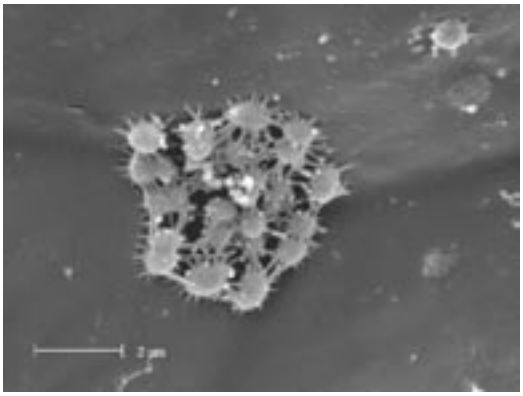


Fig. 1 - Biofilm of *Staphylococcus aureus* Sa5 cells in a cut-edge of Scarola leaf inoculated with the pathogen and incubated at 8°C for 6 days.

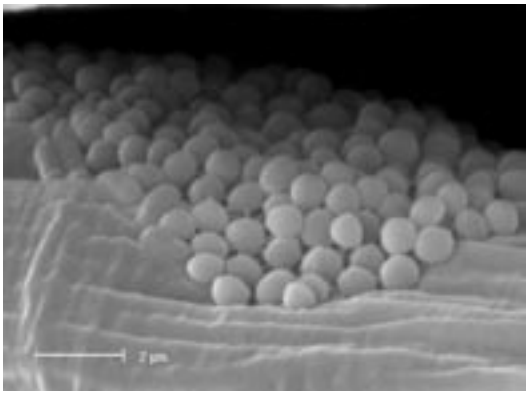


Fig. 2 - Biofilm of *Staphylococcus aureus* Sa4 cells adherent to cut-edge of Scarola leaf inoculated and incubated at 8°C for 6 days.

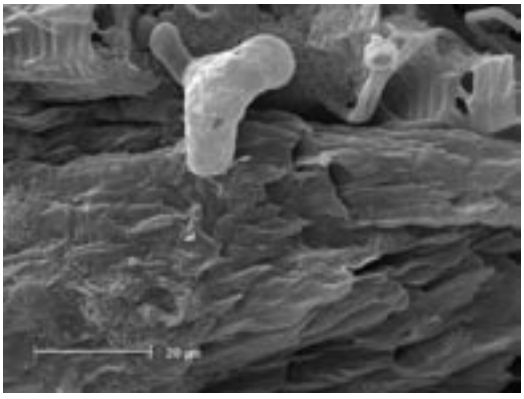


Fig. 3 - Massive growth of *Staphylococcus aureus* Sa4 cells near damaged lymphatic vessels in cut-edge of Scarola leaf.

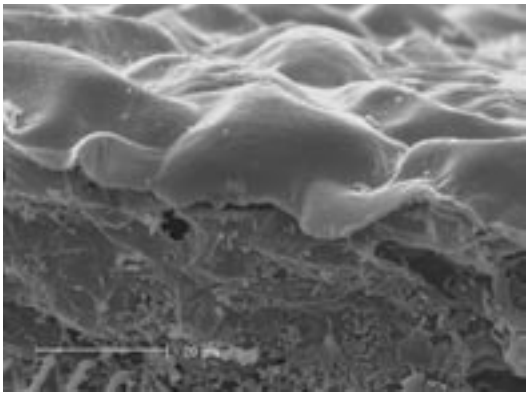


Fig. 4 - Difference in microflora density between surface and cut-edges on salad leaf inoculated with *Escherichia coli* EcV8 and incubated at 8°C for 6 days.

(Fig. 5). When sprinkled on salad leaves also *A. hydrophila* cells occupy the same preferential ecological niches (Fig. 6 and 7).

It is interesting to note that the colonizing ability of *L. casei* strain was maximal on cut-edges, stomata and trichomes, as represented in Fig. 8, 9 and 10 respectively.

The above observations suggest that the contamination sites of pathogens in fresh vegetable leaves are mainly localized on the cut-edges, damaged tissues, and in the stomata; deep penetration into these sites is also observed. This could be attributable to the inability of pathogenic bacteria to degrade the hydrophobic cuticle layer present on the leaf surface to reach the soluble nutrients. The obtained results agree with the data reported by Seo and Frank (1999), Takeuchi and Frank (2000) and Takeuchi *et al.* (2000), who also demonstrated that cellular viability in such niches is poorly affected by the sanitizing treatments.

The present research demonstrates that LAB colonization occurs in the same ecological niches occupied by pathogenic strains. This ecological aspect assumes

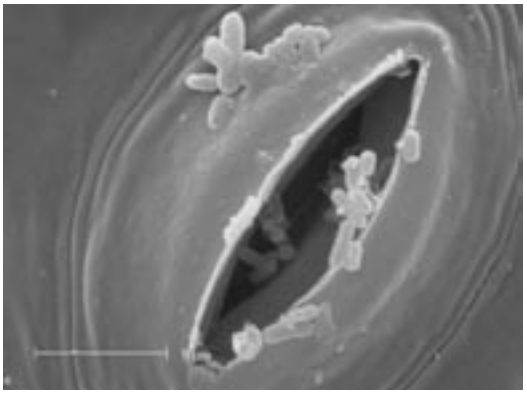


Fig. 5 - Deep internalization of *Escherichia coli* Ct1 cells in Scarola leaf stomata.

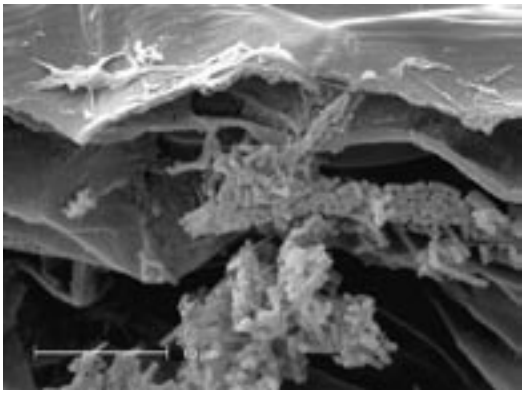


Fig. 6 - Difference in microflora density on surface and cut-edges on salad leaf inoculated with *Areomonas hydrophila* A2 and incubated at 8°C, 6 days.

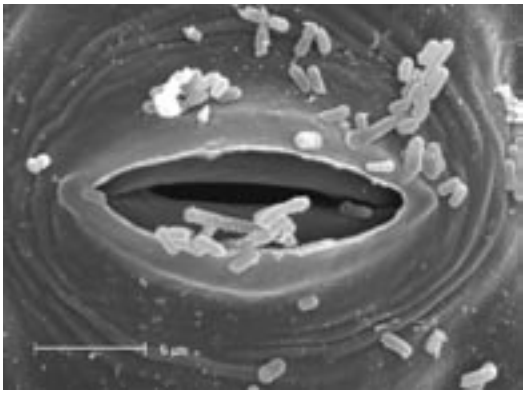


Fig. 7 - Colonization leaf stomata by *Areomonas hydrophila* inoculated cells.

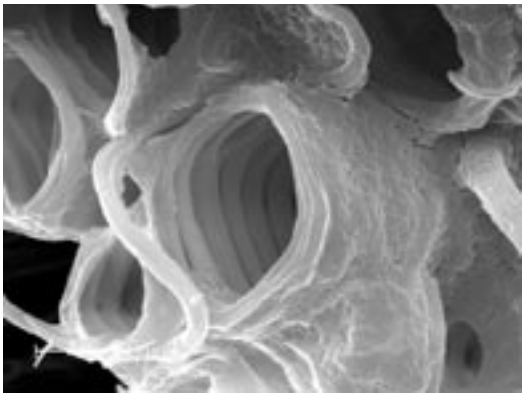


Fig. 8 - Preferential localization of *Lactobacillus casei* LC34GF strain on damaged lymphatic vessels in cut-edge surfaces of Scarola leaves 6 days after its inoculation.

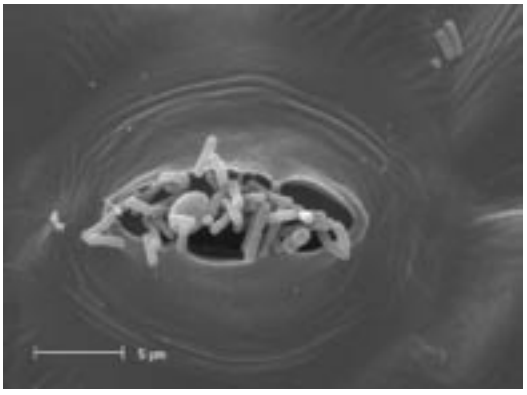


Fig. 9 - Penetration of *Lactobacillus casei* LC34GF cells in Scarola leaf stomata.

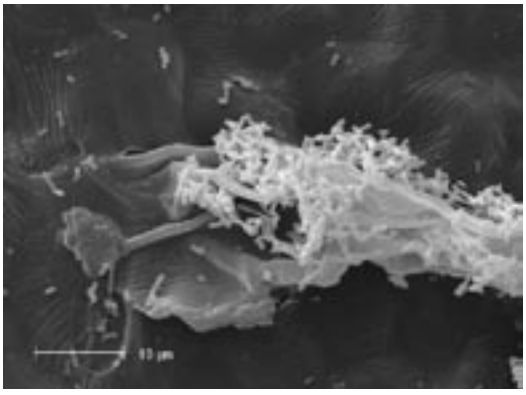


Fig. 10 - Intense growth of *Lactobacillus casei* LC34GF strain on damaged trichomes of Scarola leaf.

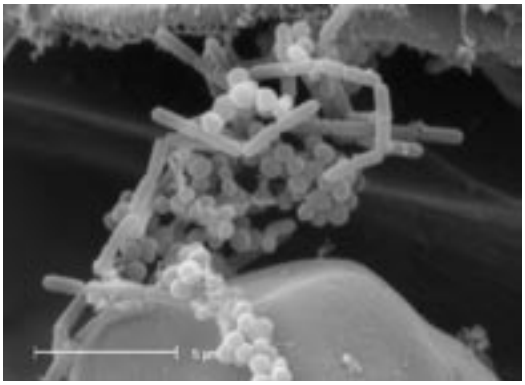


Fig. 11 - Visualization of a cut-edge on Scarola leaf inoculated with *Stapylococcus aureus* Sa4 (10^5 CFU/g) and subsequently with *Lactobacillus casei* LC34GF (10^6 CFU/g) on the first day of incubation at 8°C.

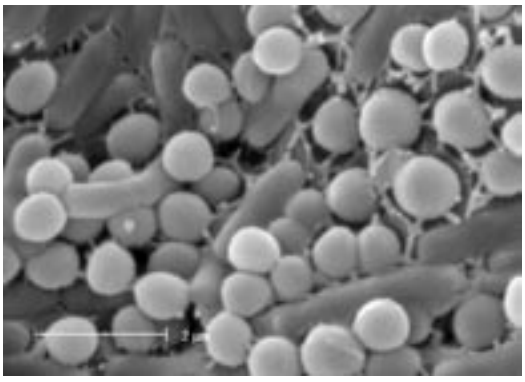


Fig. 12 - Visualization of a cut-edge on Scarola leaf inoculated with *Stapylococcus aureus* Sa4 (10^5 CFU/g) and subsequently with *Lactobacillus casei* LC34GF (10^6 CFU/g) on the second day of incubation at 8°C.

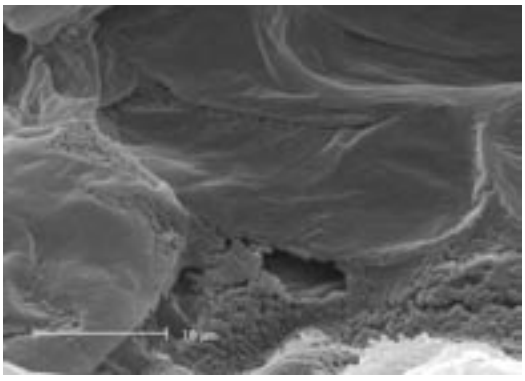


Fig. 13 - Visualization of a cut-edge on Scarola leaf inoculated with *Stapylococcus aureus* Sa4 (10^5 CFU/g) and subsequently with *Lactobacillus casei* LC34GF (10^6 CFU/g) on the fourth day of incubation at 8°C.

particular importance, as it could explain the protective effect of LAB against pathogens in ready-to-eat vegetables (present study; Vescovo *et al.,* 1996). In fact the localized multiplication of inoculated LAB cells allows the inhibiting metabolites to be maintained in a concentrated form, so assuring a strong antagonism toward the competing pathogenic microflora.

SEM observations of cut-edges, carried out on samples taken during challenge experiments between *L. casei* LC34GF and *S. aureus* Sa4 and Sa5, showed an increasing prevalence of LAB compared to pathogens on first, second and fourth day of leaf storage at 8°C (Fig. 11, 12, 13). It is noteworthy to underline that the inhibitory effect of LAB occurs even if the pathogenic strains are organized in a protective biofilm structure (Fig. 12).

These data clarify the conflicting results appearing in literature about the possible protective role of the indigenous microflora against pathogenic species (Carlin *et al.*, 1996; Francis and O'Beirne, 2002; del-Campo *et al.*, 2001). In fact, it is well known that such microflora usually colonize undamaged surface areas not available for pathogens (Takeuchi *et al.*, 2000); therefore a close inhibiting interaction with pathogens is not always possible. The last considerations could also explain the need of a high inoculum of *L. casei* Lc34 GF (6-7 log CFU/g) to get a successful control of the total aerobic microflora represented mainly by pseudomonads (data not shown).

CONCLUSIONS

The LAB protective cultures mainly grow in the same ecological niches where pathogenic cells adhere; the effectiveness of added LAB cells extends during the whole storage period contrary to the activity of sanitizing agents

which decline suddenly. Moreover the protective effect is probably enhanced by abuse temperature, making LAB addition to fresh salads particularly interesting for food safety in the prevention of risk from cold-chain failure.

Further studies are advisable to improve the washing process by the use of proper physical methods to remove the epiphytic microflora especially when it is organized in biofilm form.

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LECTURE

POLYSACCHARIDE-LIPID EDIBLE COATING AS WATER VAPOUR BARRIER: APPLICATION TO BAKERY PRODUCTS

FILM EDIBILI A BASE DI POLISACCARIDI E LIPIDI COME BARRIERE AL VAPORE D'ACQUA: APPLICAZIONE A PRODOTTI DA FORNO

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ABSTRACT

In previous investigations, a polysaccharide-lipid based edible film was developed and optimised to improve water vapour barrier properties (Peressini *et al.*, 2001; Bravin *et al.*, 2002; Peressini *et al.*, 2002). The edible film contains a structural matrix of corn starch – methylcellulose and soybean oil as moisture barrier.

Our first aim of this work was to study the effect of application technique of liquid film, drying temperature and thickness on water vapour permeability (WVP) of the optimised edible film. Lamination and atomisation at different pressures (0.20 and 0.35 MPa) of the liquid dispersion, drying temperature from 25° to 60°C and thickness ranging from 15 to 80 μm were used to obtain information relevant to the food coating.

Further aims were to establish a coating procedure of bakery products and to evaluate the effect of coating on water vapour uptake of these moisture sensitive foods. The study was conducted to determine the effectiveness of the edible coating as moisture barrier as well as its potential to extend the storage life of dry foods stored at different relative humidities (RH). Crackers were chosen as an ideal model of dry bakery product for the simple shape and regular surface. Coated and uncoated (control) samples were stored at 25°C at 65, 75 and 85% RH. Different samples were weighed at regular intervals and moisture uptake and resistance to water vapour transmission (R) were calculated. Measurement of mechanical properties of coated and uncoated products at various a_w was also performed. Shape, dimension and colour of crackers were evaluated by image analysis.

Laminated films gave better moisture barriers than sprayed films. High atomisation pressure and high thickness increased film WVP. Results identified atomisation pressure of 0.2 MPa, drying temperature of 60°C and thickness of 30 μm as the best coating conditions.

Coated crackers gave longer shelf-life and higher R than controls at all storage conditions. Increasing RH reduced sample resistance to water vapour transmission, but

the decrease in R values was lower for coated products. Mechanical properties and image analysis showed no significant differences among commercial and coated crackers.

RIASSUNTO

In lavori precedenti, è stato sviluppato ed ottimizzato un film edibile a base di polisaccaridi e lipidi con buone proprietà barriera al vapore d'acqua (Peressini *et al.*, 2001; Bravin *et al.*, 2002; Peressini *et al.*, 2002). Il film è composto da una base strutturante di amido-metilcellulosa e da olio di soia come barriera al vapore d'acqua.

Il primo obiettivo di questa ricerca ha riguardato lo studio dell'influenza della tecnica di applicazione del film allo stato fluido, della temperatura di essiccazione e dello spessore finale sulla permeabilità al vapore d'acqua (WVP) del film edibile. Al fine di ottenere utili informazioni per l'applicazione del rivestimento al prodotto sono stati investigati l'applicazione per laminazione e nebulizzazione a diverse pressioni (0,20 e 0,35 MPa), temperature di essiccazione da 25° a 60°C e spessori compresi nel *range* da 15 a 80 μm.

Successivamente è stata definita la procedura più idonea per la ricopertura di prodotti da forno ed è stato valutato l'acquisto d'acqua nel tempo di questi prodotti sensibili ad idratazione. Lo studio è stato condotto al fine di determinare l'efficacia del rivestimento edibile come barriera al vapore e, quindi, la sua potenzialità nel prolungare la shelf-life di alimenti a bassa attività dell'acqua conservati a diverse umidità relative (RH). I cracker sono stati scelti come modello ideale di prodotto da forno a bassa a_w per la forma semplice e per la superficie regolare.

I campioni ricoperti e non (controllo) sono stati conservati a 25°C a RH del 65, 75 e 85%. I diversi campioni sono stati pesati ad intervalli di tempo regolari per calcolare l'acquisto d'acqua nel tempo e la resistenza al trasferimento del vapore d'acqua (R). Sono state determinate anche le proprietà meccaniche dei cracker ricoperti e non condizionati ad a_w diverse. Forma, colore e dimensioni sono state valutate attraverso l'analisi dell'immagine.

I film ottenuti per laminazione hanno fornito le barriere migliori al vapore d'acqua. Pressioni di nebulizzazione e spessori elevati hanno determinato un aumento della WVP. Le condizioni operative più idonee alla ricopertura dei prodotti sono risultate la pressione di nebulizzazione di 0,20 MPa, la temperatura di essiccazione di 60°C e lo spessore finale del film di 30 μm.

In tutte le condizioni di conservazione i cracker ricoperti hanno mostrato una shelf-life ed una R maggiori rispetto al controllo. Un aumento dell'RH è stato accompagnato da una riduzione di R, tale riduzione è risultata nettamente più marcata per il controllo. Le proprietà meccaniche e l'analisi dell'immagine non hanno evidenziato differenze significative tra i cracker commerciali e quelli ricoperti.

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THE INFLUENCE OF USING DIFFERENT PACKAGING ON THE QUALITY DECAY KINETICS OF "CUCCÌA"

INFLUENZA DI DIVERSI IMBALLAGGI
SULLA CINETICA DI DECADIMENTO DELLA "CUCCÌA"

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ABSTRACT

The renewed interest of consumers towards traditional and typical dishes has led to an increase in the demand for "Cuccìa", which is a traditional Sicilian food made by boiling durum wheat kernels "complete" with their aleuronic and embryonic layer in water. The aim of this work was to assess the influence of using different packaging (PET and active PET packaging) on the quality decay kinetics of "Cuccìa". In particular, the performance of traditional plastic containers and that of an innovative material containing an oxygen scavenger was evaluated by monitoring the quality of bottled "Cuccìa" during storage at 15° and 37°C. For comparative purpose the quality decay kinetics of "Cuccìa" bottled in glass was also determined. Results showed that the containers made of PET can slow down the quality decay kinetics of "Cuccìa" to that of the containers made of glass.

RIASSUNTO

La "Cuccìa" è la cariosside di grano duro "integrale" quindi completa dello strato aleuronico ed embrione, tradizionalmente preparata appena bollita e condita con

- Key words: "Cuccìa", oxygen scavenger, packaging, PET, shelf-life -

sale, olio e aceto. Le caratteristiche nutrizionali e salutistiche di tale prodotto risultano estremamente interessanti. Infatti, la "Cuccìa" ha un elevato contenuto in fibra, vitamine del gruppo E e B, numerosi acidi grassi polinsanturi quali il linoleico e linolenico, e polifenoli, tutti fattori che non sfuggono ad un consumatore attento. Scopo della ricerca è stato quello di testare l'influenza di differenti imballaggi (PET e PET attivo) sulla cinetica di decadimento della "Cuccìa" in alternativa ai tradizionali contenitori in vetro attualmente utilizzati. I risultati hanno mostrato come l'imballaggio in PET ha mantenuto le caratteristiche qualitative della "Cuccìa" simili al vetro per quanto riguarda sia alcune caratteristiche sensoriali che chimiche durante il periodo di conservazione. Inaspettatamente l'active packaging non ha mostrato grossi vantaggi di efficienza per tali caratteristiche ottenendo un maggior decadimento della qualità della "Cuccìa" anche rispetto ai contenitori in vetro probabilmente a causa della bassa concentrazione di "oxigen scavenger". Si può quindi affermare che per l'imballaggio di questo prodotto è possibile utilizzare imballaggi in PET in sostituzione del vetro che, oltre ai vantaggi commerciali ed economici, consentono di conservare la "Cuccìa" alle stesse condizioni.

INTRODUCTION

"Cuccìa" is a traditional Sicilian food made by boiling durum wheat kernels "complete" with their aleuronic and embryonic layer in water and dressing it with salt and vinegar. The renewed interest of consumers towards traditional and typical dishes has led to an increase in the demand for this specific product. Due to its sensorial peculiarities, such as the refined taste, the consistency and the golden color, "Cuccìa" can be used simply as it is or to integrate minimally processed products as an alternative to corn. Moreover, the concentration of nutraceutical constituents in such product appears to be high. In fact, "Cuccìa" has a high fiber content, vitamins E and B, a great number of unsaturated fatty acids such us linoleic and linolenic, and polyphenols. "Cuccìa" is generally stored in glass containers filled with extra virgin olive oil.

The aim of this work was to assess the influence of using different packaging (PET and active PET packaging) on the quality decay kinetics of "Cuccìa".

MATERIALS AND METHODS

Materials

Three different types of bottles were used in the present investigation to run the tests: 1) commercially available glass bottles with a capacity of 1,000 cm^3; 2) commercially available PET bottles supplied by SIPA S.p.A. (Vittorio Veneto – Italy), with an average wall thickness of 0.56 mm, a diameter equal to 12.1 cm and a capacity of 1,800 cm^3; 3) commercially available PET bottles with 1% oxygen scavenger supplied by SIPA S.p.A. (Vittorio Veneto – Italy), with an average wall thickness of 0.56 mm, a diameter equal to 12.1 cm and a capacity of 1,800 cm^3. All the bottles were used as received. For the sake of simplicity,

the glass bottles will henceforth be referred to as Reference, the PET as Package A and the bottles made of PET and the oxygen scavenger will be referred to as Package B.

"Cuccìa" preparation

The tests were run on a widely used durum wheat cultivar "Simeto" collected from a Sicilian biological farms. The wheat was selected for homogeneity and flawlessness. The "Cuccìa" was prepared as follows. Wheat kernels were washed three times with tap water, drained, soaked in water at room temperature for 18 hours and then drained. During this last step, the water was changed every 3 hours. Then the "Cuccìa" was cooked in water for two hours and drained. The kernels were rapidly put into a solution composed of boiling water, vinegar, citric acid and tartaric acid, for 6 minutes both to improve the sensory characteristics and to microbiologically stabilize the product (pH≤4,2). Later the kernels were drained again and hot filled in the three types of containers. Each package contained 1.5 kg of "Cuccìa", and was filled with Sicilian extra virgin olive oil. The containers were stored at two different temperatures 15° and 37°C. The following physical parameters were monitored over 6 months: pH, acidity, peroxide number, spectrophotometric analysis, refractometric index, hardness, drag coefficient.

Sensory analysis

A sensory analysis of "Cuccìa" samples to get a complete and reliable description of all the characteristics perceived by the human senses (henceforth attributes) was carried out by a panel (30 students, 18 females and 12 males between 20 and 25 years of age from the University of Catania). Judges were selected with respect to odour identification ability, memory for odours and flavours, taste sensitivity and most important, verbal creativity and intelligence. In the preliminary session the aim of the work and the recommended behaviour before and during the tests concerning generation of attributes, discussion and reduction assessment were explained. Two sessions were carried out to define the descriptor evaluation scale and procedure of the ranking test (ISO 8587, 1998).

After extensive training 12 judges were selected able to evaluate descriptors: colour, kernel uniformity, odour, oil odour, hardness determined by force required to bite completely through sample placed between molars, masticability, savour, pericarp toughness, off-flavours and overall judgement.

In this sensory method the intensity of attributes were rated independently by assessors. The samples were presented to assessors in a random order, within each session the design was balanced for order and carry over effects (MacFie *et al.*, 1989). In the card (Fig. 1) the descriptive terms were accompanied by numerical scores on a scale from maximum (corresponding to 9) to absence of attribute (corresponding to 1) (Lanza *et al.*, 1995, 2000).

Chemical descriptors

The oil chemical descriptors (that is, pH, acidity, peroxide number, spetctrophotometric analysis and refractometric index) as well as the wheat mechanical descriptors (i.e., hardness, drag coefficient) were monitored for a period of 6 months at a frequency of 20 days. The former were determined following the official meth-

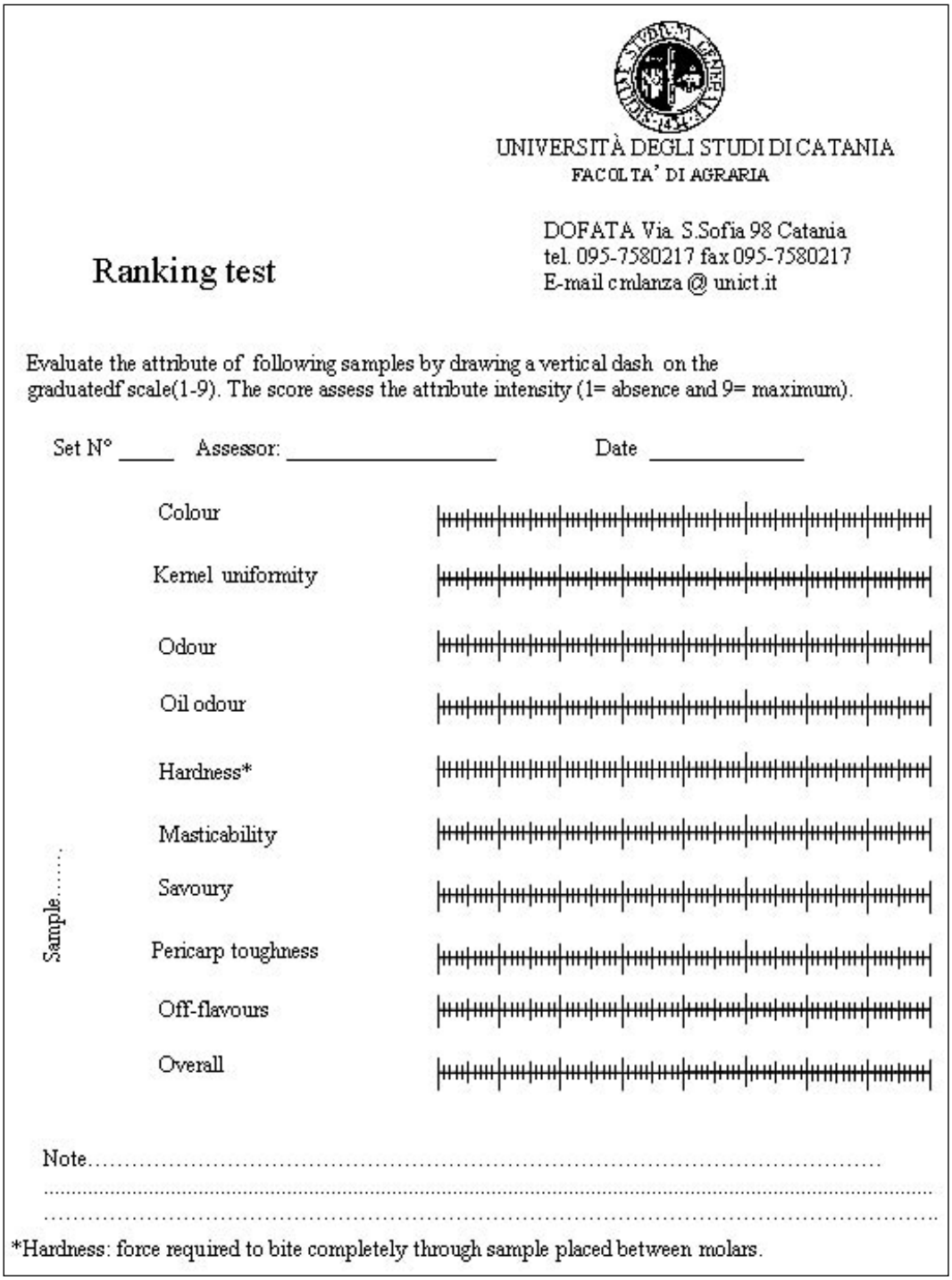


Fig. 1 - Card of sensory analysis.

ods, whereas the latter were determined by means of a digital penetrometer "TR 53205". The analysis to determine the above chemical and mechanical quantities were performed in duplicate.

RESULTS AND DISCUSSION

As reported above, the quality of the investigated food depends on several quality sub-indices, which can be clustered in three groups: sensorial, chemical and mechanical. Sensory evaluation gives an overall attribute of the "Cuccìa". On the other hand, chemical and mechanical quality indices are limited to describe the quality of the oil and that of the wheat kernel, respectively. To determine the influence of using different packaging on the quality decay kinetic is of the "Cuccìa" the above quality sub-indices were monitored during storage. The results obtained will be discussed separately.

Chemical descriptors

As an example in Fig. 2-4 the time course during storage of the oil acidity, peroxide number and ΔK of samples stored at 15°C is shown. Whereas, table 1 reports the initial and final values of the oil acidity, peroxide number and ΔK at 15° and 37°C. As can be inferred from the data shown in Fig. 2-4 and listed in Table 1, the

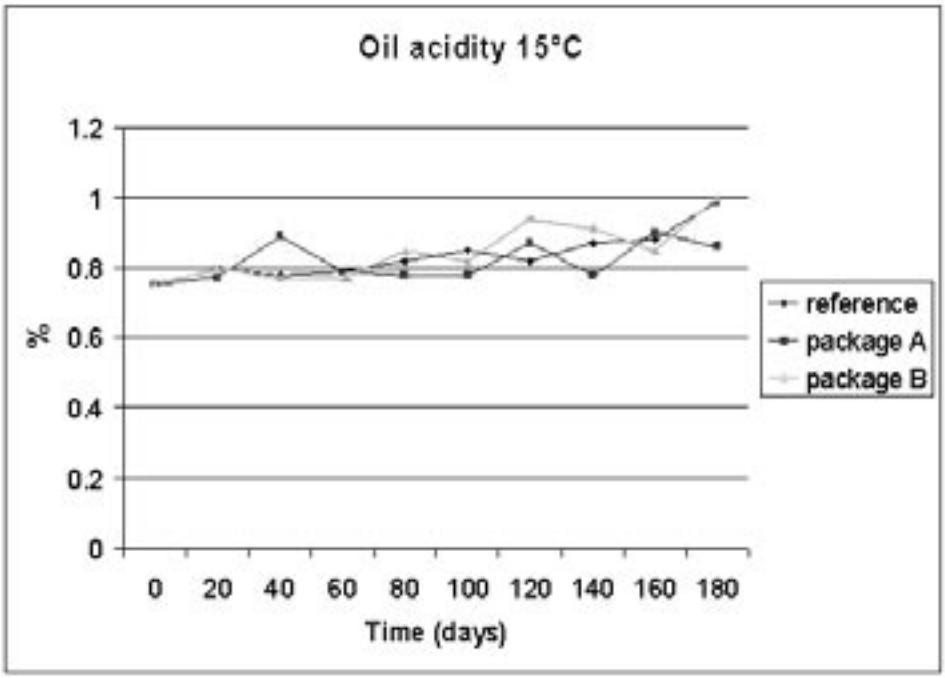


Fig. 2 - Oil acidity plotted as a function of storage time for samples stored at 15°C. The curves reported in the figure do not represent any model, they have been drawn to highlight the trend of the data.

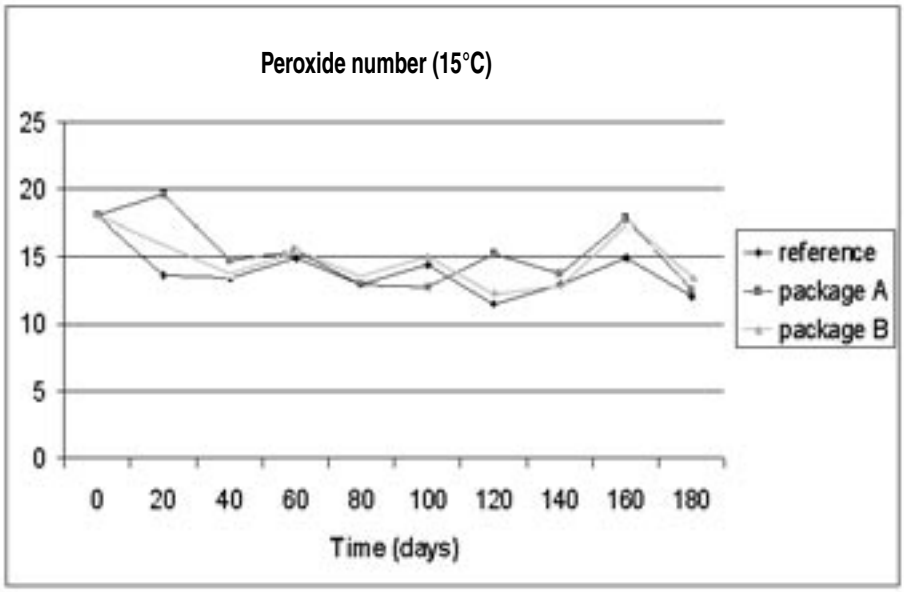


Fig. 3 - The evolution of oil peroxide number during storage for samples stored at 15°C. The curves reported in the figure do not represent any model, they have been drawn to highlight the trend of the data.

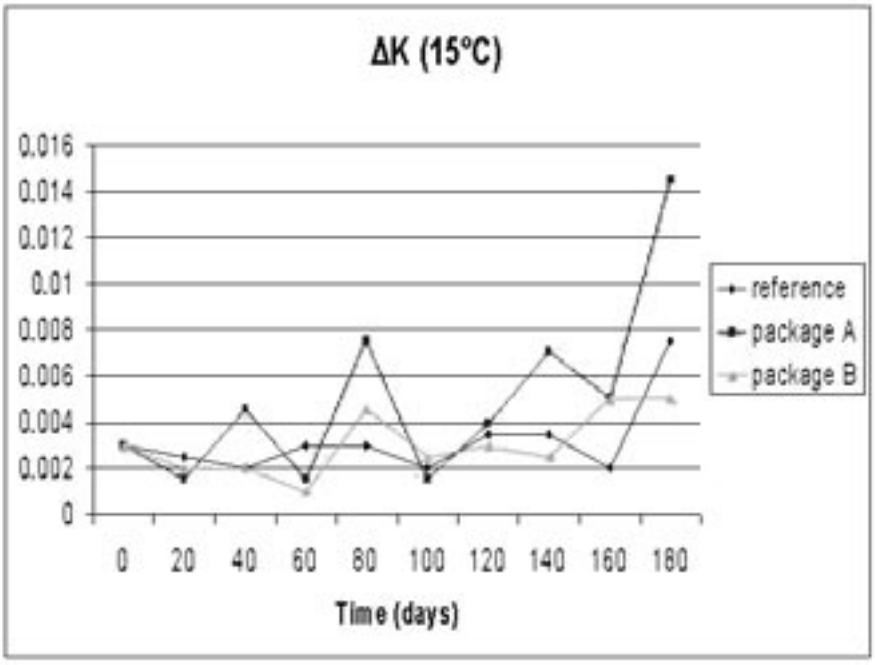


Fig. 4 - ΔK plotted as a function of storage time for samples stored at 15°C. The curves reported in the figure do not represent any model, they have been drawn to highlight the trend of the data.

Table 1 - Initial and final values of the chemical descriptors used in this investigation to describe "Cuccìa" quality. The values reported in brackets are the standard deviations.

Attributes	Initial Value	Final Value (180 days)					
		Stored 15°C			Stored 37°C		
		Reference	Package A	Package B	Reference	Package A	Package B
Oil Acidity (%)	0.75±0.01	0.99±0.01	0.86±0.03	1.00±0.00	1.35±0.00	1.39±0.01	1.50±0.00
Oil peroxide no.	18.5±1.76	12.0±2.82	12.5±0.71	13.5±0.01	10.0±0.88	8.8±0.35	6.8±0.14
Oil ΔK	0.0040±0.00	0.008±0.0	0.015±0.0	0.005±0.0	0.025±0.0	0.027±0.0	0.037±0.0

type of container used to bottle "Cuccìa" did not influence the evolution of the above mentioned physical quantities, suggesting that, with regard to chemical descriptors, PET bottles could replace glass bottle in packaging this specific food product.

Mechanical descriptors

Fig. 5-6 show the evolution during storage at 15° and 37°C of the force necessary for the thrust of the drained wheat. Whereas, Table 2 reports the initial and final values of the force necessary for the thrust. As expected this quality index increases with storage due to the diffusion of oil molecules inside the wheat kernel, which plasticizes the macromolecular matrix increasing its tenderness. As oil dif-

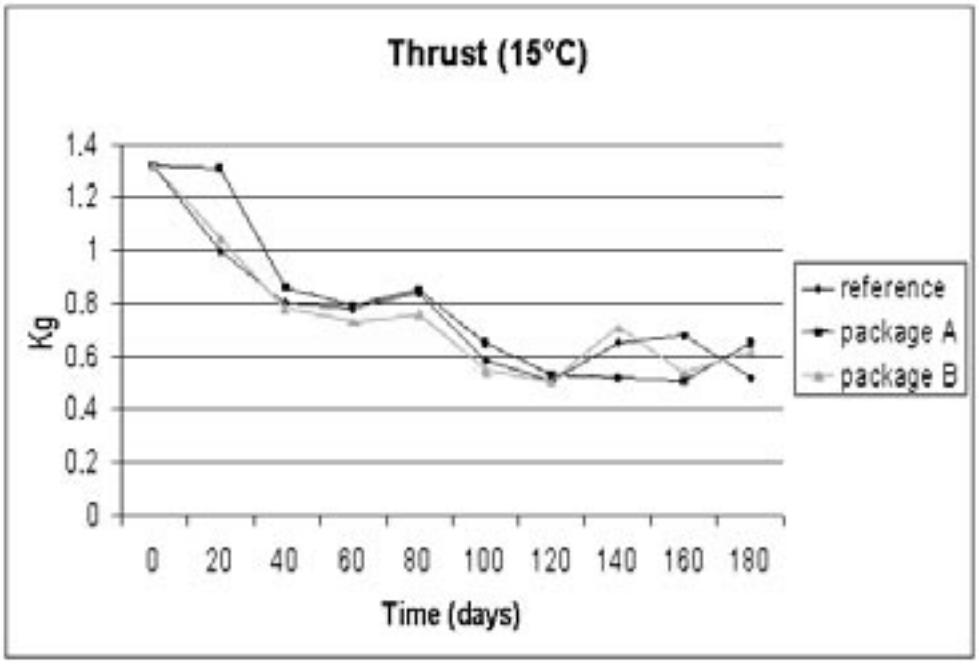


Fig. 5 - The force necessary for the thrust plotted as a function of storage time for samples stored at 15°C. The curves reported in the figure do not represent any model, they have been drawn to highlight the trend of the data.

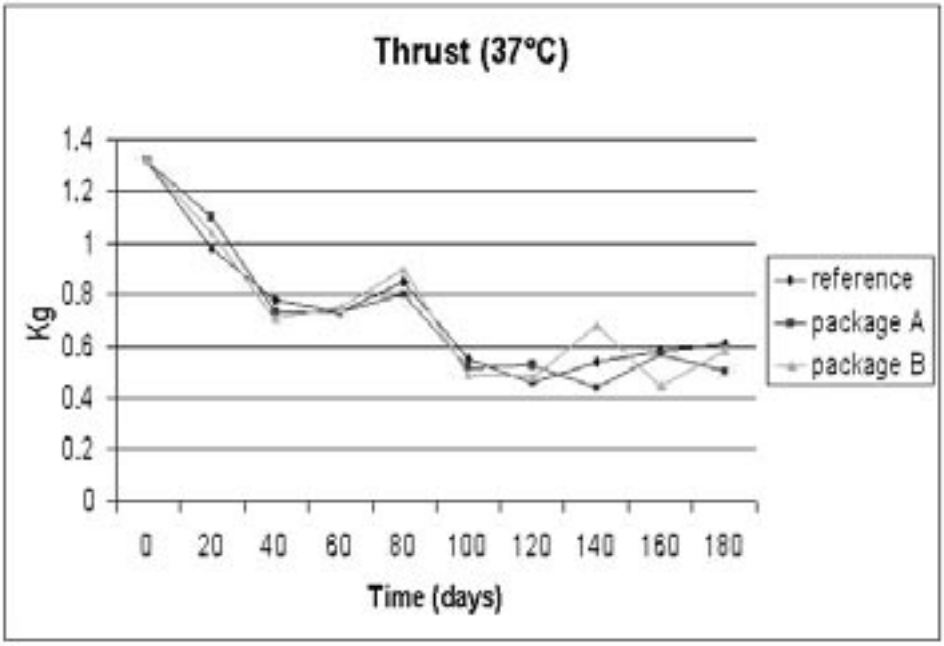


Fig. 6 - The force necessary for the thrust plotted as a function of storage time for samples stored at 37°C The curves reported in the figure do not represent any model, they have been drawn to highlight the trend of the data.

fusion inside wheat kernel is not affected by the type of package used to package the product, there is no difference between the three types of packages.

Sensory evaluation

Table 3 reports the initial and final values of the sensory attributes at 15° and 37°C. Whereas Fig. 7 and 8 show the overall of "Cuccìa" plotted as a function of storage time at 15° and 37°C. It is worth noting that intensity of this attribute first decreases then increases. The initial decrease of the "Cuccìa" whole quality

Table 2 - Initial and final "Cuccìa" mechanical descriptors. The values reported in brackets are the standard deviations.

Attributes	Initial Value	Final Value (180 days)					
		Stored 15°C			Stored 37°C		
		Reference	Package A	Package B	Reference	Package A	Package B
Wheat Hardness (kg)	1.32±0.37	0.52±0.15	0.65±0.13	0.62±0.17	0.61±0.15	0.50±0.15	0.59±0.20
Wheat cutting opposition (kg)	1.75±0.20	1.63±0.31	1.45±0.36	1.60±0.14	1.75±0.17	1.35±0.27	1.38±0.38

Table 3 - Initial and final values of "Cuccìa" sensory attributes. The value reported in brackets is the standard deviation.

Attributes	Initial Value	Final Value (180 days)					
		Stored 15°C			Stored 37°C		
		Reference	Package A	Package B	Reference	Package A	Package B
Colour	6.1±0.75	3.3±1.22	3.2±1.20	4.5±0.89	4.9±2.17	5.3±1.54	6.3±1.30
Kernel uniformity	6.0±1,85	5.9±1.89	6.0±1.41	5.8±1.39	5.2±2.29	5.4±2.09	5.4±1.36
Odour	2.4±1.25	1.2±0.61	1.2±0.58	1.3±0.62	1.2±0.39	1.3±0.87	1.25±0.62
Oil odour	5.5±1.68	4.5±1.48	3.8 ±1.30	4.9±1.64	3.4±1.53	5.2±1.75	4.8±2.05
Hardness	6.0±1.63	5.3±1.42	5.2±0.94	4.8±1.51	5.2±1.40	5.0±1.71	5.0±1.51
Masticability	6.0±1.63	5.1±1.01	4.5±1.20	4.3±1.10	4.2±1.25	4.2±1.67	4.6±1.51
Savoury	6.0±1.45	4.5±1.00	5.0±1.34	5.2±1.67	4.9±1.63	5.2±1.19	5.9±1.55
Pericarp toughness	5.1±2.02	5.5±1.31	5.0±1.63	4.7±1.29	5.0±1.08	4.7±1.39	4.8±1.34
Off-flavours	2.0±1.23	1.5±1.03	1.4±1.79	1.1±1.48	1.6±1.00	1.8±1.11	1.46±0.58
Overall	6.5±1.52	6.0±1.21	5.3±1.48	5.1±1.68	4.8±1.48	4.7±1.74	4.9±1.29

is related to the oxidative phenomena, which reduce the oil quality. Whereas the increase in the "Cuccìa" overall judgement is caused by an increase in the wheat kernel tenderness. Results shown in the above figures and in Table 3 point out that there is no substantial difference among the examined packages.

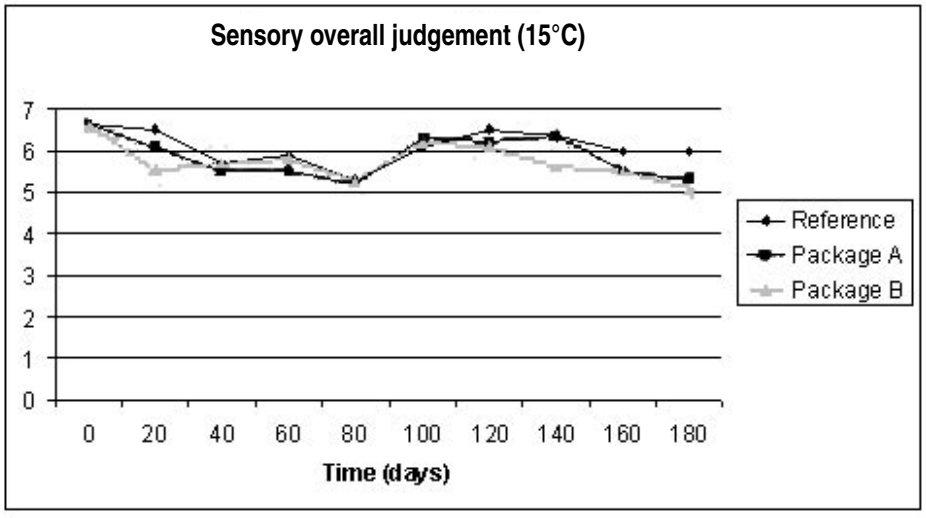


Fig. 7 -The "Cuccìa" sensory overall judgement plotted as a function of storage time at 15°C. The curves reported in the figure do not represent any model, they have been drawn to highlight the trend of the data.

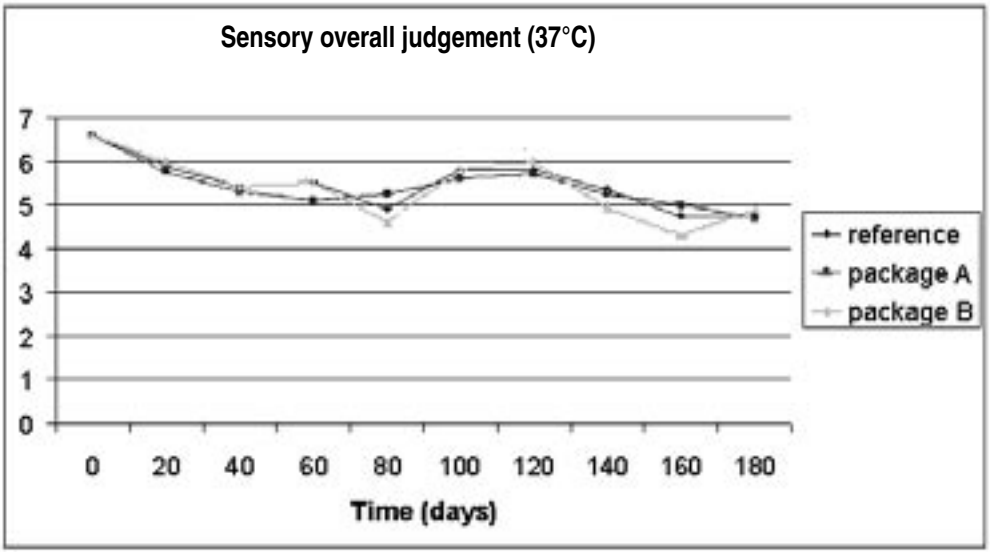


Fig. 8 - The "Cuccìa" sensory overall judgement plotted as a function of storage time at 37°C. The curves reported in the figure do not represent any model, they have been drawn to highlight the trend of the data.

CONCLUSIONS

In this study the influence of using different packaging on the quality decay kinetics of "Cuccìa" was assessed. "Cuccìa" quality was described by means of sensorial, chemical and mechanical attributes. Results show that PET containers can slow down the quality decay kinetics of "Cuccìa" to that of the containers made of glass. Due to the low concentration of oxygen scavenger, the investigated active packaging did not show any appreciable difference in the quality decay kinetics of "Cuccìa" with respect to the PET containers. The above results suggest that containers made of glass can be replaced by plastic containers in preserving the quality of this specific product during storage.

ACKNOWLEDGEMENTS

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EFFECTIVENESS OF PASTEURIZATION ON *ALICYCLOBACILLUS ACIDOTERRESTRIS* SPORES IN THE PRESENCE OF LOW MOLECULAR WEIGHT CHITOSAN

EFFETTO DELLA PASTORIZZAZIONE SULLE SPORE DI *ALICYCLOBACILLUS ACIDOTERRESTRIS* IN PRESENZA DI CHITOSANO A BASSO PESO MOLECOLARE

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ABSTRACT

An *Alicyclobacillus acidoterrestris* strain, isolated from a package of spoiled pasteurized pear juice, was grown *in vitro*, in order to verify the effectiveness of a pasteurization treatment in the presence of chitosan. A response surface model was developed for the effects of thermal treatment, low molecular weight (LMW) chitosan and pH on the survival of *A. acidoterrestris* spores. Survival depends on the interaction among the independent variables, in particular it was affected by pH, thermal treatment and chitosan concentration as individual terms and by chitosan concentration in its quadratic term. Chitosan demonstrated a significant reducing effect on the *A. acidoterrestris* spores. The model was validated against data not used in its development. Results indicated that the model is able to provide reliable prediction of the influence of chitosan and pH on heat-resistance of *A. acidoterrestris* spores.

RIASSUNTO

In questo lavoro di ricerca è stata valutata l'efficacia del chitosano a basso peso molecolare nel ridurre la termoresistenza delle spore di *Alicyclobacillus acidoterrestris* isolato direttamente da una confezione di succo di pera pastorizzato e alterato. La vitalità delle spore è stata determinata in funzione della concentrazione di chitosano, del pH e del tempo di riscaldamento alla temperatura di pastorizzazione. Allo scopo di individuare le variabili più significative e le loro combinazioni sulla

- Key words: *Alicyclobacillus acidoterrestris* spores, fruit juice, statistical model, thermal treatment -

vitalità delle spore microbiche è stato realizzato un piano sperimentale fattoriale a tre variabili e cinque livelli, sviluppato secondo un Central Composite Design (CCD). L'analisi statistica basata sull'analisi delle superfici di iso-risposta ha permesso di mettere in evidenza una significativa efficacia del chitosano nel ridurre la termoresistenza delle spore di *A. acidoterrestris*. Inoltre, è stato possibile sviluppare e validare un modello matematico capace di prevedere la sopravvivenza delle spore in funzione del pH e della concentrazione di chitosano impiegata.

INTRODUCTION

A pasteurization process should be able to guarantee the microbiological stability, at room temperature, of high-acidic foods. A similar process, indeed, ensures the inactivation of non spore-forming microorganisms, which are responsible for the spoilage of these products, while the surviving spores, generally, should not be able to germinate and grow due to the acidic substrate (pH 4.6).

In this context, the potential growth of *Alicyclobacillus acidoterrestris*, a thermoacidophilic, non pathogenic and spore-forming bacterium, has received considerable attention since it has been isolated and identified in several pasteurized and spoiled acidic foods (Splitstoesser *et al.*, 1994; Pontius *et al.*, 1998; Yamazaki *et al.*, 1996; Pettipher *et al.*, 1997).

Recently, *A. acidoterrestris* has been suggested as a target microorganism for the design of pasteurization processes in high-acidic fruit products (Vieira *et al.*, 2002; Sieve and Gibbs, 2001).

The design of a thermal process should include optimization, in order to make sure that the level of inactivation required for the target microorganism is satisfied without impairing the quality of the product (Vieira *et al.*, 2002). Moreover, the combination of antibacterial substances with thermal treatment should be used to reduce thermal damage and guarantee the microbiological stability of the products.

In recent years, chitosan has attracted notable interest for its industrial application (Muzzarelli, 1977; Knorr, 1984), antimicrobial (Kendra and Hadwidger, 1984; Sudharshan *et al.*, 1992; Yalpani *et al.*, 1992; Wang, 1992; Sekiguchi *et al.*, 1994; No *et al.*, 2002), antitumor (Suzuki *et al.*, 1986; Tokoro *et al.*, 1988) and hypocholesterolemic functions (Sugano *et al.*, 1992). Chitosan has been approved as a food additive in Korea and Japan since 1995 and 1983, respectively (Weiner, 1992; KFDA, 1995). Furthermore, chitosan is a natural, economic and largely available polymer. For this reason, in this work chitosan was used as an antimicrobial substance against a strain of *A. acidoterrestris* isolated from spoiled pear juice. The aim of this work was to study the effects of pH, chitosan concentrations and holding times to the pasteurization temperature on the survival of *A. acidoterrestris* spores. The individual effects and the interactions of these factors were analysed by mean of a quadratic response surface methodology.

MATERIALS AND METHODS

Preparation of *A. acidoterrestris* spore suspension

The *A. acidoterrestris* strain used in this study was isolated from a spoiled pear juice. The microorganism was grown on acidified Malt Extract Agar (MEA, pH 4.5 Oxoid, Milan, Italy) at 45°C.

Sporulation was achieved on acidified MEA. After incubation for 3-5 days at 45°C, spores were washed from the surface of the agar with cold sterile distilled water and centrifuged at 3,000 g for 15 min. The pellet was washed three times and suspended in sterile distilled water. To destroy the vegetative cells, the spore suspension was pasteurized for 10 min at 80°C.

The number of spores was determined by microscopy and after plate counting on acidified MEA. Spore suspensions were stored at 4°C, for a week.

Experimental design

The composition of standard medium was the following: malt extract 17 g/L and mycological peptone 3 g/L.

The values of pH, chitosan concentration and holding time at 80°C varied according to a three-factor and five-level Central Composite Design (CCD) (Box *et al.*, 1978) as reported in Table 1. The CCD reduces the number of possible combinations to a manageable size because it uses only a fraction of the total number of factor combinations for experimentation.

Different concentrations of chitosan at low molecular weight, were prepared in 1% acetic acid to give finally solution of 0.5, 1.0, 1.5 and 2.0%. Each solution was sterilized at 121°C for 15 min.

An aliquot of 1 mL of each solution, was added to 9 mL of the standard medium to achieve the final concentration as provided in the experimental design.

The pH of the synthetic medium was adjusted by using 0.1N NaOH after autoclaving to avoid any changes in pH that have resulted from autoclaving.

An aliquot of 0.1 mL of the spore suspension (approximately $2x10^7$ CFU/mL) was inoculated in each sample.

Finally, inoculated samples were subjected to the pasteurization process at 80°C, in a water bath, for different holding-times to obtain the conditions as provided in the experimental design.

The experimental runs are listed in Table 2: each combination of selected variables was replicated twice.

Measurement of spore survival

The number of surviving spores was determined by plate-counting methodology

Table 1 - Coded and natural level of the Central Composite Design (CCD).

Levels	[pH]	[t](*)	[C](**)
-2	3	5	0.00
-1	3.5	10	0.05
0	4	15	0.10
+1	4.5	20	0.15
+2	5	25	0.20

(*) holding-time at 80°C (expressed in minutes);
(**) concentration of chitosan (expressed as % w/v).

Table 2 - Composition of the various runs of the CCD.

Run	[C](**)	[pH]	[t](*)
1	0.15	4.5	20
2	0.05	4.5	10
3	0.15	4.5	10
4	0.05	4.5	20
5	0.15	3.5	10
6	0.05	3.5	20
7	0.15	3.5	20
8	0.05	3.5	10
9	0.10	4	15
10	0.10	4	15
11	0.10	5	15
12	0.10	3	15
13	0.20	4	15
14	0.00	4	15
15	0.10	4	25
16	0.10	4	5
17	0.10	4	15

(*) holding-time at 80°C (expressed in minutes);
(**) concentration of chitosan (expressed as % w/v).

on standard medium and incubation at 45°C for 2 days. Survival was calculated as $Log[N_f]/Log[N_0]$: where $[N_f]$ is the spore concentration at different holding-times and $[N_0]$ is the concentration of inoculated spores.

Statistical analysis

The stepwise multiple regression was performed to fit a second-order polynomial function to the experimental data (STATISTICA for Windows, Statsoft, Tulas, USA):

$$Y = \beta_0 + \beta_1(X_1) + \beta_2(X_2) + \beta_3(X_3) + \beta_{12}X_1X_2 + \beta_{13}(X_1X_3) + \beta_{23}(X_2X_3) + \beta_{11}(X_1)^2 + \beta_{22}(X_2)^2 + \beta_{33}(X_3)^2$$

where

Y: survival spores;

β_0: intercept of model;

β_1, β_2, β_3: coefficients of regression;

X_1, X_2, X_3: independent variables (in the following, they will be referred to as [pH] for the pH values of standard medium, [t] for the holding-time at pasteurization temperature and [C] for the concentration of LMW-chitosan, respectively).

Only the parameters having a statistically high significance ($p<0.05$) were included in the model.

The goodness of fit of the statistical model was evaluated by taking into count the coefficient of determination (R^2) and F-test.

In order to evaluate the applicability and the limits of statistical model a measure of model bias and model accuracy were carried out by computing the median relative error (MRE) related to each experimental run and mean absolute relative error (MARE) respectively (Delignette-Muller *et al.*, 1995). With this aim the percent relative error (RE) was calculated by using the following equation:

$$RE = 100 \times \frac{Yp - Yo}{Yp}$$

where Yp is the survival spores prediction and Yo observed values of survival spores. The MARE parameter was computed by using the following equation:

$$MARE = \frac{1}{n} \times \sum_{i=1}^{n} \left| RE \right|_i$$

where n is the number of experimental cases.

Bias and accuracy factors were also calculated according to Ross (1996): the indices asses the level of confidence one can have in the predictions of the model and whether the model displays any bias which could lead to “fail-dangerous”.

Moreover, in order to individuate the experimental outlier measures, the analysis of residuals was also performed: nothing outlier data was individuate because each residual was close in ± two standard deviation.

With the aim of evaluating the effects of each independent variable on the survival of the *A. acidoterrestris* spores, the surfaces of iso-response were obtained

by fixing one independent variable for time to its central value scheduled in the central composite design (Table 1).

RESULTS AND DISCUSSION

In Table 3 the best-fit equation of the proposed statistical model, describing the changes of the spore survival as a function of the significant variables among those investigated is reported.

Figs. 1, 2 and 3 show the three-dimensional plots corresponding to the following combined effects: [t] x [C], [pH] x [C] and [pH] x [t], respectively. As shown in Fig. 1 and 2, relative to the effects of [t] x [C] and [pH] x [C], minimum survival occurred at a chitosan concentration about 0.14%, holding-time of 25 min and pH 4. It is possible to individuate a chitosan concentration corresponding to the minimum survival, indeed, as indicated by the negative sign of individual term and positive sign of quadratic term, the surviving spores decrease up to a certain threshold with increasing chitosan concentration after which they increase.

Fig. 3 shows the three-dimensional plot relating [pH] and [t] to the *A. acidoterrestris* spore survival: it was at its minimum level when both pH and holding-time were at their highest level.

Table 3 - Best-fit equation for the effects of pH, holding-time at 80°C and LMW-chitosan concentration on *A. acidoterrestris* spore survival.

Best-fit equation	R^2	P	SE
$Log[N_t]/Log[N_0] = 6.9160 - 0.0545\ [pH] - 0.0049\ [t] - 2.4915\ [C] + 8.9152\ [C]^2$	0.755	<0.001	0.268
(*) [pH], [t], [C]: independent variables (i.e. pH of standard medium, holding-time at 80°C expressed in minutes and concentration of chitosan expressed as % w/v, respectively); (°) $Log[N_t]/Log[N_0]$: survival of the *A. acidoterrestris* spores.			

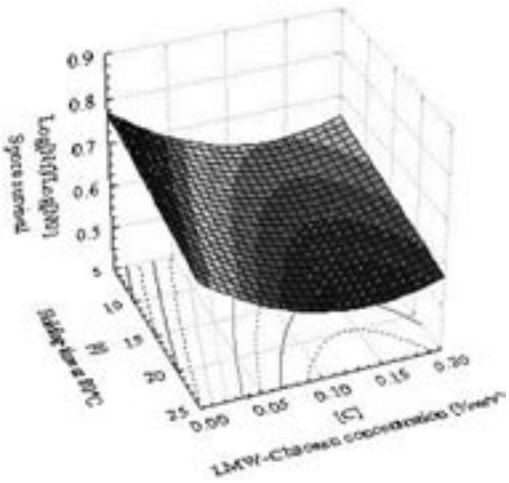


Fig. 1 - Three-dimensional plot of the interaction [t] X [C] on the survival of *A. acidoterrestris* spores.

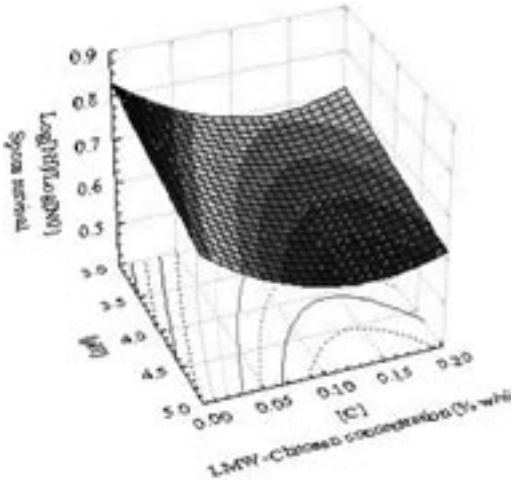


Fig. 2 - Three-dimensional plot of the interaction [pH] X [C] on the survival of *A. acidoterrestris* spores.

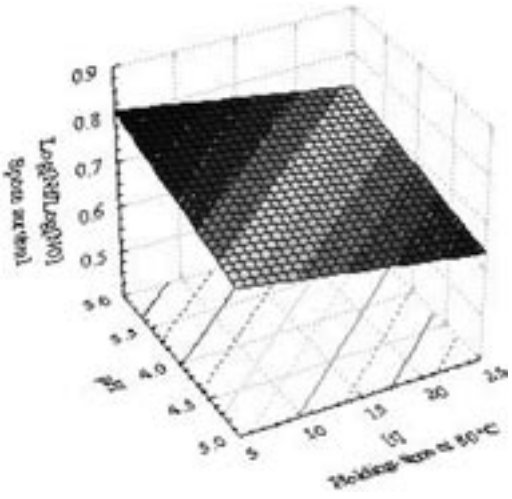


Fig. 3 - Three-dimensional plot of the interaction [pH] X [t] on the survival of *A. acidoterrestris* spores.

It could be hypothesized that, at low concentration of chitosan, the electrostatic interactions between the negative charges of the proteins on the cellular membrane and polcationic chitosan can cause the agglutination of the microbial spores (Fereidoon, 1999) and, probably, a greater heat-resistance. Moreover, at high concentration, the chitosan could transfer a greater positive charge to the microbial cells and consequently it could keep them in suspension and reduce their heat-resistance.

The analysis of the iso-response surfaces shows that approximately 0.14% could be the optimal concentration of chitosan in reducing the heat-resistance of the *A. acidoterrestris* spores during the thermal treatment. Nevertheless, as expected, at each chitosan concentration,

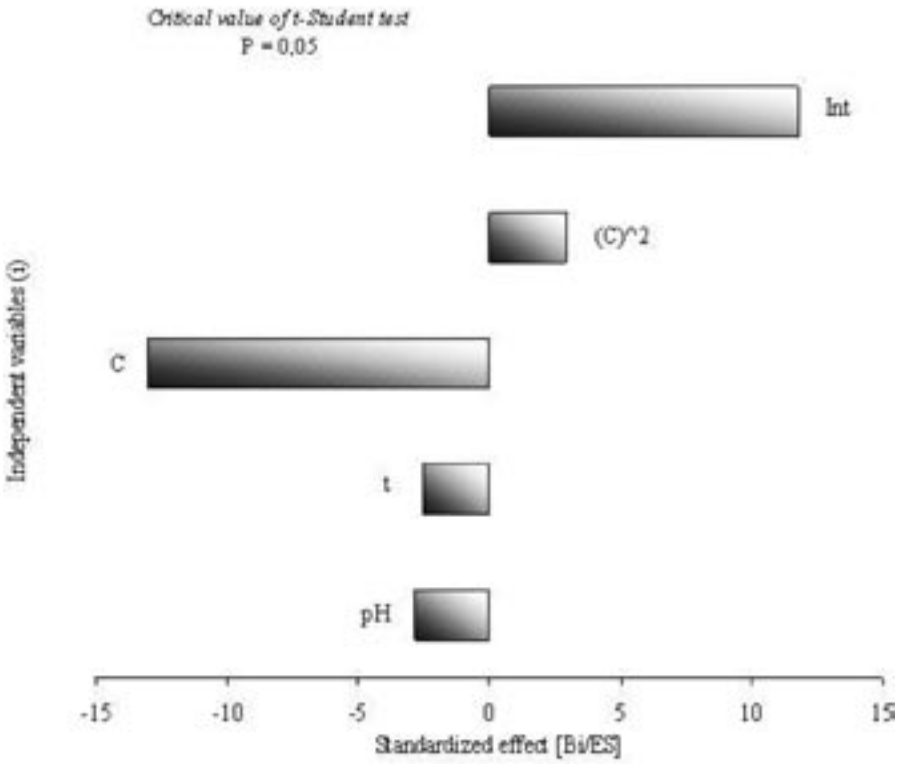


Fig. 4 - Pareto diagram. Effects of the greatest significant independent variables on the survival of *A. acidoterrestris* spores.

the number of surviving spores decreases due to the increase of the holding-time during the thermal treatment (Fig. 1).

Moreover, in our experimental conditions it can be observed that, by increasing of the pH value, the *A. acidoterrestris* spores get more sensitive to the thermal treatment for all chitosan concentrations. The same effect always appears in terms of the heat-resistance of the *A. acidoterrestris* spores also concerning the combined effect [pH] x [t], and it is noted for each time of treatment.

With the aim to better show the standardized effect of the coefficients of regression, a Pareto diagram is reported in Fig. 4 (the standardized effects were obtained by computing the ratio [Bi/SE] between the coefficient of regression [Bi] and the standard error [SE]). It could be observed that [C] is the independent variable having the greatest influence on the viability of spores, afterwards there are [t] (holding-time at 80°C), [pH] and [C^2] parameters, respectively.

Table 4 shows the MRE and MARE parameters for the proposed statistical model. It can be observed that MARE and MRE parameters have a very low value, i.e. 4.871% (±0.067) and 0.457% (±0.067) for the experimental data used to develop the model. The corresponding parameters with respect to the data used for model validation also have very low values. It can be emphasized that the proposed model is adequately not biased and accurate.

A scatter plot of RE (%) indicates a random distribution of relative error around 0% and thus a lack of systematic prediction bias (Fig. 5).

The bias and accuracy factors were also calculated according to Ross (1996), on average, the model is "fail-safe" (Fig. 6). Indeed, the predicted values of surviving spores exceed 4.9% only with respect to the experimental data used for the model development. In the same way, the predicted values exceed 4.4% only with respect to the experimental data used for the model validation.

CONCLUSIONS

In our experimental conditions, the chitosan having low molecular weight was shown to be more effective in reducing the heat-resistance of the *A. acidoterrestris* strain.

Table 4 - Indices of performance for statistical model development and validation.

	Cases	Delignette-Muller *et al.* (1995) method			
		Relative Error (%)			
		MARE ± dev.st	MRE	Minimum	Maximum
Model development	17	4.871±0.067	0.457	-12.809	12.098
Model validation	17	4.409±0.068	-0.943	- 9.414	11.146
		Ross (1996) method			
		Accuracy factor		Bias factor	
Model development	17	1.049		1.002	
Model validation	17	1.044		1.008	

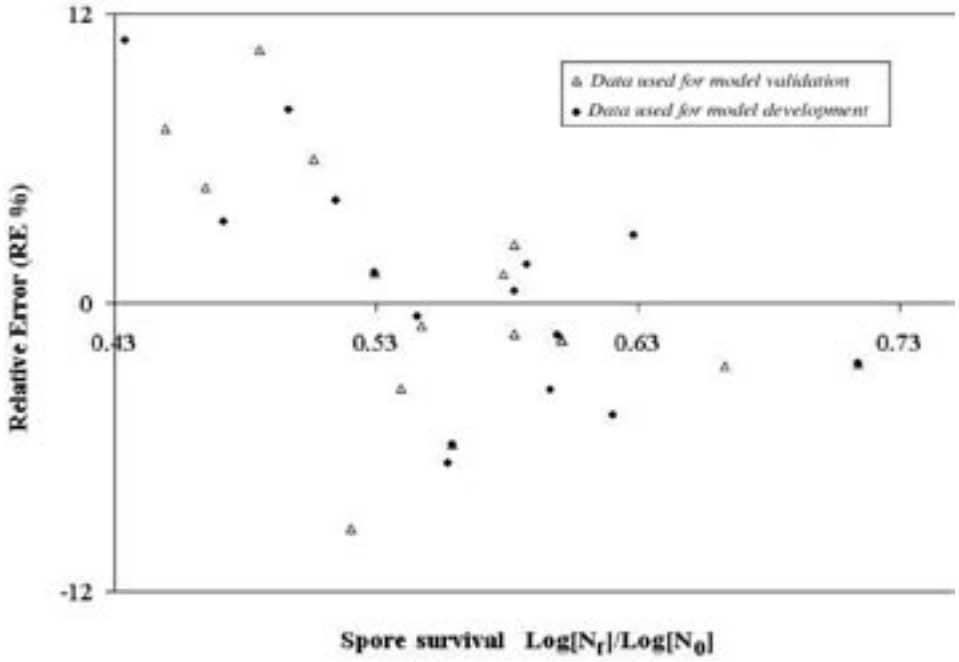


Fig. 5 - Scatterplot of RE of spore survival prediction for data used in model development and data used for model validation.

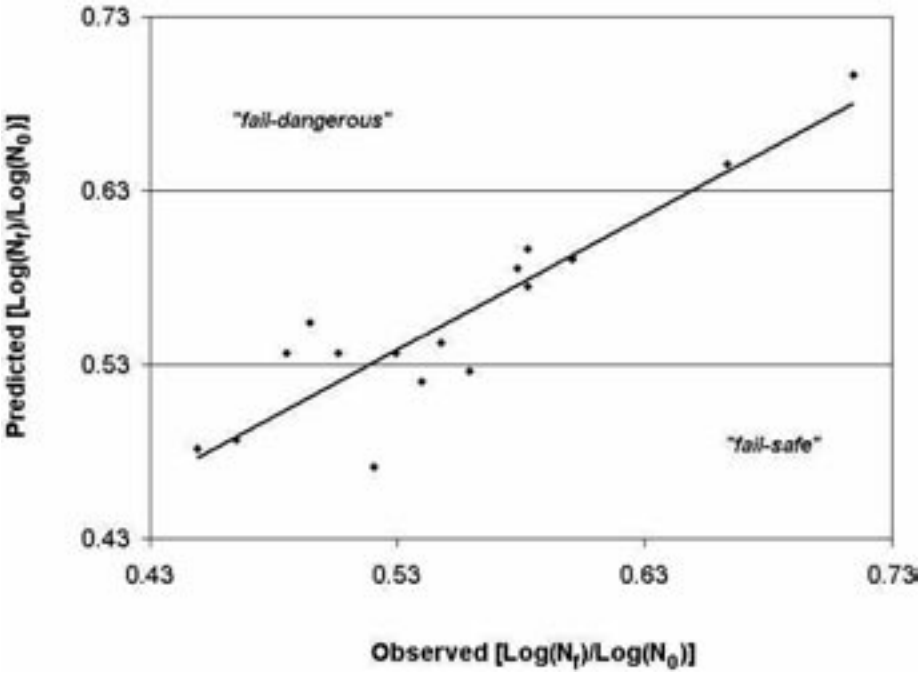


Fig. 6 - Prediction of statistical model for surviving spores of *A. acidoterrestris* during thermal treatment in the presence of LMW chitosan.

Moreover, the Response Surface Methodology allows accurately to evaluate and predict the influence of some independent variables (i.e. substrate pH, holding-time at the pasteurization temperature and chitosan concentration) and their reciprocal interactions on the thermal death of *A. acidoterrestris* spores.

It can emphasized that thermal treatment is more effectiveness when the pH-value of substrate increases. In particular, chitosan shows the greatest effectiveness when it is at the optimal concentration, i.e. 0.14%.

On the basis of these initial results, further studies are necessary to investigate the effectiveness of thermal treatments in the presence of chitosan taking into account different variables (such as water activity and temperature) and directly on real foods with the aim of reducing the minimal sterilizing effect required to achieve the safety and reducing their thermal damage. As mild-technology the proposed combined treatment could be used in the food industry to preserve the sensory and nutritional quality of foods that require a thermal treatment to achieve the microbiological stability.

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LECTURE

SUPERFICIAL TREATMENT USING PLASMA PROCESSES ON POLYMER FILMS USED FOR PACKAGING

TRATTAMENTI SUPERFICIALI MEDIANTE PROCESSI AL PLASMA SU POLIMERI UTILIZZATI NEL PACKAGING

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ABSTRACT

Thin barrier films of SiO_x were deposited on a polymer by plasma treatment with a hexamethydisiloxane-O_2-Ar mixture from a parallel–plate reactor. Several measurements were performed with Optical Emission Spectroscopy to search for the contribution of the excited species in the gas phase and with Fourier Transform Infrared Spectroscopy to analyse the signals relating to different functionalities present on the film. To understand the capacity of the film to stop the gas molecules, permeation rate measurements were also made. These measurements showed that SiO_x barrier films on a polymer can reduce the permeation rate of oxygen (OTR) and water vapour (WVTR) by several orders of magnitude.

RIASSUNTO

Il plasma è un gas parzialmente o totalmente ionizzato e si ottiene applicando ad un gas un campo elettrico DC o AC. Il plasma è popolato da differenti densità di molecole, atomi, radicali, ioni ed elettroni, e tutte queste specie sono presenti in differenti stati energetici. È possibile catalogare i plasmi sperimentali in base alla pressione e alla temperatura in plasmi termici (pressioni > 10 kPa, sistemi in equilibrio termodinamico) e plasmi freddi o glow discharge (pressione < 10 kPa, sistemi che non sono in equilibrio termodinamico). In questi ultimi anni le glow discharge hanno avuto un ruolo rilevante nella scienza della materia grazie soprattutto alla possibilità di depositare mediante Plasma Enhanced Chemical Vapour Deposition (PECVD) sottili film su qualsivoglia substrato. I materiali sottoposti ai processi via plasma acquistano particolari proprietà e composizione superficiali senza che nel contempo siano modificate le proprietà del bulk e soprattutto senza produrre degradazione termica. Questa versatilità ha permesso di progettare tutta una nuova serie di materiali con specifiche proprietà superficiali.

- Key words: Gas Transmission Rate, Plasma Enhanced Chemical Vapour Deposition, permeability, shelf-life -

INTRODUCTION

Polymers represent materials of the future, due to their low density, flexibility, manufacture by injection moulding, and cost-effectiveness. However, their surface properties often do not meet the demands regarding scratch resistance [1,2], wettability [3,4], biocompatibility [5,6], gas transmission [7,8], adhesion [9,10], or friction [11,12]. Hence additional surface modifications are required to achieve the desired properties. Flexible-polymer materials as PET or PE, with barrier properties, are the object of increasing interest in food-packaging, pharmaceutical and optical industries. The permeation of gases (e.g. oxygen, carbon dioxide) and vapours (e.g. water vapour, aromas and flavours) through polymers represents an important problem in their use as packaging materials. It is well known that permeation through a polymeric membrane is a solubility/diffusion-controlled process, and extensive determinations of the mass transport rates have been made [13]. Growing demands for increased shelf-life [14] of food products and chemical inertia of the contact surface have stimulated developments of polymers with improved high-barrier properties. Transparent materials such as silicon oxide (SiOx) deposited on polymer substrates are increasingly often used as gas barrier films for food and medical-device packaging. These transparent thin films were deposited on the polymer substrates by various methods, for example, the sol-gel methods[15], physical vapour deposition[16], electron beam deposition [17], Plasma Enhanced Chemical Vapour Deposition (PECVD) [8], and magnetron sputtering [18]. Roll-to-roll coaters used to deposit gas barrier films such as SiO_x on polyethyleneterephthalate (PET) substrates are reported [19,20]. In this paper, the characterisation of SiO_x film prepared by PECVD is reported.

Transport properties of polymers

The medium in which gas permeates can be categorized as being porous or dense. A dense medium is either rubbery or glassy in nature, which is defined by its glass transition temperature (T_g). Gas permeation through a medium is dependent on the gas solubility and diffusivity in the medium and the nature of the medium. The "Solution-diffusion Model" is used as model gas permeation in rubbery materials, the same model is used to describe gas permeability in glassy polymers but with opportune approximations. Transport of external molecules in rubbery polymer systems is generally governed by two simultaneous phenomena: (A) adsorption-diffusion phenomenon in which the mass flux is driven exclusively by a concentration gradient and (B) a relaxation phenomenon where the major driving force is locally the distance of the system from the equilibrium state [21]. If mass transfer takes place in a substantially unperturbed matrix, then the diffusion phenomenon controls the process rate and relaxation phenomenon can be neglected. Conversely, if mass transport takes places in a swelling system, then the relaxation phenomenon controls the process rate. Both these phenomena can be interpreted by a "Solution-diffusion Model", where the driving force is the chemical potential gradient of the diffusant across the organic matrix[22,23]. This mode of diffusion can be reformulated into more practical forms by exchanging the chemical potential gradient with measurable forces. Thus the chemical potential gradient across the membrane can be expressed only as a function of concentration [24-27]. When these conditions are met, the "Solution-diffusion Model" can be used to calculate the gas through a membrane. Flux is defined as the amount of material flowing through a given cross-sectional area in a given amount of time. When the chemical potential and Fick's law are used to describe gas permeation, the system

is at equilibrium and the gas permeation process is simplified to a one-dimensional flux [24-26]. In this case an adequate description of gas transport phenomena can be described with equation (1) [24].

$$J = - \frac{D}{RT} Cext \frac{\partial \mu}{\partial \chi} \quad (1)$$

where J is the mass flux, D is the diffusion coefficient, R is the gas constant, T is the absolute temperature, Cext is the penetrant concentration in the external phase and

$$\frac{\partial \mu}{\partial \chi}$$

is the penetrant chemical potential in the polymer phase. Equation (1) can be written in terms of penetrant concentration, Cp, as follows:

$$J = - (D \cdot S) \cdot \frac{\partial \left(C_p / S \right)}{\partial_\chi} \quad (2)$$

Where the solubility parameter, S, describes how the penetrating compound is distributed in the polymer/external phase system. It is usually defined as:

$$S = \frac{Cp\infty}{Cext} \quad (3)$$

where Cp∞ is the equilibrium penetrant concentration in the polymer. The permeability coefficient P is defined by means of the product D and S. P is a product of a kinetic diffusion coefficient D, and a thermodynamic quantity known as the solubility coefficient S. The permeability coefficient can be calculated with a technique known as the Time Lag Method [28]. The gas permeation in a glassy polymer is a complicated phenomenon because of its nonequilibrium state. This state of nonequilibrium causes the properties of a glassy polymer to change with time. These properties change in order to lower the free energy of the polymer, and come to a state of equilibrium. However, polymers never reach equilibrium, and their time dependent properties are characterized by physical aging. In addition to this time dependent behaviour, polymers are also characterized by free volume, which is know as excess free volume. This excess free volume consists of "intersegmental packing defect" that is literally frozen into the polymer structure when the temperature drops below their T_g. This polymer can be modelled with the "Dual Mode Sorption Model". The Dual-Mode Sorption Theory successfully models the sorption of small molecules in many polymers [29,30]. The success of the Dual-Mode Sorption Theory resides in the proposition of the existence of two distinct sorption modes, these modes were stated earlier as a Henry's Law and Langmuir mode.

Polymer structure and permeability

Movement of molecules through the membranes can be defined by means of Gas Transmission Rate (GTR) and by means of permeability. GTR represent the gas quantity which goes through one area of a film with a defined thickness for unit of time. It is

expressed in [mL(stp)/m^2 24h bar]. Permeability is one property of polymers. The value of permeability is expressed in GTR for one polymer with a pre-defined thickness standard. In a film, it can be imagined that molecules, as water or oxygen fill empty spaces. After that, the diffusion process takes place transferring molecules from one site to another. If a polymeric chain or more of these are in the same direction of shift vector, the molecule can stop in the site for a necessary time in order for the natural vibration of the chain to open a passage. If an oligomer has high flexibility, the movement of a part of the chain spreads along the same chain and the passage opens more frequently. For a small or voluminous rigid polymeric molecule, the movement is slow and not frequent. Diffusion through amorphous polymers is generally high and consequently these polymers have weak barrier proprieties, for this reason it is necessary to coat them with thin inorganic films [31]. Every mechanism which reduces oligomer mobility also reduces the diffusion and therefore variations of the percentage of crystallinity or cross-linking of a polymer, allow for a better barrier for the small gaseous molecules. In polymers with high crystallinity and density gaseous molecules can't become soluble nor spread inside the structure. A polymer which has 100% crystallinity would be a perfect barrier. These structures are not feasible. All crystalline polymers, in fact, contain amorphous zones where permeability occurs. The following table (1, 2) shows how permeability varies as a function of the degree of crystallinity (in %).

In addition to crystallinity, the other factors which influence barrier properties are:

- the nature of intermolecular bonds;
- the packing efficiency of chains.

Table 2 shows how Nylon 6.6, in spite of its lower crystallinity with respect to polyethylene, has a lower GTR since there are greater interchain bonds.

Plasma polymerisation is a very powerful technique for preparing very thin and highly cross-linked polymeric films. As in the case of classical polymers, gas permeation performance of plasma polymers is strongly related to their chemical composition and structural properties (density, free volume, etc.). Among the different plasma polymer precursors reported in literature, organosilicon monomers allow plasma polysiloxane layers to deposit. This seems to be promising material for gas separation processes [32-35].

Plasma processing of polymers

Plasma is a fully or partially ionized gas, which can be generated by applying a DC or AC electric field. In plasma there are different densities of molecules, atoms, radicals, ions and electrons, and all these species are present in various states and energy conditions. The plasma state is often referred-to as the fourth state of matter. Much of the visible matter in the universe is in the plasma state. This is true because stars, as well as all visible interstellar matter, which are omnipres-

Table 1 - Correlation between crystallinity and Gas Transmission Rate for oxygen (O_2) for Polyethylene at low and high density.

Polymer	% Cristallinity	O_2TR [mi/m^2 day bar]
LDPE	50	5,000
HDPE	80	1,146

Table 2 - Correlation between chemical characteristics of polymers, crystallinity and the Gas transmission rate for oxygen (O_2TR) for Polyethylene and Nylon 6.6.

Polymer	% Crystallinity	O_2TR [mi/m^2 day bar]
HDPE	80	1,146
NYLON 6.6	20	83

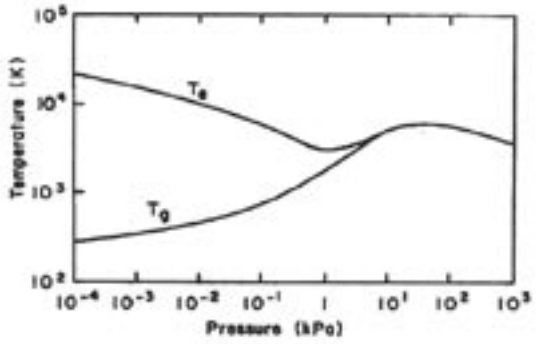


Fig. 1 - Trend of the electronic temperature Te and gas temperature Tg as a function of pressure.

ent in the universe, are in the plasma state. It is possible to categorize laboratory plasmas into thermal and cold plasmas. This subdivision is typically related to the pressure and temperature in the plasma. The first type given at high gas pressure (>> 10 KPa) are systems in thermodynamic equilibrium (it is possible to define only one temperature). High gas pressure implies many collisions in the plasma, leading to an efficient energy exchange between plasma species, and hence, equal temperatures. Cold plasmas or glow discharges, given at low pressure (<10 KPa) are in non-thermodynamic condition, in this case it is possible to define two different temperatures, T_g and T_e, where T_g is the gas temperature and Te is the electronic temperature.

This point is particularly important and deserves some explanation: electrons acquire energy from the external electric field, then redistribute the energy to molecules by means of collisions. A low pressure can be seen, low number electron-molecule collisions, then high mean free path of the particles. Therefore electrons keep most the energy gain from the accelerating field (very high values of Te between 2-10 eV), while heavy particles are at room temperature. As a conclusion a good fraction of the energy is transformed into an internal degree of freedom of the molecules, for instance excitation and formation of reactive species. In these years, glow discharges have had a great impact on materials science, having largely utilised for PECVD, and a diversity of treatments, such as grafting and etching of thin polymer films. These processes confer them a tailored surface composition and enhanced resulting properties without producing thermal degradation. This has allowed to design an interlay new class of materials with variable steichiometry and specific properties, which have contributed to solve some of most important problems of materials, mostly without affecting their bulk properties. The study of plasma processing of polymers is based on the combined use of in situ and continuous plasma diagnostics, e.g. optical emission spectroscopy (OES), mass spectrometry, and of film diagnostics, e.g. ESCA and FT-IR, as well as on the comparison with the adapteds analysis of the performance of materials, e.g. wettability, gas transmission rate of O_2 an H_2O, etc., depending on particular applications. This approach has allowed us to find the linkage between: a) species in the gas phase, b) surface composition, c) material performance. While understanding the connection between a) and b) it is essential to understand the mechanism of the various processes, the a) vs. c) connections provide very unique and direct tools controlling the performance of processes. This latter issue is of great industrial importance in that it can solve some of the most frequent constraints in the industrial use of PECVD technology.

SiO_x-like barrier films deposited on PET by PECVD

PECVD is one of the techniques that allows industrial-scale deposition of high-quality barrier coatings with good uniformity and good adherence to the substrate [36, 8, 37]. In food packaging, a few tens of nanometer silica-like films deposited onto PET

can be obtained using silicon-containing organic compounds such as hexamethyldisiloxane (HMDSO) alone or with O_2 or with O_2/Ar mixture in PECVD processes. These coatings have some important advantages, over other processing techniques, they are optically clear, microwaveable allow recycling of the coated polymer, while providing a good permeation barrier. As an example, O_2 transmission rates better than 1 cc day^{-1} atm^{-1} m^{-2} [14, 38, 39,40] can be obtained with PET coated with a SiO_2-like layer. This is a useful feature for increasing the shelf-life of packed food products. Though the growing interest in gas barrier technology has catalysed a great deal of work, some important issues remain vague due to the complexity of the plasma process when organosilicons are utilised in the plasma medium. The principal point for the rationalisation of a process is the identification of the precursors and the correlation between the species density in the plasma and their composition. Optical Emission Spectroscopy (OES) and Fourier Transform Infrared Absorption Spectroscopy (FTIRAS) are certainly suitable techniques for the characterisation of plasma feed by HMDSO and O_2.

The population of such plasmas is quite broad and complex: atoms (e. g. H, O, Si), low molecular weight species (e.g. CH, OH, CO, CO_2, SiO), heavier fragments and oligomers, as well as powder and undissociated monomer.

Experimental

The deposition process was carried out in a stainless steel reactor with a parallel plate configuration and a 5 cm inter-electrode gap. The upper RF driven electrode (diameter = 10 cm) is covered with a quartz plate to avoid film contamination by electrode material sputtering during discharge; the lower ground steel electrode (diameter = 16 cm) holds the substrate to be processed. The reactor was pumped by a turbomolecular and a rotary pump. The flow rate of the gases and the HMDSO vapour were controlled by means of MKS mass flow meters, and the pressure was monitored with a MKS capacitive gauge. The discharge was run at different feed gas composition and power values, keeping constant the other parameters, as reported in Table 3. The plasma phase investigation was carried out by optical emission spectroscopy. The plasma emitted radiation was sampled through a quartz window mounted on the reactor by means of an optical fibre and analyses by Jobin-Yvon HR 320 spectrometer (Fig. 3). SiO_x films were deposited onto polished Si substrates for ex situ FTIR chemical characterization performed by means of a Bruker IR Fourier Equinox 55 spectrometer under N_2 purging and in the presence of a desiccant to reduce water adsorption onto the samples.

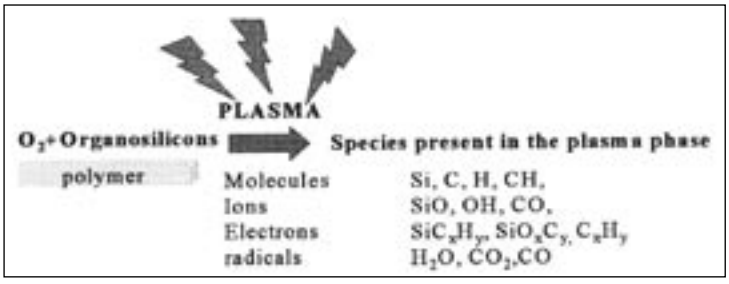


Fig. 2 - Schematic picture of a plasma-chemistry reaction and their products.

The resolution of the infrared spectra of the coatings was 4 cm^{-1}. SiO_x film were also deposited onto 12 micron RNK12 polyethyleneterephtalate (PET) films for performing gas transmission rate measurements (GTR). Film thickness was measured by means of a Tencor Instru-

ment alpha-step 100 profilometer. Table 3 shows the parameters which were varied during the experiment.

Permeability reader and gas chromatograph

Permeation characteristics of the polymers coated with thin films of SiO_x were investigated for oxygen, water vapour, methane, using the permeability reader LYSSY GPM 200.

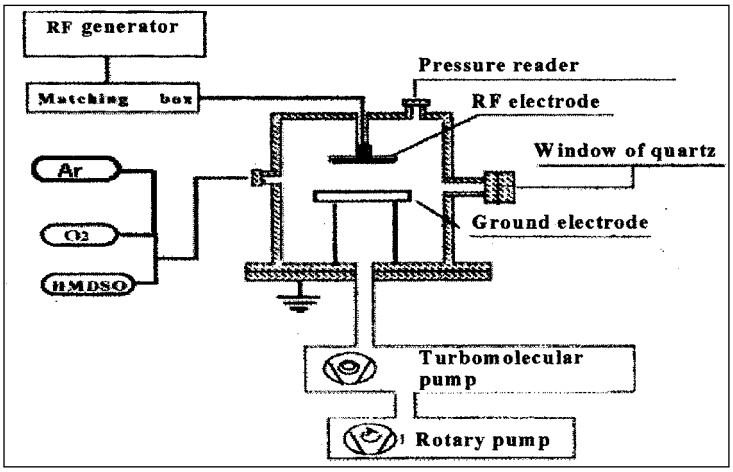


Fig. 3 - Schematic representation of the apparatus for the SiO_x-like film deposition.

It is composed of a sampling cell and of a permeation cell. The film to be tested is inserted between the cells. The gas whose GTR is to be measured flows in the upper cell, while the lower cell is collected at one container with 30 mL of helium, that circulates by one membrane pump. This operation consists in measuring out the gas quantity which permeates in the lower cell through the film, after a prefixed time. This measuring out has been carried out by taking one quantity of gas which circulates in the lower cell and by sending it to the gas-chromatograph. The gas-chromatograph (GC 8000 Top by TermQuest) has a thermoconductivity gauge (HWD Carlo Erba Instruments). The typical chromatograms which have been obtained for these gases are subsequently reported.

RESULT AND DISCUSSION

SiO_x film characterization deposition rate, has been measured on wafer of silicon. If the other parameters were constant, the deposition rate increases with power.

The values obtained of GTR for oxygen, methane and water vapour are reported in the following Table 4.

Table 3 - Deposition conditions.

External parameter	Variation range of parameters
Pressure	100-300 [mtorr]
RF Power	50-200 [Watts]
Thickness	300-7,000 [Å]
Flow O_2	15-95 [sccm]
Flow HMDSO	2,5-3 [sccm]
Flow Ar	0-30 [sccm]

It can observed that permeability decreases as power increases as thickness decreases. Characteristics FT-IR absorption bands of plasma deposited films are listed in Table 5.

The signals found in the spectrum do not correspond exactly to those reported in table because:

1. the amorphous structure causes dispersion around frequency average values, resulting in a broadening effect of the bands;

2. near signals overlapping.

In Fig. 8, the spectra show the typical Si-O-Si bending mode [41-48] around 820 cm^{-1} and stretching mode in the range 1,050-1,070 cm^{-1}, the spectrum shows, also, the absorption bands at 2,970 and 1,260 cm^{-1}, related to the stretching of CH_x and the bending modes of methyl groups in $Si(CH_3)_x$. Stretching bands of $Si(CH_3)_x$ at 840-885 cm^{-1} as well, mark the presence of organic components in the film. Another important feature is the absorption of the bands characteristic of Si-OH bonds [41-51], OH stretching in H-bonded silanol around 3,500 cm^{-1} and the bending mode at 930 cm^{-1}. Observing in

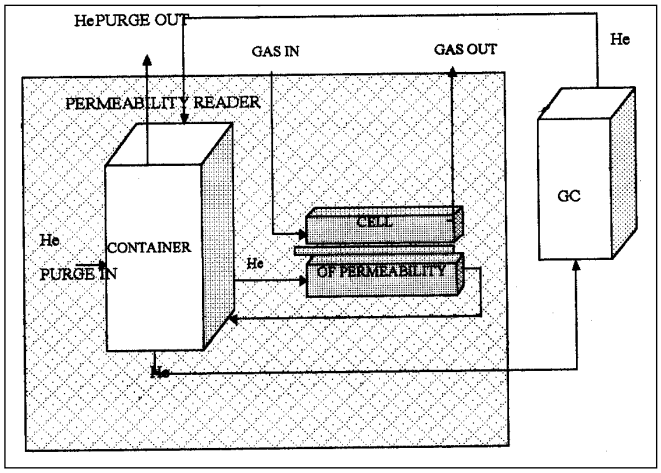


Fig. 4 - Schematic representation of the permeability reader and gas chromatograph.

Table 4 - GTR for oxygen water and methane in various conditions. The table shows the medium values and their error.

PET with SiO_x film							
Power [Watts]	Flow of O_2 [sccm]	Flow of Ar [sccm]	Flow di HMDSO [sccm]	Thickness [Å]	OTR [mL/m^2 day bar]	WVTR [mL/m^2 day bar]	CH_4TR [mL/m^2 day bar]
100	20	25	3	2,300	58±1	24±4	6±1
125	20	25	3	1,550	45±8	23±2	14±1
150	50	0	3	1,400	35±3	21±1	2.5±0.5
200	20	25	3	800	22±4	0.8±0.5	2.5±0.5
ORIGINAL PET					123±18	41±4	10±1

Table 5 - Absorption Bands Characteristics of SiO_x films.

Frequency [cm^{-1}]	Chemical bond	Vibration mode
3,650-3,500	O-H	stretching
3,000-2,900	C-H	stretching
1,270	Si-C	bending
1,075-1,050	Si-O-Si	stretching
940-900	Si-OH	bending
810	Si-O-Si	bending

detail the zone 4,000-2,500 cm^{-1} (Fig. 9) a reduction of the OH band can be seen at high power levels, while the stretching mode of CH around 2950 cm^{-1} is observed only at 50 W. In the range 1,300-800 cm^{-1} (Fig. 10) it presents the Si-C signal (1,270 cm^{-1}), it decreases as a function of the delivered power, while the stretching of Si-O (1,075^{-1}-1,050 cm^{-1}), moves to high wavenumber when the power increases.

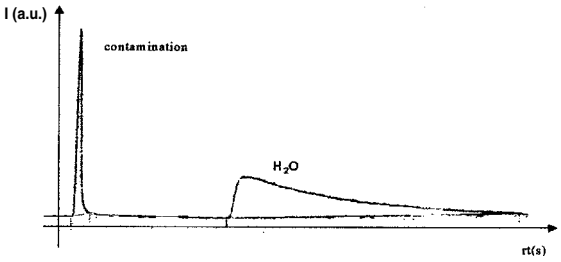


Fig. 5 - Water vapour with air contamination chromatogram.

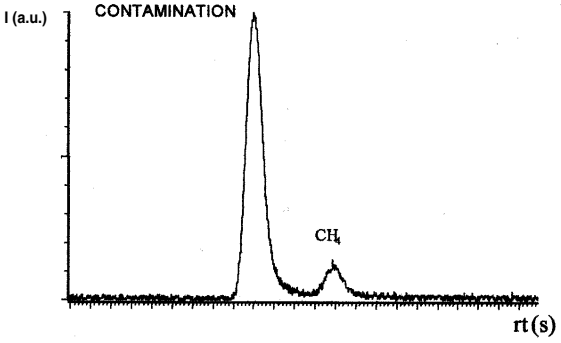


Fig. 6 - Methane with air contamination chromatogram.

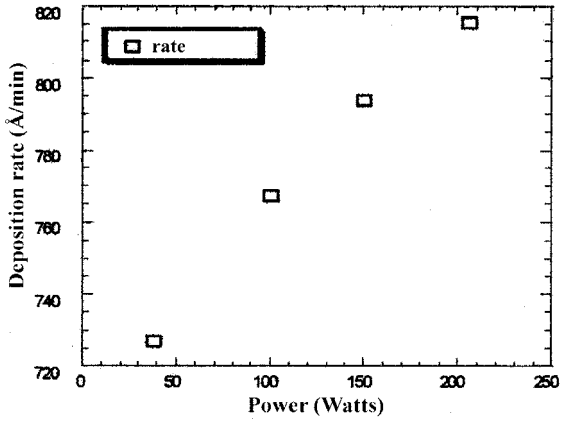


Fig. 7 - Trend of the deposition rate as a function of the power (flow O_2 20 sccm, flow Ar 25 sccm, flow HMDSO 3 sccm, pressure 100 mTorr).

All these observations have suggested that, when power increases, the "inorganic" character of the coatings increases [52-54]. Moreover, when power increases, the concentration of Si-OH group decreases (the bending mode of Si-OH at 900-940 cm^{-1} disappears) and the deposited films are more cross-linked, compact and stable because the silanol group interacts with water vapour. The comparison between the spectrum of films obtained with the same power are shown in Fig. 14, as a function of the O_2/monomer ratio.

It can be observed that, at low dilution conditions, the film's infrared spectrum shows the characteristic of carbon-containing moieties, in fact the signal of Si-C at 1,270 cm^{-1} is present in all three mixtures, but in the case of the film obtained under high O_2/monomer ratio, the signal has a low intensity and the position of Si-O stretching mode is shifted to higher wavenumbers. This confirms its greater "inorganic" character. This is in agreement with the kinetics described by Lamendola *et al.* [14], which states that under low dilution conditions the deposition precursors are mainly SiC_xH_y and CHx radicals, the film stoichiometry can be expressed as $SiC_xH_yO_z$. As the O_2/monomer ratio increases, the higher homogeneous oxidation efficiency produces more SiO radicals and volatile molecules CO_2 than Si and CH, leading to a marked inorganic character of

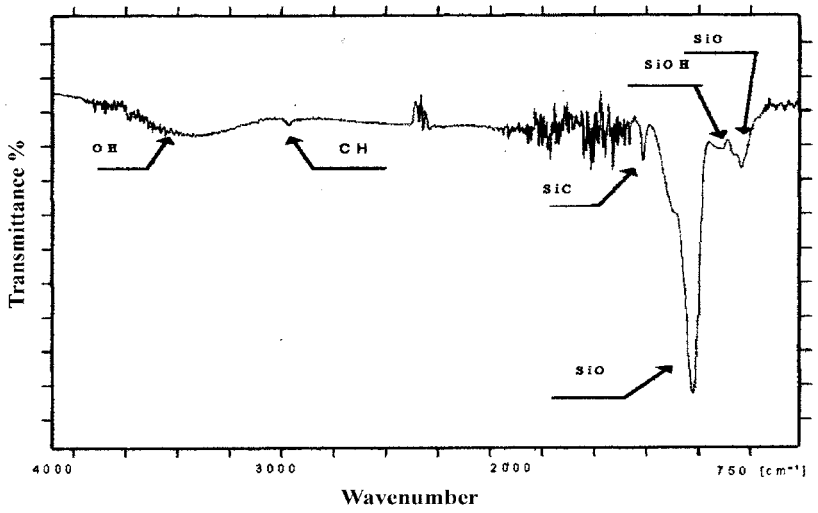


Fig. 8 - Typical spectra of SiO_x film.

the film (Si-O-Si functional groups prevail over $Si(CH_3)_x$), thus the film chemistry approaches SiO_2. In Fig. 12 the ratio of the integrated areas of SiOH (39,900-2,830 cm^{-1}) and SiO (1,270-980 cm^{-1}) bands, $SiOH/SiO^{(film)}$, has been reported in order to quantify the influence of power on the chemistry of the film. It could be observed that the silanol content decreases as a function of the delivered power. Furthermore, comparing the effect of the RF power onto the $SiOH/SiO^{(film)}$ ratio with the OTR values of SiOx coated PET, a good correlation between the chemical composition and the barrier performance occurs.

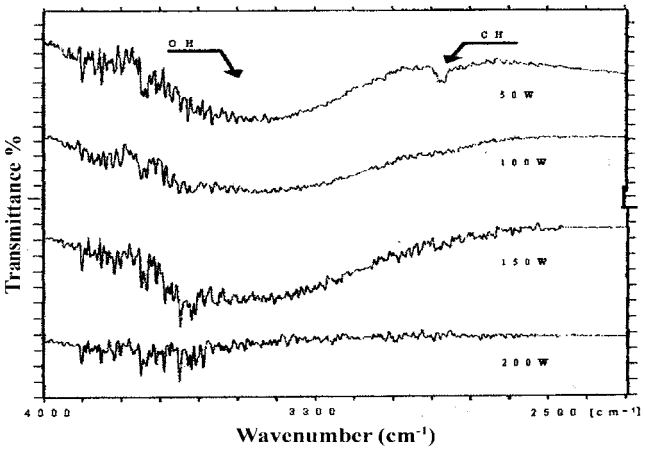


Fig. 9 - Spectra of SiO_x in the 4,000-2,500 cm^{-1} range in function of power.

Plasma phase characterization

The Si, O, SiO, CO, CH and OH emission intensity has been measured by means of optical emission spectroscopy. The presence of these species in HMDSO fed plasmas has been demonstrated in various papers [44, 55]: the characteristics of these emissions are reported in the following table.

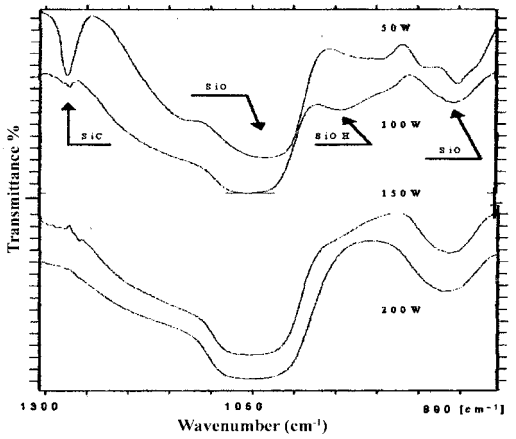


Fig. 10 - Spectra of SiO_x in the 1,300-800 cm^{-1} range in function of power.

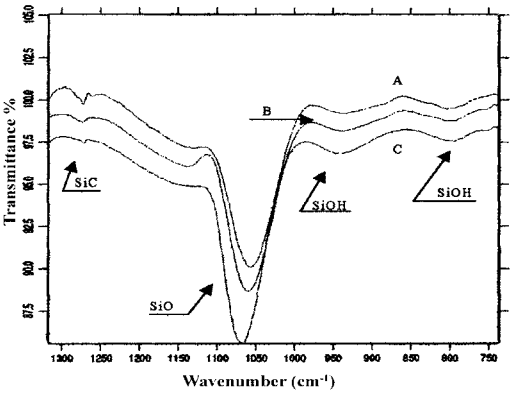


Fig. 11 - Spectrums of SiO_x film deposited at different O_2/monomer ratios. (A) 15 sccm O_2, 30 sccm Ar; 3 sccm HMDSO; (B) 20 sccm O_2, 25 sccm Ar, 3 sccm HMDSO; (C) 50 sccm O_2, 3 sccm HMDSO.

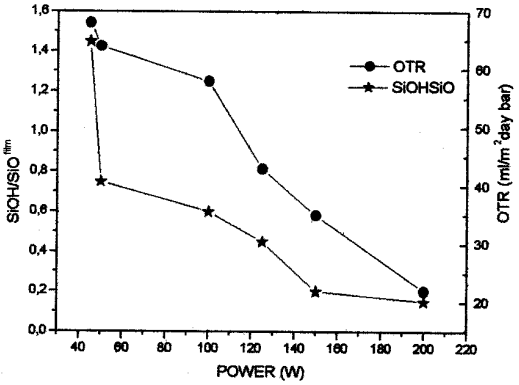


Fig. 12 - SiOH-to-SiO surface ratio (FT-IR) of SiO_x film and OTR of SiO_x coated PET samples as a function of the RF power.

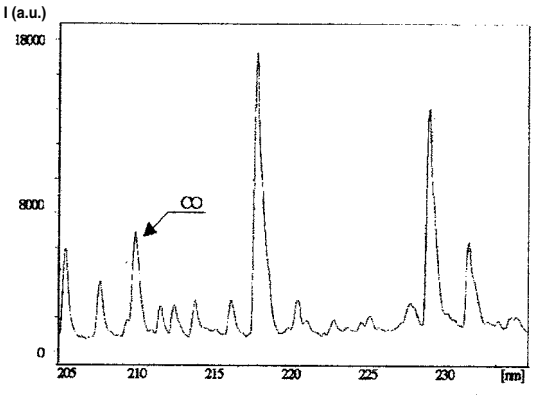


Fig. 13 - Band of CO.

Table 6 - Wavelength and transition of species.

Species	System of transition	Wavelength [nm]
CO	$A^1\prod$-$X^1\Sigma$	206.76
SiO	$A^1\prod$-$X^1\Sigma^+$	243.60
Si	$3p^2\,^3P$-$4s\,^3P^0$	251.61
OH	$A^2\Sigma^+$-$X^2\prod$	306.36
CH	$A^2\Delta$-$X^2\prod$	431.32

CONCLUSION

The effect of the feed gas composition and RF power input has been investigated in order to optimise the plasma deposition of SiO_x films, by means Optical Emission Spectroscopy and Fourier Transform Infrared Absorption Spectroscopies.

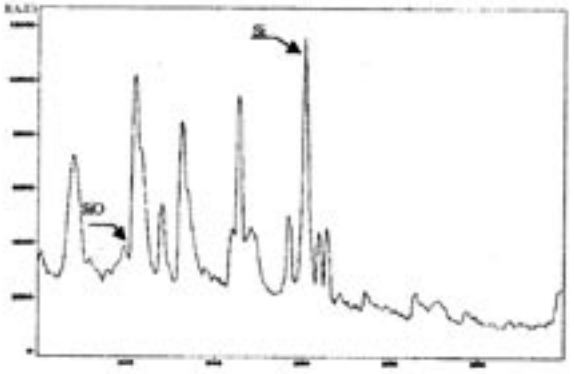


Fig. 14 - Band of SiO and atomic line of Si.

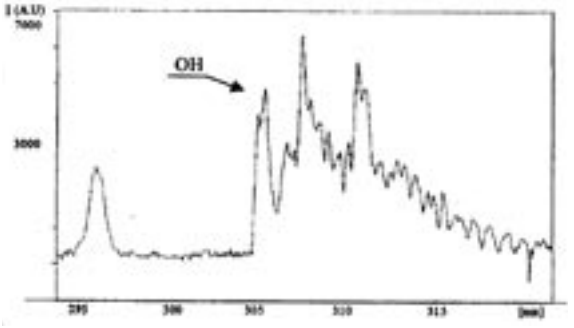


Fig. 15 - Band of OH.

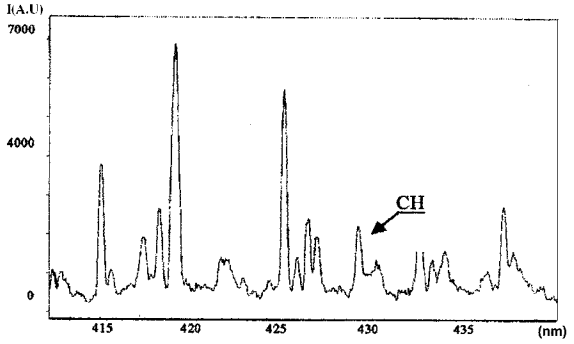


Fig. 16 - Band of CH.

In combining the permeability measurement with those of OES and FT-IR it is possible to assert that there is a correlation between the properties of the film, its composition and distribution of the reactive species in the gas phase. By increasing either the oxygen content in the feed gas mixture or the RF power the carbon content in the deposited film decreases. This is due to a higher efficency of the oxidation reactions yielding CO, CO_2, water and other oxidized volatile molecules. The silanol concentration in films is substantially reduced as the RF power input increases. Infrared Absorption spectroscopy has shown that the silanol content in the coating is correlated with its gas barrier properties. It can be concluded that, in experimental conditions when the O_2/HMDSO ratio and RF power increases permeability decreases (films become a better barrier to O_2, H_2O and CH_4) because of the reduction of the silanol group density in the coating. The latter decreases as power increases, likely because of the condensation of vicinal silanol groups which create Si-O-SiO bonds with loss of water.

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STUDY AND DEVELOPMENT OF AN ANTIMICROBIAL PACKAGING SYSTEM BASED ON THE RELEASE OF SILVER IONS

MESSA A PUNTO DI UN SISTEMA
DI CONFEZIONAMENTO ANTIMICROBICO A RILASCIO DI IONI ARGENTO:
FILM NANOCOMPOSITI AG/PEO-LIKE DEPOSITATI VIA PLASMA

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ABSTRACT

A comparative study on the effectiveness of Ag^+ based antimicrobial film in slowing down the growth of spoilage microorganisms is presented. With the aim of determining the effectiveness of proposed active film, three different growing media were used to determine the microbial growth curve of *Alicyclobacillus acidoterrestis*, a food spoilage microorganism, both with and without contacting the media with the active film. Results suggest that the developed active film successfully slows down microbial growth both in the case of a food model system (Malt Extract broth) and in the case of apple juice.

RIASSUNTO

Una delle linee di ricerca nel campo dei sistemi di *packaging* attivo studia e mette a punto film capaci di esplicare un effetto antimicrobico nella conservazione di alimenti e bevande. Un esempio interessante è rappresentato dai film antimicrobici basati sull'adesione di particelle di argento-zeolite alla superficie di laminati a contatto con gli alimenti. I sistemi di rilascio di ioni Ag^+ sono in grado di bloccare lo sviluppo e la crescita microbica quando la frazione acquosa dall'alimento idrata la struttura porosa della zeolite. L'attività antimicrobica degli ioni Ag^+ è legata alla loro capacità d'interferire con il metabolismo cellulare di microbi e batteri attraverso

- Key word: antimicrobial, apple juice, silver ions -

l'inibizione di un gran numero di enzimi e di modificare i meccanismi di replicazione del DNA. Pur risultando tossici per diversi batteri (*Escherichia coli, Pseudomonas aeruginosa* e *Staphylococcus aureus*), gli ioni argento non sono citotossici e sono tollerati dall'uomo entro ampi limiti di concentrazione.

I plasmi di non equilibrio modificano la superficie dei materiali tramite processi di deposizione (PE-CVD) di film sottili, di trattamento (*grafting* di gruppi funzionali), e di *etching* (ablazione). Nel campo del *packaging*, per esempio, si usano processi PE-CVD in grado di depositare su PET film a composizione SiO_x con ottime proprietà barriera all'ossigeno, al vapor d'acqua e agli aromi. Diversi sono gli studi effettuati sui processi plasmochimici. In particolare si sono ottimizzate le condizioni di deposizione di film sottili idrofili a matrice polietilenossido (PEO)-like contenenti cluster di argento di dimensioni nanometriche. Questi film, detti Ag/PEO-like, sono depositati in un reattore plasmochimico a piatti paralleli, asimmetrico, a radiofrequenza (RF, 13.56 MHz), dove viene fatto avvenire simultaneamente, in condizioni opportune, un processo PE-CVD da miscele gassose Ar/DEGDME (dietilglicol-dimetiletere) e un processo di sputtering dal catodo ricoperto di argento del reattore. I film nanocompositi Ag/PEO-like coniugano le proprietà *non fouling* dei materiali PEO convenzionali, con le note proprietà antibatteriche dell'argento, e si sono rivelati efficaci in alcune applicazioni del campo dei biomateriali, come coating antibatterici di dispositivi biomedici.

Questo contributo presenta i risultati preliminari ottenuti utilizzando coating Ag/PEO-like depositati via plasma su polietilene, come sistemi antibatterici di probabile impiego nell'ambito delle tecnologie di *packaging* attivo applicabili nel settore alimentare.

INTRODUCTION

Developing antimicrobial films intended for food packaging applications is one of the research activities in the fields of both applied microbiology and packaging. The active component of these antimicrobial systems can either be essentially organic or inorganic. In particular, the inorganic systems are based on metal ions and the most common used are silver, copper and platinum ones. Recent studies have shown that silver ions (0.04 mg/L) in combination with copper ions can be an effective method for controlling *Legionella pneumophila*, recovered in water distribution systems (Lin Y.E. *et al.*, 1996). These ions bond to the negatively charged bacterial cell wall changing cell wall permeability. This action coupled with protein denaturation induces cell lysis and death (Lin Y.E. *et al.*, 1996). It was suggested that the prime molecular target for silver ion resides in cellular -SH groups, which are of importance in the activity of many enzymes and in protein structure. Silver was reported to inhibit dehydrogenation processes and phosphate uptake and also promote the efflux of phosphate and potassium ions and other substrates (Russell A.D. *et al.*, 1994). Silver was shown to cause abnormalities in the size, cytoplasmic contents, cell membrane and outer cell layers of sensitive cells (Russell A.D. *et al.*, 1994). Silver was also found to interact with isolated nucleic acids although the significance of such interactions in in vivo systems is not clear (Russell A.D. *et al.*, 1994). Moreover, the antimicrobial activity of silver ions is linked to their ability to modify the DNA replication mechanisms.

Though resulting toxic for various bacteria (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis and Staphylococcus aureus*), silver ions are not cytotoxic and are tolereted by man within a wide range of concentrations (Feng Q.L. *et al.*, 1998). Various studies have shown that Gram-negative bacteria are more suscep-

tible to the toxic effects of silver due to their thinner murein wall, which allows more rapid absorption of the ions into the cell (Gray J.E. *et al.*, 2003).

Non equilibrium plasma techniques modify the material surface through the deposition (PE-CVD, Plasma Enhanced Chemical Vapour Deposition) of thin films, treatment (i.e., grafting of chemical functionalities) and etching processes (Favia P. *et al.*, 2001). In particular, the conditions of deposition of thin hydrophile films, with polyethyleneoxide-like matrix containing silver clusters of nanometric dimensions have been optimized. These films, known as Ag/PEO-like, have been deposited by Radio Frequency (13.56 MHz), asymmetric, parallel plate plasma reactor, where it simultaneously takes place, under certain conditions, a PE-CVD process from gaseous mixture Ar/DEGDME (diethyleneglycol-dimethylether) and a sputtering process from a silver RF electrode (cathode). The nanocomposite films Ag/PEO-like conjugate the non-fouling conventional properties of PEO polymers with known bacterial repellency for silver. They have been shown to be effective in some applications in the field of biomaterials, such as antimicrobial coating for biomedical devices.

This contribution gives the preliminary results obtained using Ag/PEO-like coatings deposited via plasma on polyethylene, as a possible antimicrobial system against *Alicyclobacillus acidoterrestris*, which is a Gram-positive, sporeforming bacterium already recognized as a cause of spoilage in acidic beverages (i.e. fruit juices, tomato juice, etc.). It has been considered a target in the quality control of such foods because of its thermal and acidity resistance (Murakami M. *et al.*, 1998). Few data are available in literature regarding these methabolic characteristics of *A. acidoterrestris* and no reports were found about the sensitivity of this bacterium to some antimicrobial substances normally used in food production. The aim of this study is to investigate the effectiveness of Ag/PEO-like film against a strain of *A. acidoterrestris*, isolated from spoiled pear juice and cultived in different conditions.

MATERIALS AND METHODS

Microorganism

A strain of *Alicyclobacillus acidoterrestris* ricovered from pear juice and cultured in vitro onto MEA (Malt Extract Agar, Oxoid) at refrigeration temperature, was used. The strain was revitalized in MEB (Malt Extract Broth, Oxoid) and incubated at 44°C for 48 hours.

Culture Media

In order to evaluate the antimicrobial effectiveness of the nanocomposite Ag/PEO-like films, the following media are used:

A) a model system suitable to make *A. acidoterrestris* grown, MEB, modified (pH 4.5);

B) orange juice from retail pH 3.67, with a sugar concentration equal to 11.5°Brix;

C) apple juice from retail, with characteristics similar to the above orange juice (pH 3.5, sugar concentration equal to 11.1°Brix).

Monitoring of cell growth

Samples were periodically taken from the batch cultivation for a period 4 days. The viable cell number was determined by counting the colony forming units (cfu) on MEA (Oxoid, Italy). The plates were incubated at 44°C for 48 hours.

Film preparation

A RF (13.56 MHz) asymmetric parallel plate plasma reactor with a silver RF electrode (cathode), sketched in Fig. 1, has been utilized for depositing Ag-containing PEO-like coatings through a simultaneous sputtering/deposition process. The experiments are run under conditions that develop, due to its smaller area respect to the ground, a bias-induced ion-bombardment (mostly due to Ar+ ions) at the cathode, that is enough to prevent the deposition of any coating at that electrode, and to drive the sputtering of Ag atoms. Such discharges have been fed with 0.25 sccm of diethyleneglycol-dimethylether vapors ($CH_3O(CH_2CH_2O)_2CH_3$, DEGDME, 162°C boiling point) and argon (7.5-30 sccm), and sustained at different values (5-30 W) of RF power and at 50-100 mTorr of pressure. DEGDME vapors were fed in the discharge from a reservoir through a needle valve and a steel line, all heated at 35°C. In these such experimental conditions Ag/PEO-like films of CxHyOz matrix composition, with variable Ag content (clusters), are deposited at the ground electrode of the reactor. More details on this plasma deposition process are available in ref. [9].

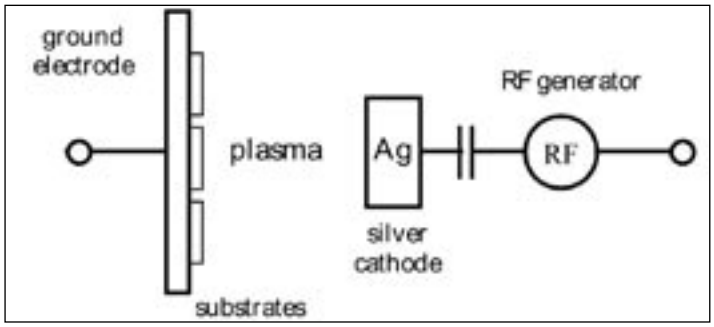


Fig. 1 - Sketched of an asymmetric parallel plate plasma reactor with a silver RF electrode (cathode).

For this work, coatings to be utilized as sources of anti-bacterial Ag^+ ions in liquid foods have been deposited using the following experimental conditions: 0.25 sccm of DEGDME; 30 sccm argon; 30 W RF power input; 100 mTorr of pressure. 100 mm polyethylene (PE) has been used as substrate.

The surface composition of the films has been investigated by means of Electron Spectroscopy for Chemical Analysis (ESCA) with a PHI 5300 ESCA spectrometer. Unmonochromatized MgKα radiation has been used (1253.6 eV), overall (0 - 1100 eV of Binding Energy, BE) and detailed (C1s, O1s, Ag3d ESCA; AgMVV Auger) spectra have been acquired at an electron take-off angle of 45° (sampling depth of about 70 Å). The following ESCA atomic composition has been found for the coatings utilized in this work: Ag 7%; C 73%; O 20%.

A value of 53±4° has been found for the advancing Water Contact Angle (WCA) of the coatings. This measure of their hydrophilic character has been obtained with a Ramé Hart 100 goniometer.

RESULTS AND DISCUSSION

To test the effectiveness of developed active film in inhibiting microbial growth three different media were used to grow the investigated food spoilage microorganism: Malt Extract broth, orange juice and apple juice. For the sake of simplicity, the tests conducted using the above-mentioned media will henceforth be referred to as Test A, Test B and Test C, respectively.

Fig. 2 shows the microbial growth curves obtained for Test A. The two sets of data shown in the above figure were obtained by immerging the developed active film in the media, to simulate the contact between package and food model system (Sample A), and

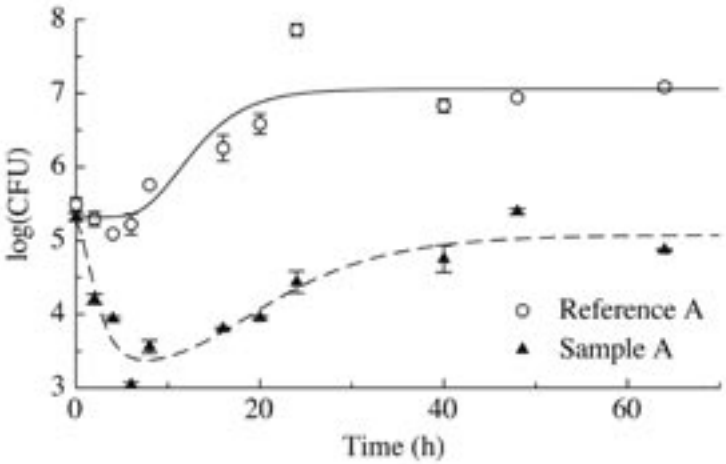


Fig. 2 - The evolution of microbial population during storage for Test A. The curves reported in the figure does not represent any model, they have been drawn to highlight the trend of the data.

by growing the investigated food spoilage microorganism in Malt Extract broth without the active film (Reference A). As shown in Fig. 2 there is a marked difference between Reference A and Sample A. In particular, Reference A shows a typical growth curve for a batch cultivation, where there is a lag phase followed by an exponential growth phase and a stationary phase. On the contrary for Sample A, a rapid decrease in the viable cell concentration is observed right after inoculation, then the microbial population increases to reach the stationary phase. It is worth noting that the viable cell concentration measured for Sample A at the stationary phase is two orders of magnitude lower than that of Reference A, suggesting that the use of the proposed active film is effective in slowing down the growth of investigated food spoilage microorganism in Malt Extract broth.

Fig. 3 shows the evolution during storage of the decimal logarithm of the viable cell concentration obtained in the case of Test B. As for Test A the two sets of data shown in the above figure were obtained by immerging the developed active film in the medium (orange juice) to simulate the contact between package and food (Sample B), and by growing the investigated microorganism without the active film (Reference B). Contrary to what detected for Test A, the two sets of data show a similar trend. In fact, both Sample B and Reference B show a lag phase, then an increase to reach the stationary phase. Results suggest that the active film did not influence the growth of the investigated microorganism if the growing medium is orange juice.

Fig. 4 shows the time course during storage of the decimal logarithm of the viable cell concentration detected for Test C. As above, Sample C was obtained by immerging the developed active film into apple juice, whereas Reference C was obtained by growing the investigated microorganism without the active film. The data obtained for Test C show an intermediate behavior between that of Test A and that of Test B. In particular, for Sample C a slow decrease in the viable cell concentration was found during the entire period of observation. On the other hand, Reference C showed a growth curve typical for a batch cultivation. The viable microbial count detected for Sample A at the stationary phase is about two log cycles lower than that of Reference A, suggesting that the developed active film is effective in inhibiting microbial growth if apple juice (a limpid juice) is used as growth medium.

As reported above the developed antimicroabial films successfully slow down

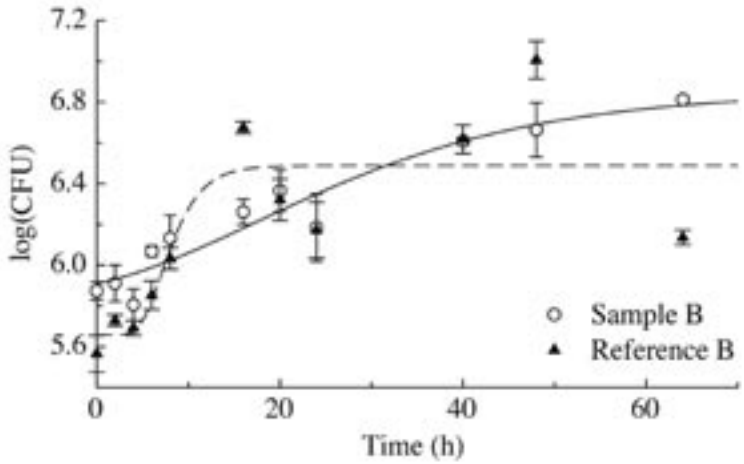


Fig. 3 - The evolution of microbial population during storage for Test B. The curves reported in the figure does not represent any model, they have been drawn to highlight the trend of the data.

microbial growth in a model system (MEB) and in the case of apple juice, whereas they do not influence the microbial growth in the case of orange juice, suggesting that the effectiveness of the proposed active film depends, among others, on the growth media. To point out the influence of the growth media on the time course during storage of the viable cell concentration of the investigated microorganism, the growth curves obtained for References A, B and C are shown in Fig. 5. Results suggest that there is not a marked influence of the medium on the growth of the investigated food spoilage microorganism. In fact, there are many other factors that can affect the efficiency of the developed film, such as: the ionic strength of the media, and the amount of suspended organic matter. In particular, the amount of Ag^+ released in the media, and consequently the antimicrobial activity of the film, depends on the ionic strength of this latter. Whereas, the presence of suspended organic matter in the growing media absorbing Ag^+ reduces its concentration in the media. Unfortu-

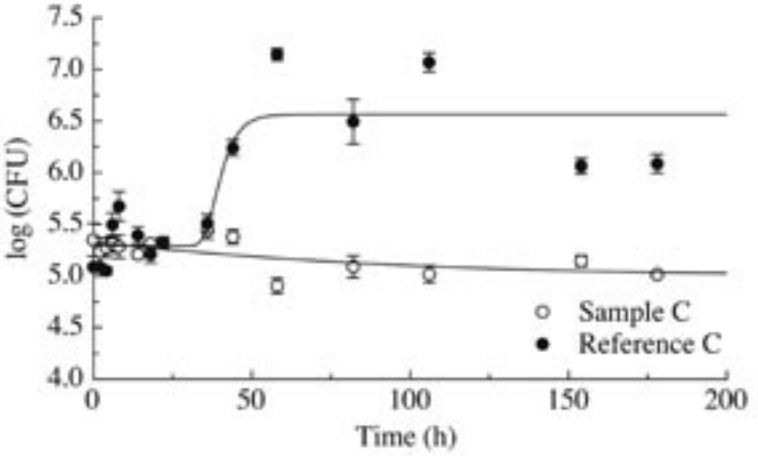


Fig. 4 - The evolution of microbial population during storage for Test C. The curves reported in the figure does not represent any model, they have been drawn to highlight the trend of the data.

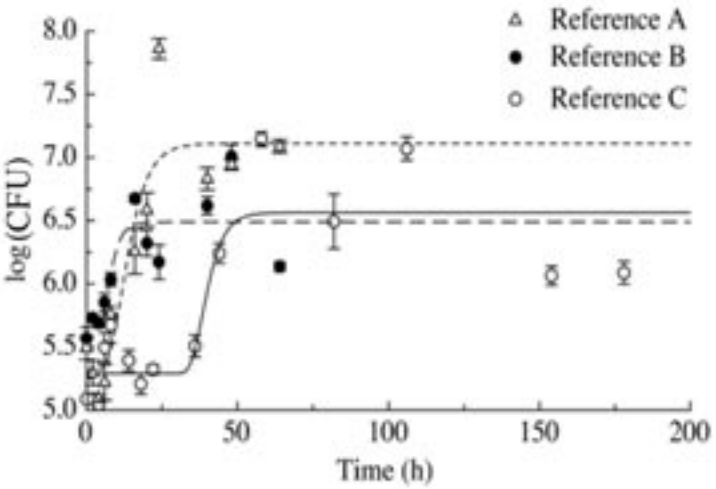


Fig. 5 - The evolution of microbial population during storage for Reference A, B and C. The curves reported in the figure does not represent any model, they have been drawn to highlight the trend of the data.

nately, at this stage of the research it is not possible to determine which of the above mentioned factors is responsible for the ineffectiveness of the antimicrobial active film in reducing microbial growth.

CONCLUSIONS

The effectiveness of Ag^+ based antimicrobial film in slowing down the growth of spoilage microorganisms was assessed. Viable cell concentration was monitored during storage using three different growing media. Tests were run both by contacting the active film with the growing media, and without contacting the active film with the media. Results suggest that the developed active film successfully slows down microbial growth both in the case of a food model system (Malt Extract broth) and in the case of limpid apple juice. However, it has also been observed that the effectiveness of the active film depends on the type of growing media. Unfortunately, at the present stage of research, it was not possible to establish which of the possible causes is responsible for the ineffectiveness of the active film in reducing microbial growth.

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POTENTIALITY OF PEEKWC AS NEW MATERIAL IN FOOD PACKAGING

POTENZIALITÀ DEL PEEKWC COME NUOVO MATERIALE NELL'IMBALLAGGIO ALIMENTARE

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ABSTRACT

The potential of the polyetheretherketone (PEEKWC), as a new film material in food packaging, has been investigated. Its food suitability has been proven by migration experiments, using different food simulants. Furthermore, poly-α-pinene (PαP), admitted by the Food and Drug Administration, has been added to the pure PEEKWC to improve its barrier and handling properties. The addition of PαP has significantly changed the film morphology and, consequently, gas and water permeability and selectivity. Both PEEKWC and PEEKWC/PαP films proved to be suitable in modified atmosphere packaging of some fresh products according to their respiration rates.

RIASSUNTO

L'obiettivo dell'imballaggio alimentare è quello di allungare la shelf-life degli alimenti mediante la creazione e il mantenimento di un'atmosfera ottimale all'interno dell'imballaggio capace di prevenire il contatto tra l'alimento e l'ambiente circostante, evitando qualsiasi alterazione non desiderata conservandone la freschezza del prodotto.

In questa ottica, i materiali utilizzati nel packaging alimentare devono essere in grado di combinare la velocità di respirazione di prodotti freschi con la velocità di trasporto di ossigeno e anidride carbonica (reagente e prodotto di diversi processi di respirazione) tra l'esterno e l'interno dell'imballaggio. In genere si utilizzano preferenzialmente film polimerici multistrato. Tali film presentano, però, lo svantaggio di non essere completamente riciclabili; per questo motivo molta ricerca è volta alla preparazione di film costituiti da un unico materiale, più facilmente riciclabile.

In questo contesto, è stato studiato un nuovo film polimerico costituito da un poliariletereterchetone modificato (PEEKWC).

- Key words: gas permeability, modified atmosphere packaging (MAP), poly-α-pinene, polyetheretherketone (PEEKWC), water vapour transport -

Tale materiale è un polimero amorfo che possiede eccellenti proprietà termiche e meccaniche e un'elevata resistenza agli agenti chimici. È, inoltre, solubile in diversi solventi (caratteristica che lo rende adatto alla lavorazione) a differenza del tradizionale PEEK. Nel presente lavoro, al fine di verificare l'idoneità alimentare del materiale PEEKWC, con e senza additivo (resina terpenica poli-alfa-pinene - PαP, ammessa dalla FDA), sono state condotte misure di migrazione con diversi simulanti alimentari (acqua e soluzioni idro-alcoliche e acide), in determinate condizioni operative (temperatura e umidità relativa) in accordo con le Direttive della Commissione Europea 90/128/EEC (2001). I valori ottenuti, essendo inferiori rispetto al limite (60 ppm) ammesso dalla Direttiva Europea, confermano l'idoneità di questo polimero ad essere usato nel packaging alimentare.

I film sono stati preparati usando la tecnica di evaporazione del solvente e sono state ottenute differenti morfologie al variare del solvente. Mediante microscopia a scansione elettronica (SEM) sono state visualizzate le sezioni trasversali di film in PEEKWC, con e senza additivo.

INTRODUCTION

The aim of packaging is to prolong the food shelf-life by avoiding undesired alterations and keeping the freshness of the product. Materials for active packaging have to be suitable to create an optimum atmosphere, combining the respiration rate of the specific fresh food (i.e. fruits and vegetables) with the mass flows exchanged between the external and internal environments (Cameron *et al.* 1995, Petersen *et al.* 1999). By a mass balance on oxygen and carbon dioxide, respectively, reagent and product of the respiration process, it has been possible to determine the appropriate transport properties of the packaging material, once the optimum storage conditions were determined. The flow of such gas species and the reaction rate are influenced by their concentration inside and outside the packaging environment and also by temperature (Cameron *et al.* 1994). Multi-layer polymeric films are used due to the difficulty to have a single material that meets the fresh food needs in terms of optimal gas selectivity and permeability. However, these films have the drawback of not being recyclable and the actual total amount of packaging waste in Europe is at least 61 million tonnes per year (Avella *et al.* 2001).

In this context, as new membrane material, a modified polyaryletheretherketone (PEEKWC) has been studied. This polymer has excellent chemical, thermal and mechanical properties, and has the advantage, compared to traditional PEEK, to be soluble in several common organic solvents, facilitating the membrane preparation (Liu *et al.* 1987, Zhang *et al.* 1987, Drioli and Zhang 1989).

The transport properties of PEEKWC film have been modified by adding different amounts of a terpenic resin, poly-α-pinene (PαP), admitted by the Food and Drug Administration (FDA). Moreover, thermal, mechanical and transport properties of PEEKWC and PEEKWC/PαP films have been evaluated to prove their suitability in food packaging applications.

MATERIALS AND METHODS

PEEKWC was supplied by the Chan Chung Institute of Applied Chemistry, Academia Sinica. The polymer powder was purified by Soxhlet extraction using

methanol and dried in a vacuum oven. Chloroform was purchased from Merck and used as solvent without further purifications. The additive, a terpenic resin, poly-α-pinene (PαP) was purchased from Aldrich. The repeating units of the polymer and the additive are shown in Fig. 1.

Membrane preparation and characterisation

The purified polymer, with (up to 50 wt%) and without additive (PαP), was dissolved at room temperature in chloroform at a concentration of 12 wt.%. The solution was magnetically stirred for at least 1 day to allow a complete dissolution of the polymer. The solutions were cast uniformly onto a glass plate by means of a hand-casting knife (BRAIVE Instruments) with a knife gap set at 100 and 250 μm. All membranes were prepared starting from a dope solution containing different polymer/additive ratios, from 100/0 to 50/50.

The films were left to evaporate over night at room temperature and set in an oven under vacuum for 24 h to remove completely the solvent.

The morphology of the dried films was observed using a Scanning Electron Microscopy (SEM), Cambridge, Stereoscan 360, at 20kV. The membrane samples were freeze-fractured in liquid nitrogen for cross-section analysis. All samples were evacuated and then sputter-coated with gold under argon atmosphere before SEM analysis.

The membrane samples thickness was measured by a digital micrometer (Carl Mahr D 7300 Esslingen a.N.).

Calorimetric measurements on films were carried out using a Diamond Pyris DSC Perkin Elmer Instruments. The samples were first heated from 0° to 250°C at a heating rate of 10°C/min; they were then cooled down to 0° at 10°C/min and reheated at the same scan rate. The glass transition temperature, T_g, was determined considering the second scan profile. Mechanical experiments were realised using an Instron 4301 dynamometer. Film samples in rectangular form were cut using a manual punch cutter. Tensile tests were performed at a crosshead speed of 5 mm/min at room temperature. Tensile modulus, stress and strain at maximum load and at break, were evaluated on pure and added PEEKWC. The mechanical parameters were determined on ten identical specimens and the results are reported as the average value.

The food compatibility of the investigated material was tested by overall migration experiments with three food simulants (3 wt.% acetic acid and 15 wt.% ethanol in water, water) in a climatic oven (Angelantoni 320E) at 40°C for 10 days, according to Commission Directive 90/128/EEC (1990).

The static contact angle of distilled water was determined using a CAM 100 device produced by KSV Instruments, Ltd. The results are reported as the average value of at least 10 measurements on each sample.

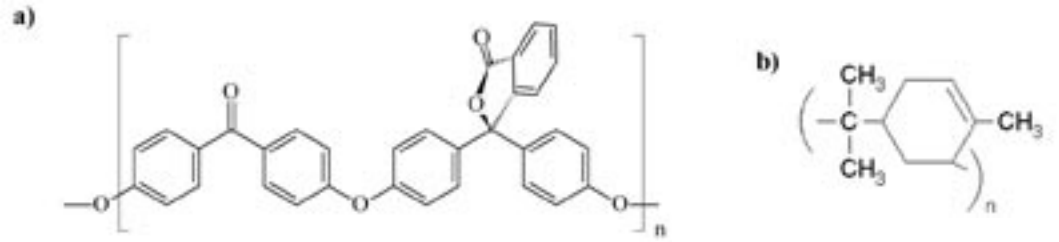


Fig. 1 - Chemical structure of PEEKWC (a) and PαP (b).

The water vapour transmission rates (WVTR) were measured on the films at three different temperatures (5°, 24°, 45°C) using the "cup method", 4818 UNI part 26.

The pure gas permeance was measured on a testing instrument constructed by GKSS Forschung, Germany. The instrument consists of a thermostated permeation cell, where the membrane is set, connected via a series of pneumatic control valves, at the feed side, to different gas cylinders and to a calibrated permeate volume, downstream. The method is based on a pressure increase measurement promoted by the gas passage through the membrane toward the fixed volume on the permeate side, which is completely evacuated before the start of the experiment. The permeation rate tests were carried out using three different gases (nitrogen, oxygen, carbon dioxide in the order), operating at atmospheric pressure on feed side and a 10-3 mbar vacuum on permeate side.

RESULTS AND DISCUSSION

The cross-section micrographs of the PEEKWC films, with and without Poly-α-pinene additive are shown in Fig. 2. PαP concentration significantly affects the membrane morphology. In particular, increasing the amount of additive, the structure changes from a dense (pure PEEKWC) to a symmetric open cell network, in which the presence of additive aggregates is clearly distinguished. Their number and size increase with the additive concentration. A few cavities are randomly distributed inside the polymer matrix for low additive concentration (95/5 sample); their number substantially increases in correspondence to a 90/10 PEEKWC/PαP ratio up to a 20 wt.% additive concentration where the cells become open but still only partially interconnected. The presence of additive agglomeration is mainly due to the phase separation occurring during the solvent evaporation. Furthermore, membranes were transparent at a ratio of PEEKWC/PαP lower than 20 wt.%, whereas they were opaque at higher additive concentration. Moreover, according to the additive plasticiser nature, a higher elastic character was observed.

The membrane surfaces for all investigated samples were also analysed by SEM. They were homogeneous and defect-free.

Thermal and mechanical properties (Tg) of PEEKWC and PEEKWC/PαP films are reported in Table 1.

The addition of PαP to the PEEKWC causes a strong decrease in the glass transition temperature (Tg), with respect to the PEEKWC. In fact, Tg shifts from 228°C (pure PEEKWC) to 170°C (PEEKWC/PαP 80/10) while it remains constant at higher PαP content (Table 1).

PEEKWC is characterised by a Young's Modulus of about 2,100 MPa. A 10 wt.% of additive does not modify the modulus values, while it increases to 2,400 MPa for 80/20 PEEKWC/PαP. At 50/50 PEEKWC/PαP, the tensile modulus decreases with respect to 80/20 PEEKWC/PαP remaining higher than pure PEEKWC.

PEEKWC presents different values for the maximum strength and the stress at break. Both values decrease, still differing between each other, when 10 wt.% of PαP was added.

At higher PαP concentration the stress at maximum load and at break coincide, indicating a lower toughness of the material. The same behaviour was observed for the strain evaluated at maximum load and at break. These results indicate that a low PαP concentration (10-20 wt.%) induced an increase in the elastic properties of the material, but a high PαP concentration (50 wt.%) determined a drastic loss of toughness.

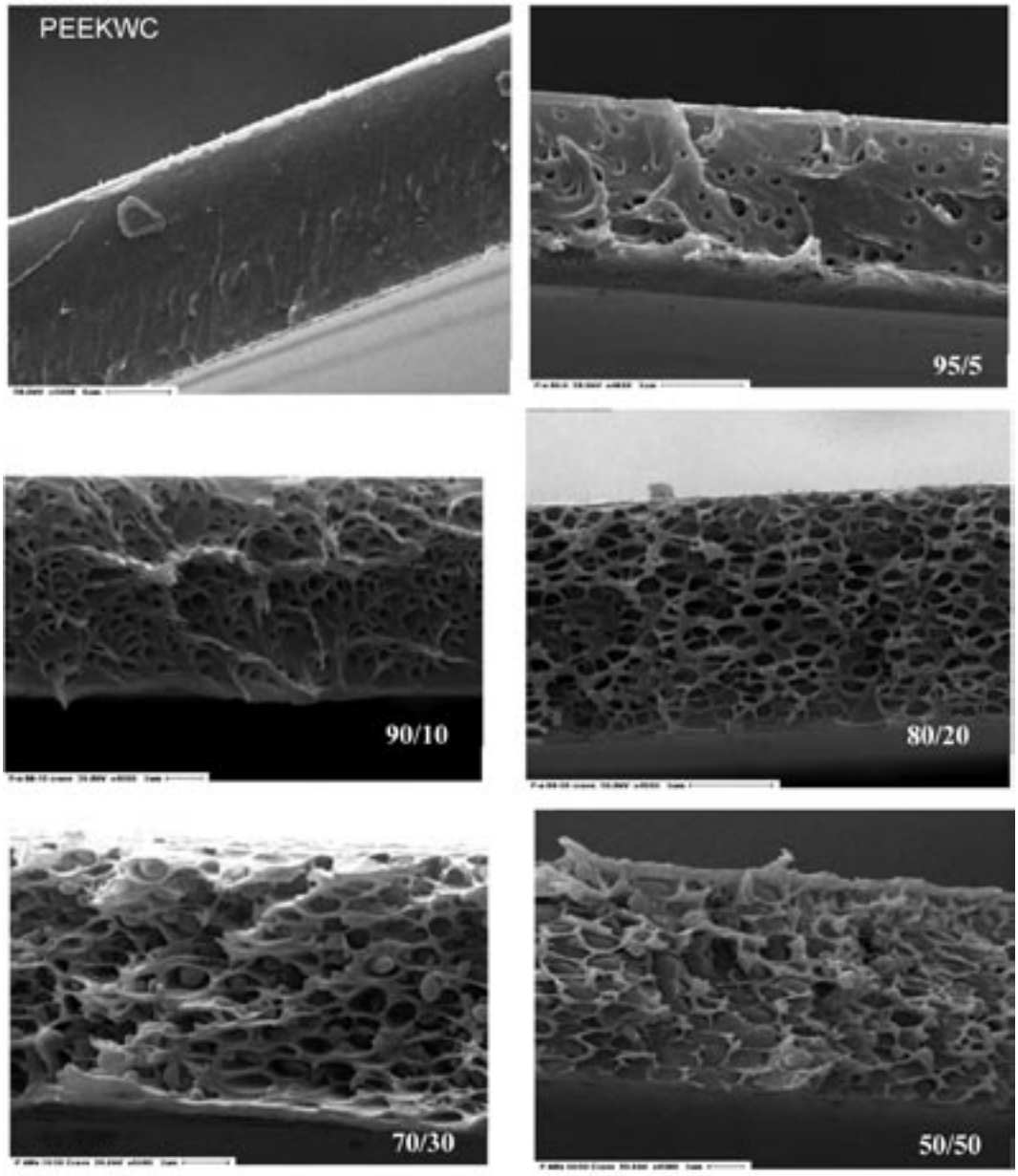


Fig. 2 - SEM pictures of the cross section of PEEKWC and PEEKWC/PαP films.

A comparison of the tensile modulus for different polymers already employed in the packaging field is reported in Fig. 3 (Van Krevelen 1990).

PEEKWC tensile modulus is higher than the average value of traditional packaging polymers, closer to the one of PA 66. The addition of PαP (80/20 PEEKWC/PαP) further increased its value, approaching the PVC one (Fig. 3).

The PEEKWC tensile strength at break was similar to PET, PVC and PS. A

Table 1 - Mechanical and thermal properties (Tg) of PEEKWC and PEEKWC/PαP.

PEEKWC/PαP	Tg (°C)	Thickness (μm)	Young's Modulus (MPa)	Max Stress (MPa)	Stress at break (MPa)	Strain at break (%)	Elongation at max load (%)
100/0	228	16	2,113±161	63.3±3.6	54.24± 7.1	7.93±2.7	5.38±0.7
90/10	170	24	2,141±146	54.47±5.6	48.34±14.4	3.68±0.6	3.61±0.6
80/20	-	21.3	2,418±144	56.77±4.9	56.72± 5	3.04±0.3	3.01±0.3
50/50	165	21	2,267±143	52.98±6.6	52.98± 6.6	3.07±0.5	3.07±0.5

proper PαP addition improved this value. On the other hand, the elongation at break was significantly lower than the commercial packaging polymer but was still higher than polystyrene (PS).

The food compatibility of PEEKWC and PEEKWC/PαP 50/50, was verified by overall migration experiments using different food simulants (Czerniawski and Pogorzelska 1997, Directive 90/128/EEC).

The results, shown in Table 1, are lower than the maximum level admitted by European Commission Directive (60 ppm), and they confirm the suitability of such a polymer for food packaging. The amount of compound, released into each food simulant solution, was comparable for the two investigated films. It indicates how the additive PαP, even at the highest concentration, did not compromise the suitability of the material.

The migration values for distilled water and acetic acid were comparable, indicating that the additive did not affect the food acid resistance of PEEKWC. Moreover,

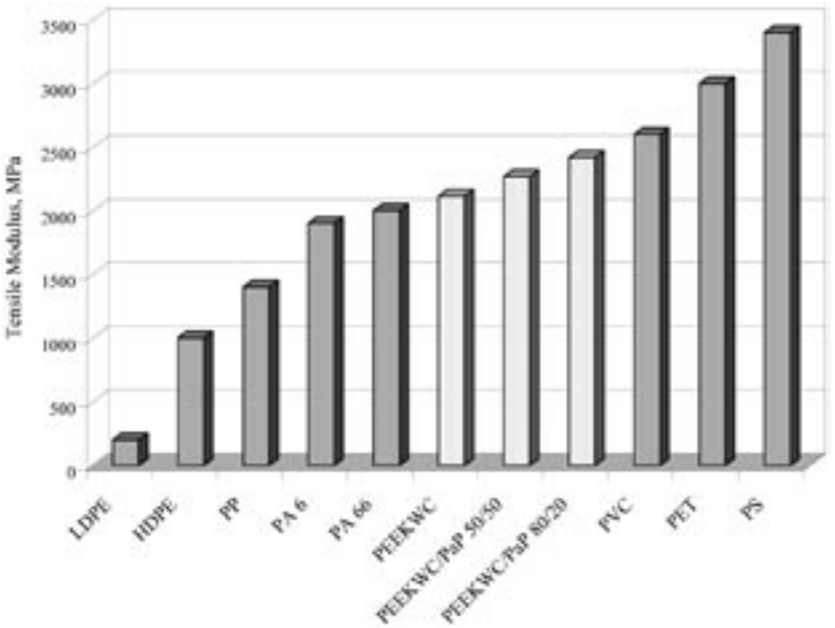


Fig. 3 - Comparison of the tensile module (MPa) of the investigated membranes with the traditional packaging polymer materials.

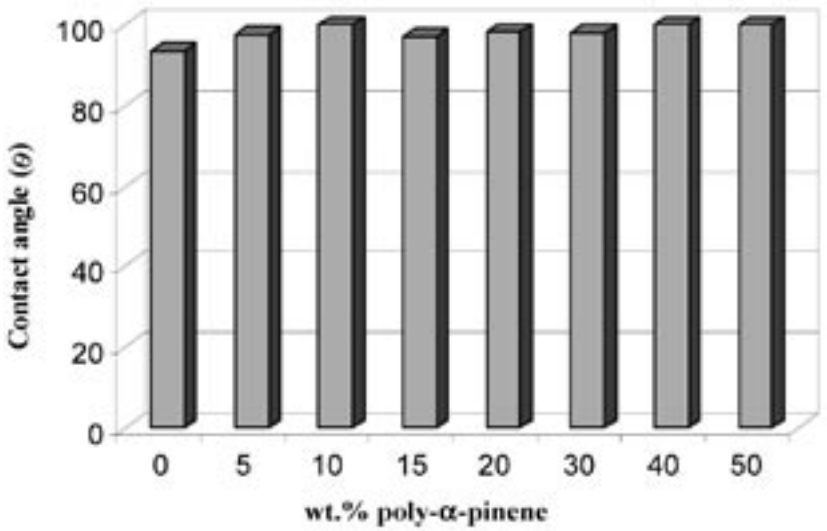


Fig. 4 - Contact angle measurements of PEEKWC and PEEKWC/PαP films.

the alcohol solution slightly increased release with respect to water, probably due to some impurities still present in the polymer.

Surface properties were investigated by the contact angle instrument as depicted in Fig. 4. These experiments showed an increase in hydrophobicity already at low additive concentration (5 wt%). Further increment of additive concentration did not influence the hydrophobic character of the membrane. The contact angle results were in agreement with the hydrophobic nature of the employed additive.

In Fig. 5, water vapour transmission rate (WVTR) experiments are presented. The WVTR of PEEKWC-based films decreased at high PαP concentrations for the all investigated temperatures (5°, 24° and 45°C).

In particular, a strong WVTR decrease from pure PEEKWC to 90/10 PαP was

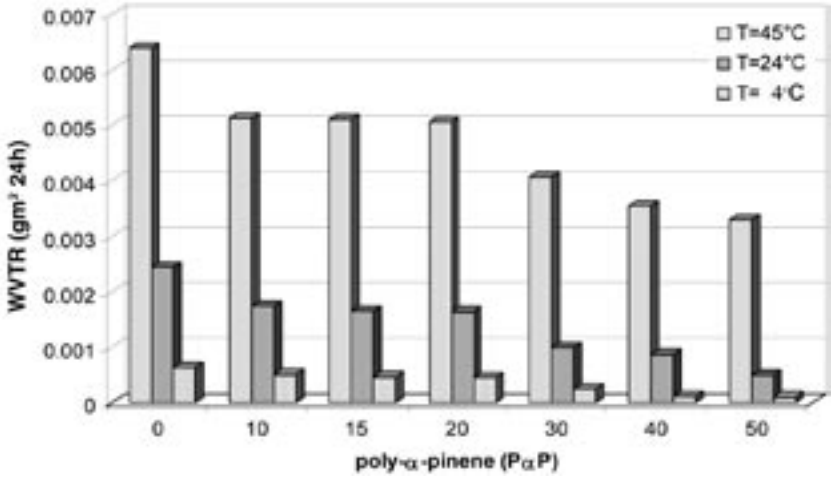


Fig. 5 - Water vapour transmission rate (WVTR) through PEEKWC and PEEKWC/PαP films.

observed; while in the additive concentration range, 10 to 20 wt.%, the WVTR value remained constant before having a further decrease to 50 wt.% of additive.

This is in agreement with SEM observations, in which the structure changed from a dense (pure PEEKWC) to an interconnected, open structure containing additive clusters. This effect is more marked above the 20 wt.% additive concentration.

This effect can be explained in terms of enhanced polymer chain mobility at high temperature for samples containing an increasing amount of additive. For all investigated temperatures, the WVTR value for 50/50 PEEKWC/PαP sample was about the half of the pure polymer.

Gas permeation experiments, using N_2, O_2 and CO_2 in this order, on the pure PEEKWC and PEEKWC/additives films were also carried out in a 20°-70°C temperature range. In Fig. 6, the O_2 permeability and WVTR of the traditional polymers used in food packaging, as bottles, films, small tanks and bags, are summarised [Miller and Krochta (1997), Panuzzo (2001)]. PEEKWC has a WVTR close to the one of PS and EVOH but significantly higher than low and high density PE and PP. The O_2 permeability was substantially higher than PET, PA 6 and EVOH. When the additive concentration increased, PEEKWC transport properties shifted to the region of the barrier materials.

Therefore, since the PEEKWC and PEEKWC/PαP have transport properties (PO_2 and WVTR) comparable to those of commercial polymers, the potential of these materials for modified atmosphere packaging fruits and vegetable of fruits and vegetables were analysed.

Oxygen and carbon dioxide were the reagent and the product respectively of fresh food respiration processes, for this reason the CO_2/O_2 selectivity trend for all PEEKWC - based films was investigated (Fig. 7). The selectivity was calculated as the

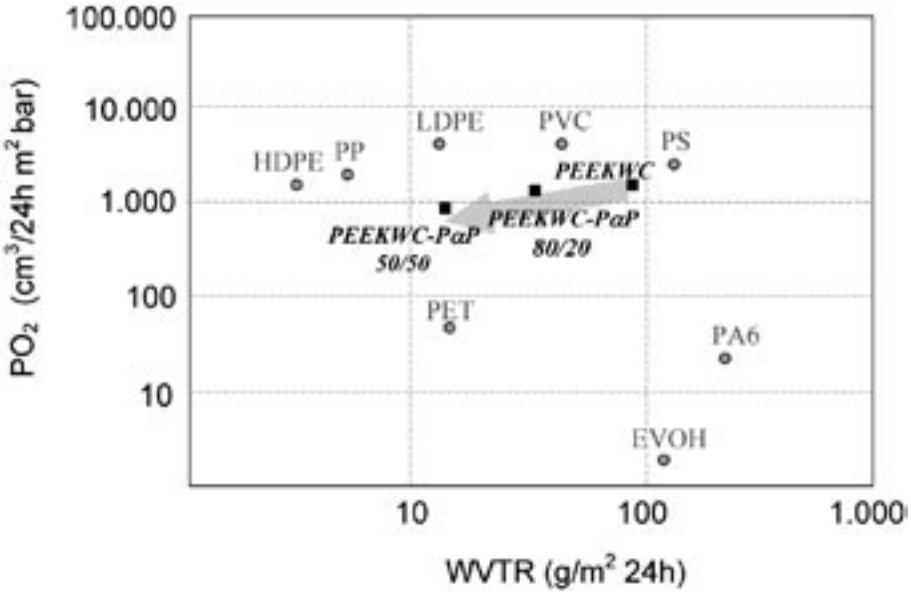


Fig. 6 - Comparison of PEEKWC and PEEKWC/PαP films with the traditional packaging films with respect to their O_2 permeability (PO_2) and water vapour transmission rate (WVTR).

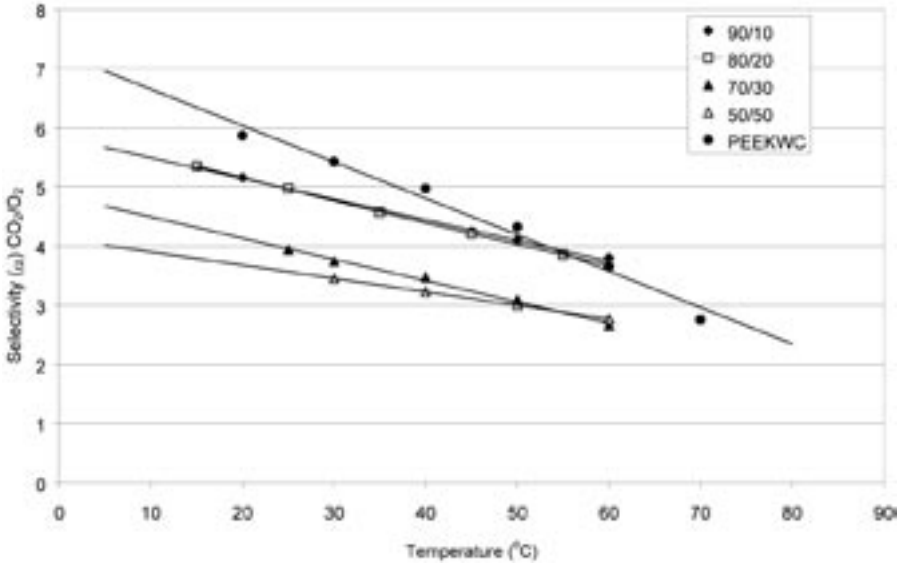


Fig. 7 - CO_2/O_2 permselectivity of PEEKWC and PEEKWC/PαP films at different temperatures.

ratio between the permeability values of the two species that obey the Arrhenius' law in the analysed temperature range, as shown in Fig. 8 and 9. In the same figures, the activation energy values for the permeation of O_2 and CO_2, respectively, are obtained calculating the slope of the straight lines depicted. CO_2/O_2 selectivity decreases with temperature according to the different activation energies of the two components. The separation performance of the polymer changes at higher additive concentration: its barrier properties increase, as already shown in Fig. 6,

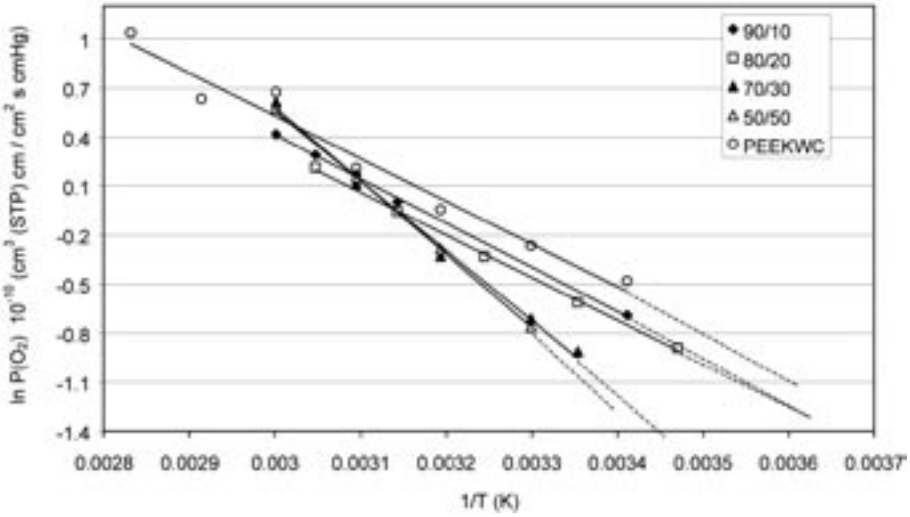


Fig. 8 - Arrhenius plot of the O_2 permeability through PEEKWC based membranes. ΔP = 1 atm.

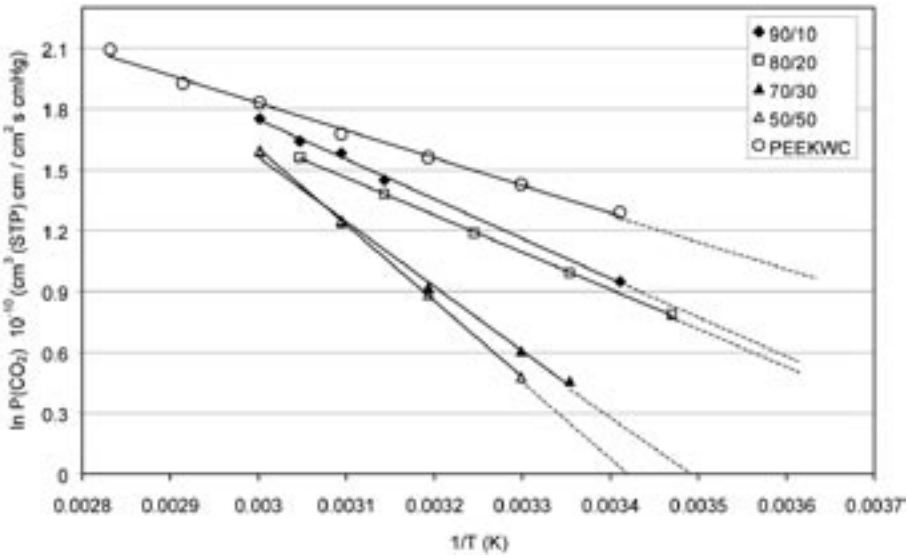


Fig. 9 - Arrhenius plot of the CO_2 permeability through PEEKWC based membranes. ΔP = 1 atm.

Table 2 - Global migration tests on PEEKWC and PEEKWC/PαP (50/50) films at 40°C for 10 days.

	Distilled water (ppm)	Acetic Acid 3% (w/v) (ppm)	Ethanol 15% (v/v) (ppm)	Limit value (ppm)
PEEKWC	18	20	32	60
PEEKWC/PαP (50/50)	20	25	30	60
* Commision Directive 90/128/EEC (2001).				

while CO_2/O_2 selectivity decreases (Table 3) (Exema *et al.* 1993). CO_2/O_2 selectivity, measured through the PEEKWC films, is comparable to the one of LDPE and silicon rubber films. The O_2 permeability is about one third lower than LDPE but two orders of magnitude lower than silicon rubber. PEEKWC/PαP 50/50 films are characterised by a CO_2/O_2 selectivity of 4.7 similar to the one of HDPE and Nylon 6. In this case, the O_2 permeability of PEEKWC/PαP 50/50 results 8.5 and 170 times, respectively, higher than that measured in the previous commercial polymers. Oxygen and carbon dioxide permeability of PEEKWC are similar to cellulose acetate ones. At low additive concentration (≤30%), the permeability of the two investigated species are still lower than pure PEEKWC. In Table 3, an increase of the activation energy for CO_2 permeation is also observed when the additive amount rises. On the other hand, the activation energy, calculated for oxygen, is constant up to 20% PαP concentration, it increases significantly at 30% of additive, remaining almost constant up to 50/50 PEEKWC/PαP ratio. The CO_2/O_2 selectivity, calculated for pure and added PEEKWC films, suggests the use of this material for some fresh products packaging, see Table 4 (Exema *et al.* 1993).

Table 3 - Oxygen and carbon dioxide permeability and activation energy (Ea) of PEEKWC and traditional packaging polymeric films. Reviewed table (Exema *et al.* 1993).

Polymers	PO_2 (Barrer)	PCO_2 (Barrer)	EO_2^P (kJ*mol-1)	ECO_2^P (kJ*mol-1)	α (CO_2/O_2) (5°C)
PEEKWC	0.36	2.4	21.7	11.3	6.7
PEEKWC/PαP (80/20)	0.30	1.7	21.7	15.3	5.7
PEEKWC/PαP (70/30)	0.13	0.52	35.6	26.5	4
PEEKWC/PαP (50/50)	0.12	0.56	37.4	31.3	4.7
Silicon rubber	100	65	8.4	-	6.5
Natural rubber	8.7	61.4	31.4	25.5	7.1
Polybutadiene	7.7	71	29.7	21.8	9.2
Poly(butadiene-styrene)	6.7	59.6	30.5	23.8	9.2
LDPE	1	7.4	43.1	34.2	6.7
HDPE	0.014	0.07	35.1	30.1	4.8
PA 6	0.007	0.035	43.5	40.6	4.7
Saran	0.0004	0.0042	66.5	51.5	10.2
PET	0.013	0.045	26.8	25.9	3.4
Cellulose acetate	0.34	2.9	20.9	29.7	8
PVC	2.1	12.7	36.9	27.6	6.1

Barrer = 10^{-10} (cm^3 (STP) cm/cm^2 s cmHg)

Table 4 - Oxygen and carbon dioxide permeability and selectivity required for various fruits and vegetables in typical market size packages (Exema *et al.* 1993) compared with PEEKWC based films. Film thickness 25 μm and 4°C.

Fresh Products	P^RO_2	P^RCO_2	α^R (CO_2/O_2)	α (CO_2/O_2) of PEEKWC films
Strawberry	22.4	23.9	1.1	
Bruxelles Sprouts	12.7	76.6	6	(90/10) and (80/20) PEEKWC/PαP (α=5.7), PEEKWC (α=6.7)
Mushrooms	10.6	13.1	1.3	
Lettuce	4.5	42.94	9.5	
Turnip	2.2	7.6	3.5	(70/30) PEEKWC/PαP (α=4)
Carrot	1.54	5.7	3.7	(70/30) PEEKWC/PαP (α=4)
Apple	1.51	9.5	6.3	(90/10) and (80/20) PEEKWC/PαP (α=5.7), PEEKWC (α=6.7)
Celery	1.26	4.04	3.2	
Green Pepper	0.67	4.03	6	(90/10) and (80/20) PEEKWC/PαP (α=5.7), PEEKWC (α=6.7)
Tomato	3.65	19.2	5.3	
Blackberry	6.4	20.1	3.14	

Barrer = 10^{-10} (cm^3 (STP) cm/cm^2 s cmHg).

Concerning the permeability of PEEKWC films, with and without additive, it is too low with respect to the fresh fruit needs. The same limitation is valid for a wide number of typical commercial food packaging polymers. However, the development of thinner films or, as an the presence of non-selective holes, carefully distributed in the bag, are the main strategies followed in the commercial fresh product packaging (Paul and Clarke 2002).

CONCLUSIONS

A new polymer, PEEKWC, pure and loaded with a terpenic resin, PαP, admitted by FDA was investigated for food packaging applications. Migration tests confirm the food suitability of PEEKWC-based films. The mechanical properties of these films also satisfy the needs of a packaging material. The CO_2/O_2 selectivity values observed for PEEKWC and PEEKWC/PαP films meet the requirements of some fresh products. At standard thickness (25 μm) the permeability data of these new materials are lower than the appropriate values for the same fresh foods. However, the possibility to prepare asymmetric membranes with a selective thin layer allows the permeation rate limitations to be overcome. Furthermore, the comparison of PEEKWC based films with the main commercial polymers used in food packaging film, places these new films in a region characterised by medium-high values of oxygen permeation rate and water vapour transmission rate. The addition of PαP causes a decrease of both rates approaching the barrier material area.

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STUDY OF AN INNOVATIVE PET (POLYETHYLENE TEREPHTHALATE) PACKAGING FOR MAYONNAISE AND EVALUATION OF PRODUCT SHELF-LIFE

STUDIO DI UN PACKAGING INNOVATIVO IN PET (POLIETILENTEREFTALATO) PER MAIONESE E VALUTAZIONE DELLA SHELF-LIFE

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ABSTRACT

The aim of this experimental study was to assess the effects of various plastic-based packagings on quality changes in mayonnaise during storage. The plastic packaging materials examined were PET, PET with an oxygen scavenger incorporated during extrusion (PET with Amosorb), and PET coated with an oxygen and carbon dioxide barrier. Packed mayonnaise was stored at 20°C and removed monthly for chemical, microbiological, sensory and rheological analysis during storage over a period of six months. The ranking of oxidative stability in mayonnaise was glass = PET-Amosorb > PET-coating > PET. A sensory panel was unable to distinguish samples stored in glass from samples stored in PET-Amosorb or PET-coating. There was no significant difference in the linear viscoelastic properties of mayonnaise packaged in glass or plastic materials during storage.

RIASSUNTO

Lo scopo del presente lavoro sperimentale è stato quello di valutare l'effetto di diversi materiali plastici di confezionamento sulla qualità della maionese durante la conservazione. I materiali utilizzati nella realizzazione dei contenitori sono stati PET, PET con incorporato un oxygen scavenger (PET con Amosorb) e PET rivestito

- Key words: mayonnaise, oxygen scavenger, packaging materials, shelf-life -

con una barriera all'ossigeno e all'anidride carbonica. La maionese confezionata è stata conservata a 20°C e sottoposta mensilmente ad analisi chimiche, microbiologiche, reologiche e sensoriali per un periodo di sei mesi. La stabilità ossidativa della maionese confezionata ha presentato l'ordine decrescente vetro = PET-Amosorb > PET-coating > PET. L'analisi sensoriale non ha evidenziato differenze tra il campione conservato in contenitore di vetro e quelli di PET-Amosorb e PET-coating. Durante lo stoccaggio non sono state individuate differenze significative nelle proprietà viscoelastiche lineari dei diversi campioni.

INTRODUCTION

Food quality during storage depends on the packaging material selected, as well as on processing, since gas may permeate through the packaging material and cause degradation of colour, flavour and nutrients. Containers made from materials other than glass are widely used for packaging. Polyvinyl chloride (PVC) was the first thermoplastic polymer to be used for the large-scale production of bottles, then new opportunities arose from the application of injection stretch blow moulding (ISBM) to the manufacture of polyethylene terephthalate (PET) bottles for water and soft drinks. Bi-oriented PET containers manufactured using this technique possess improved barrier properties and are effective for conserving tea, edible oil, isotonic drinks, spirits and some fruit juice-based products (TOPALLAR *et al.*, 1997; AYHAN *et al.*, 2001; BRODY, 2001). PET containers combine the benefits of plastic materials (lightness and unbreakability) with those of glass (transparency). Emerging gas-barrier technologies include oxygen scavengers and glass or silicon oxide, which are applied as thin coatings and possess very good gas-barrier properties (VERMEIREN *et al.*, 1999; BRODY, 2000). Oxygen scavengers serve to minimise quality changes in oxygen-sensitive foods (MALOBA *et al.*, 1996). Generally, oxygen-scavenger technologies utilise iron powder oxidation, ascorbic acid oxidation, photosensitive dye oxidation, enzymatic oxidation, unsaturated fatty acids and immobilised yeast (VERMEIREN *et al.*, 1999; BRODY, 2000).

Recently, new barrier technologies have been tested to assess their suitability for oxygen-sensitive food applications (beer, peanut butter, sauces, jams). These technologies use a range of techniques, including oxygen absorbers mixed with PET granules during container manufacture, coloured pigments or additives as barriers to light, UV and IR, as well as paints applied to the outer walls of the finished container (coating) (SALAME, 1998).

Currently, glass jars are the most popular type of packaging for mayonnaise. Mayonnaise is a semi-solid, oil-in-water emulsion comprising vegetable oil, acidifiers (vinegar and lemon juice), eggs and optional ingredients such as salt, stabilisers and thickeners. The physical differences of glass and plastic packages may cause variation in the emulsion stability of mayonnaise subject to mechanical stresses. When vibration is applied to the packaged sample, the forces are completely transferred to the product because of the rigidity of glass, whereas in plastic (PET) containers, flexing absorbs some of the shock and enhances stability of the mayonnaise (MACKSON and SINGH, 1991).

Rancidity is one of the most important causes of quality loss from spoilage in mayonnaise. It is due to off-flavours associated with oxidative and hydrolytic reac-

tions (MISTRY and MIN, 1993). One method used to retard lipid oxidation reactions is the reduction of oxygen concentration in the food by packing under vacuum or nitrogen, and using packaging materials with good oxygen-barrier properties (COUPLAND and McCLEMENTS, 1996). Mayonnaise must be protected against wavelengths shorter than 470 nm to prevent light-induced lipid oxidation. The incorporation of small amounts of polyethylene naphtalate (PEN) greatly improves the light-barrier properties of PET (LENNERSTEN and LINGNERT, 2000).

Despite the potential advantages of this method, the literature reveals few attempts to determine the shelf-life of mayonnaise packaged in plastic containers. The present study was undertaken to evaluate the preservation over time of mayonnaise packaged in containers other than glass. The plastic packaging materials examined were PET, PET incorporating an oxygen scavenger, and PET coated with an oxygen and carbon dioxide barrier.

MATERIAL AND METHODS

Packaging materials

Four different packaging containers were evaluated for their potential effect on mayonnaise quality during storage. The four packages include: a 446 mL glass container, a 473 mL PET container, a 473 mL PET-oxygen scavenger (3% Amosorb, Amoco Chemicals, USA) container and a 473 mL PET container coated with an oxygen and carbon dioxide barrier. PET and PET-Amosorb containers were produced using a injection stretching blowing machine (model CIB LAB 01/92, SIPA S.p.A, Vittorio Veneto, Italy). The oxygen scavenger was blended with PET granules to incorporate it into the sidewall of the jar. After production, the plastic containers were gamma ray-sanitised.

Glass and plastic jars were sealed with screw caps made from tinplate and polypropylene, respectively, the latter type featuring an aluminium foil heat-sealed onto the top of the jar after filling to ensure tighter sealing. Prior to jar filling, aluminium foil-wrapped polypropylene caps were fluent steam-sanitised for five minutes at 90°-98°C. The caps for the glass jars underwent the same treatment as the jars themselves (30 min in fluent vapour tunnel at 95°C).

Mayonnaise

The mayonnaise was an industrial product supplied by an Italian company. The emulsion was aseptically transferred to the sterilised containers and the packed samples were then stored at 20°C. Three containers were removed monthly for chemical, microbiological, sensory and rheological analysis over a period of six months. The ingredients and stabilisers in the mayonnaise were: sunflower oil (60.7%), water, egg yolk, lemon juice, wine vinegar, modified maize starch, salt, sugar, thickening agents (carob seed flour and guar), xanthan gum, mustard, flavours and potassium sorbate.

Determination of oxygen permeability

The permeability to oxygen ($cm^3 \cdot package^{-1} \cdot day^{-1}$) of the plastic containers was measured using an Oxtran 2/20 oxygen permeability tester (Mocon Controls, USA).

Chemical analyses

Mayonnaise pH, total acidity, vitamin A and E, peroxide value, p-anisidine and aliphatic aldehyde contents were determined. The tests were run every month for six months, to record any changes in the mayonnaise deriving from the packaging type.

The lipid phase was extracted using ether, which was removed under low-temperature nitrogen flow (30°C) and washed with water to remove short-chain organic acids. The water was removed with anhydrous sodium sulphate and the lipid phase extracted under nitrogen flow at 30°C.

Peroxide value (meq/kg) and p-anisidine content (µg/kg) were evaluated according to NGD Db6-1989 and NGD Db8-1989 respectively. Aldehydes were analysed as 2,4-dinitrophenyl hydrazones aldehydes by reverse phase HPLC according with Lampi et al. (1997). Vitamin A and E were assessed by HPLC according to procedures AOAC 992.04 and 992.03, respectively (1995).

Rheological analysis

Rheological tests were carried out in a controlled stress rheometer (StressTech Rheometer, Reologica Instruments AB, Sweden) using a serrated plate and plate sensor system (40 mm diameter; 1.5 mm gap). All measurements were made at 25±0.2°C. Special care was taken to minimise emulsion softening when the sample was initially loaded on the plate. Excess sample protruding from the edge of the sensor was carefully trimmed off with a thin blade. Before measurements were taken, the mayonnaise was allowed to stand for 5 min, to enable stresses induced during sample loading to relax. A dynamic frequency sweep was conducted by applying a constant sinusoidal strain of 0.5% within the linear region over a frequency range of 0.1 to 10 Hz. The rheological parameters used for this study were the storage (G') and loss (G") moduli.

Microbiological analyses

To verify that the type of packaging material did not affect the microbiological shelf-life of mayonnaise, lactic bacteria, yeasts and mould counts were determined in all samples during storage. The jar was incubated at 28±1°C for 14 days before microbiological tests, and regularly inspected for any exterior sign of alteration (swelling of the jar, spilling, etc.). At the end of the incubation period, 100 g of product was collected under sterile conditions and diluted with physiological salt solution at a ratio of 1:1. Portions of approximately 100 µL were inoculated in selective culture mediums, which were examined for lactic bacteria, yeasts and moulds. The culture media were sodium acetate and sorbitan mono oleate agar (MRSA) for lactic bacteria, and malt extract agar (MEA) for yeasts and moulds.

Sensory analysis

Sensory evaluation consisted of a triangular test conducted in a sensory test room according to ISO 8589 (1988), using a panel of twelve judges. Three individually labelled mayonnaise samples (two identical and one different) were presented to assessors, who were asked to identify which sample was different. In each test

session, the mayonnaise packaged in glass jar was compared to a mayonnaise stored in plastic jar. The number of tests was determined on the basis of the test sensitivity level.

Sensitivity is defined by the values selected for α, β and pd. The first value (hazard α: first-order error, or significance level) indicates the probability of perceiving a difference where no difference exists (this is the so-called first-order error, or significance level). Hazard β (second-order error) is the probability of perceiving no difference where at least one exists. Finally, pd represents the portion of the judging population capable of distinguishing the two products (PORRETTA, 2000). In this study, the above parameters α, β and p_d scored respectively 0.20, 0.20 and 40%.

Statistical analysis

Data are reported as the mean of three measurements. Significant differences between samples were verified by one-way analysis of variance (ANOVA), followed by Tukey's test with $P<0.05$ (SPSS software package).

RESULTS AND DISCUSSION

Packaging materials

The permeability to oxygen of different kinds of plastic material clearly affects the oxidative spoilage of foods such as mayonnaise because of the dependence of reaction rate on oxygen permeating through the container wall. Table 1 presents the thickness and permeability to oxygen of the plastic jars tested. PET-Amosorb and PET-coating containers exhibited oxygen-barrier properties respectively twelve and two times lower than PET-only containers.

Fig. 1 shows changes in oxygen permeability of plastic jars as a function of time. Permeability of the PET-coated jar remained constant during storage, whereas a significant increase was observed after six months for the PET-Amosorb jar. This demonstrated that an active packaging material can scavenge residual oxygen penetrating into the container for a period of only six months. The effect of different packaging materials on mayonnaise quality was investigated over a period of six months, to take into account the storage life of the oxygen scavenger dispersed into the container wall.

Table 1 - Oxygen permeability of plastic containers.

Packaging materials	Thickness * (mm)	Oxygen permeability ** ($cm^3.package^{-1}.day^{-1}$)
PET	0.48±0.01	0.030
PET-coating	0.48±0.01	0.016
PET-Amosorb	0.47±0.01	0.0025

* Mean ± standard deviation;
** Method ASTM 1927-98 (23°C; 50% RH).

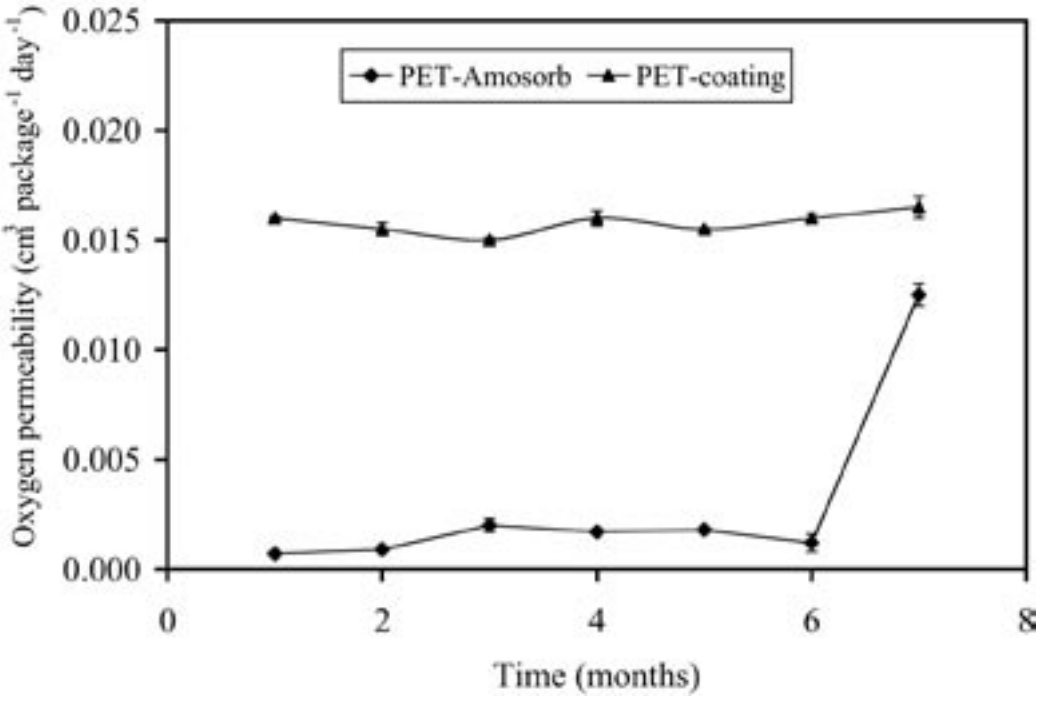


Fig. 1 - Oxygen permeability of PET-Amosorb and PET-coating containers as a function of time.

Mayonnaise

In mayonnaise, the most frequent form of spoilage is emulsion breakdown due to a combination of creaming and coalescence of the oil droplets and oxidation. Because of its low pH and high fat content, mayonnaise is relatively resistant to microbial spoilage (DEPREE and SAVAGE, 2001). No microbiological growth was recorded for samples in any of the packaging containers tested over the entire six-month storage period. Table 2 shows initial quality parameters for the industrial mayonnaise sample. Acidity (as acetic acid) and pH values are similar to those generally proposed for this product (XIONG *et al.*, 2000; MANCINI *et al.*, 2002). Vitamin E (tocopherols) is the principal antioxidant in vegetable oil. TAWFIK and Huyghebaert (1999) found that the vitamin E content of sunflower oil was 50.69 mg/100 g, which is in agreement with the present study. No statistically significant variations ($P>0.05$) in pH, acidity or vitamins A and E were recorded for mayonnaise samples in any of the packaging materials tested during storage. Tawfik and Huyghebaert (1999) investigated

Table 2 - Initial quality parameters of mayonnaise.

Determinations	Values
pH	3.46±0.13
Acidity (g /100 g as acetic acid)	0.39±0.01
Vitamin E (mg/100 g)	40.57±0.55
Vitamin A (µg/100 g)	15.50±1.73
Peroxide value (meq/kg)	8.93±1.53

Mean ± standard deviation (n=3).

the effect of packaging material on sunflower oil stability after 20 and 60 days' storage at 24°C. The Authors did not observe any differences in loss of vitamin E from glass or PET bottles, which is in accordance with our results. Otherwise, it is known that there are significant differences between lipid oxidation in bulk fats and emulsified fats (DEPREE and SAVAGE, 2001).

Various indexes have been used to evaluate the protective effect of the three plastic materials against lipid oxidation, including peroxide value (PV), p-anisidine and aliphatic aldehydes. PV was constant and did not differ significantly for samples stored in different containers during the first four months ($P>0.05$) (Fig. 2). A sharp increase in PV was observed at a storage period of five months for mayonnaises in PET and PET-coating jars. Oxidation was greater for the PET jar than for the PET-coating jar. The ranking of oxidative stability in mayonnaise was glass = PET-Amosorb > PET-coating > PET, which reflect the main role of permeable oxygen (Table 1) (KAYA *et al.*, 1993). We obtained a linear correlation between oxygen permeability of the material and PV of mayonnaise packaged for five months ($R2 = 0.989$) (Fig. 3). Published results demonstrated that the incorporation of the oxygen scavenger into the container wall can successfully slow down sample oxidation (VERMEIREN *et al.*, 1999). The PET-Amosorb container was effective as glass at inhibiting oxidation.

Reaction products of the latter stages of lipid oxidation (p-anisidine and aliphatic aldehydes) remained constant over time and did not show any signifi-

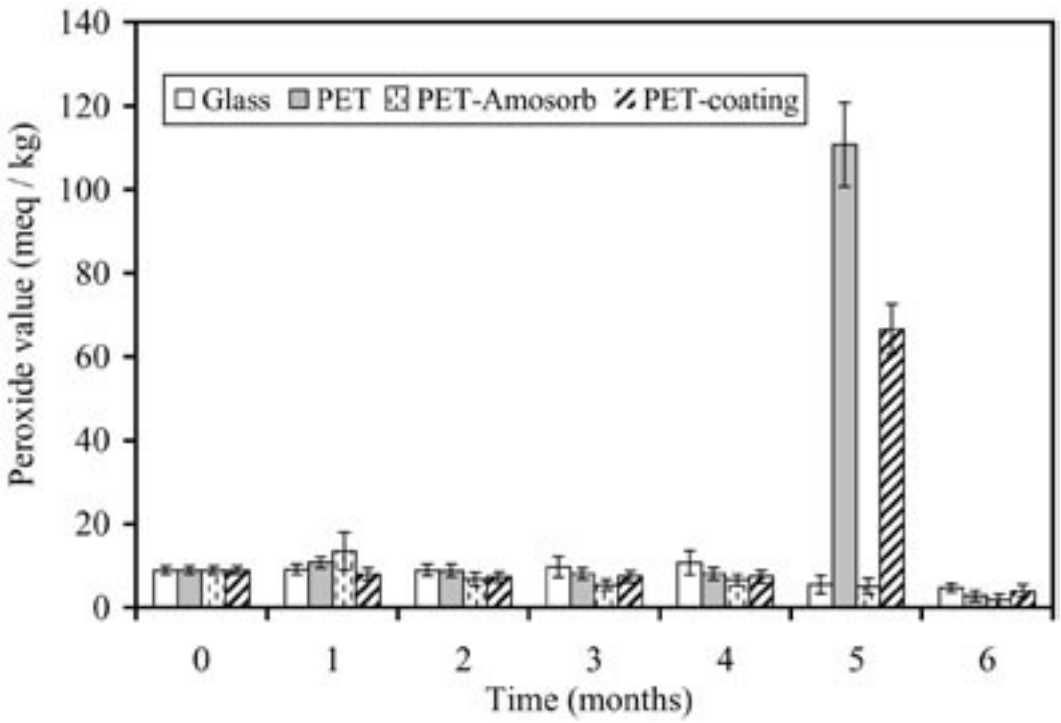


Fig. 2 - Peroxide value as a function of storage time for mayonnaise packaged in different containers.

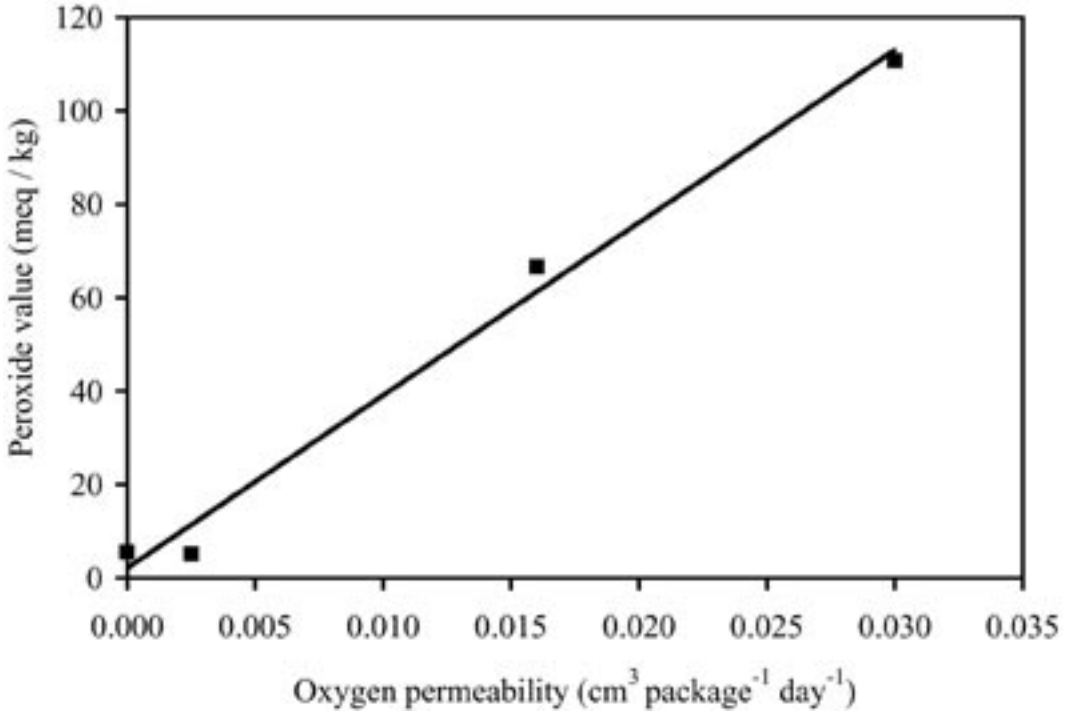


Fig. 3 - Linear correlation between peroxide value of mayonnaise at five months of storage and oxygen permeability of different containers.

cant differences between mayonnaise stored in glass and plastic containers (data not shown).

Unlike glass containers, plastic containers tend to build up electrostatic charges, which could promote the breakdown of some sauce or spread types. Physical emulsion instability was evaluated by dynamic mechanical analysis, which is considered a valuable and powerful method in the characterisation of complex structures (PERESSINI *et al.*, 1998; GUNASEKARAN and AK, 2000). Fig. 4 shows the storage modulus (G') at 1 Hz as a function of storage time for mayonnaise packaged in different containers. No statistically significant differences ($P>0.05$) in G' were observed for mayonnaise samples in any of the packaging materials during storage. The loss modulus (G") showed the same behaviour as G' (data not shown). Plastic materials did not, therefore, influence emulsion stability. The storage modulus may be considered as a measure of emulsion texture, which was found to be more solid-like when the mayonnaise was formed of smaller droplets, and more liquid-like when the mayonnaise was formed of larger droplets (LANGTON *et al.*, 1999). Coalescence is indicative of emulsion instability where droplets coagulate to form larger droplets, which in extreme cases may result in emulsion breakdown. In creaming, droplets flocculate and can promote coalescence. Several Authors have observed that the increase in particle size of emulsions with ageing induces a decrease in the magnitude of viscoelastic parameters because of the coalescence of these particles (FISCHBACH and KOKINI, 1987; PAREDES *et al.*, 1989). The

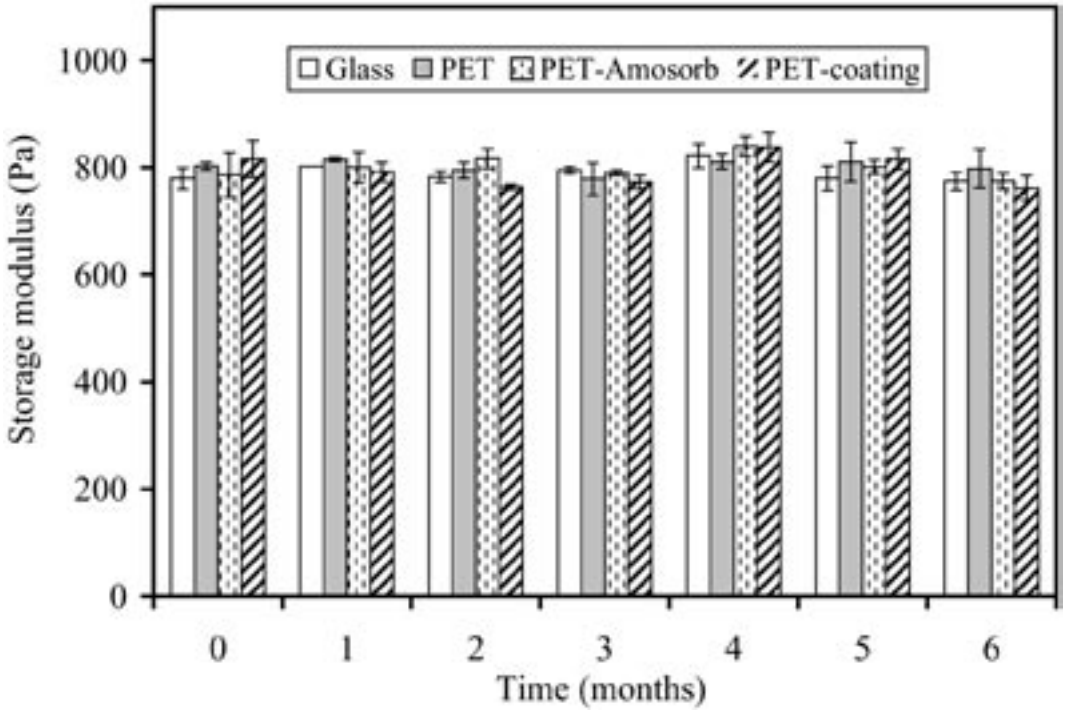


Fig. 4 - Storage modulus as a function of storage time for mayonnaise packaged in different containers.

absence of changes in G' values for mayonnaise during storage indicated high emulsion stability against creaming and coalescence, which is probably due to the stabilising effect of thickening agents (Fig. 4). In particular, xanthan gum possesses unique properties that have led to its application in food systems as a thickener and emulsion stabiliser. Xanthan is adsorbed at the oil/water interface, lowering the surface tension and decreasing the droplet size, and forms a microgel matrix in the water phase, which stabilises the emulsion by physically trapping the emulsion droplets (HENNOCK *et al.*, 1984). Mayonnaise stabilised with polysaccharides may become rancid before the emulsion breaks down (DEPREE and SAVAGE, 2001). The absence of changes in emulsion structure during storage implies that differences in lipid oxidation are due only to the barrier properties of the various containers and not to changes in the distribution of oil particle size.

Fig. 5 shows the results of sensory analysis. The panelists were able to perceive differences between glass and plastic containers only in the case of mayonnaise packaged for two and six months in the PET container. No differences were identified for samples packaged in glass containers and PET-Amosorb or PET-coating according to chemical results.

There are static models that calculate the probability of sensory failure (PSF) as a function of storage time. This parameter expresses the percentage of product likely to lose its original properties within a given period of time. The Weibull model is especially useful for investigating the shelf-life of foods. Table 3 shows the pa-

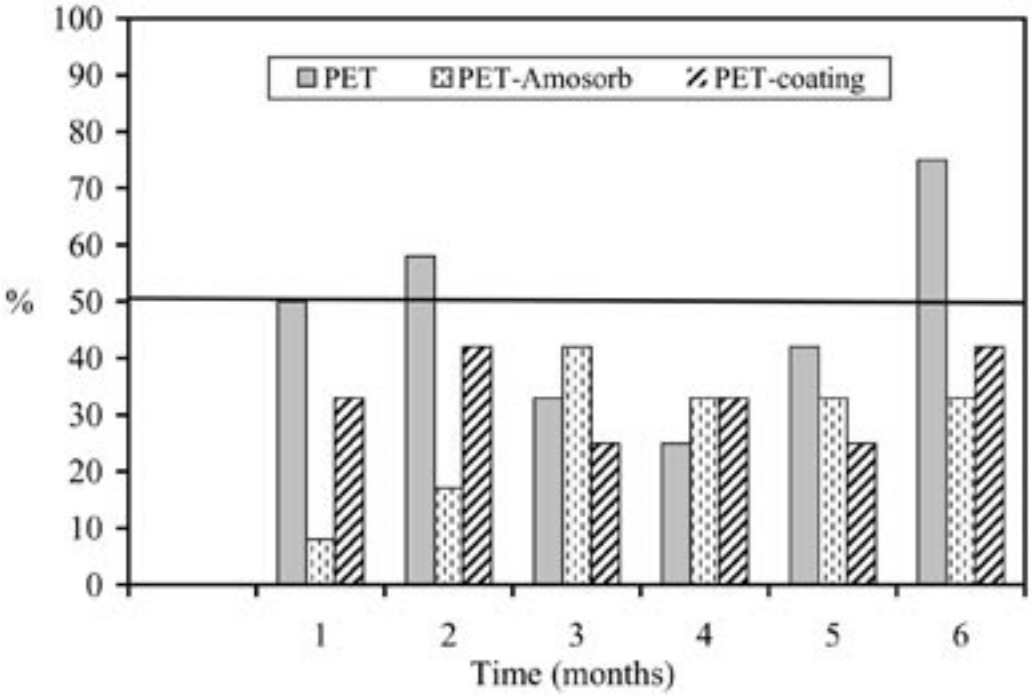


Fig. 5 - Percentage of assessors able to perceive differences between mayonnaise packaged in glass and mayonnaise packaged in plastic containers.

Table 3 - Parameters used to compute "Probability of Sensory Failure" for mayonnaise packaged in PET container.

Reverse order (k)	High quality loss	Storage time (days)	Risk (h=100/k)	Cumulative risk
6	Yes	49	16.6	16.6
5	No	73	-	-
4	No	104	-	-
3	No	141	-	-
2	Yes	168	50.0	66.6
1	Yes	170	100.0	166.6

rameters used to compute PSF in relation to high quality failure for mayonnaise. The panelists were able to perceive consecutively twice the differences between glass and plastic containers only in the case of mayonnaise packaged in the PET container after 168 and 170 days.

CONCLUSIONS

Containers made from PET incorporating Amosorb as an oxygen scavenger can provide an attractive and suitable form of packaging for mayonnaise as they provide

protection against lipid oxidation. Results for PET-coating containers showed that this form of packaging has promising potential. However, further research is needed to develop more effective ways to improve the oxidative stability of mayonnaise.

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PREDICTION OF WATER PERMEABILITY OF FLEXIBLE MULTILAYER FILMS INTENDED FOR FOOD PACKAGING APPLICATIONS

PREVISIONE DELLE PROPRIETÀ BARRIERA
ALL'ACQUA DI FILM FLESSIBILI MULTISTRATO
USATI NEL SETTORE DELL'IMBALLAGGIO ALIMENTARE

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ABSTRACT

In this paper the water barrier properties of a multilayer film as a function of the water activity at the upstream and downstream side of the film is presented. In order to prove the ability of the used expression to predict the water permeability of the multilayer film, water permeation tests were conducted at 25°C on both single layer and on multilayer film.

RIASSUNTO

In questo lavoro sono state studiate le proprietà barriera all'acqua di un film multistrato, in funzione dell'attività dell'acqua a monte e a valle del film. Per provare la capacità predittiva dell'espressione utilizzata, test di permeabilità all'acqua sono stati effettuati sia sul film multistrato sia su ciascuno dei singolo strati.

- Key words: food packaging, modelling, multi-layer films, water permeability -

INTRODUCTION

The barrier properties of flexible films play a major role in determining the shelf-life of packed foodstuffs. In fact, polymeric films controlling the rate at which small molecular weight compounds permeate into or outside the package can slow down the detrimental phenomena responsible for the unacceptability of the packaged product. Generally, the barrier properties of polymeric films are simply determined by evaluating the permeability coefficient of a given polymeric-diffusant system.

This approach can be successfully used wherever the permeability coefficient does not depend on the boundary conditions. In fact, in these cases the permeation coefficient is constant since the permeating molecules do not cause much change in the free volume of the polymeric matrix.

The above approach fails wherever the permeability depends on the diffusant partial pressure at the upstream and downstream side of the film, as in the case of water vapor transmission through moderately hydrophilic polymers. In fact, water molecules acting as plasticizers increase the macromolecular mobility of the polymer. As a consequence, both solubility and diffusivity coefficient, and consequently the permeability coefficient, depend on the local water concentration. In these cases, the permeability coefficient cannot be determined by a single measure, instead a more accurate analysis of the permeation process is necessary to properly determine the barrier properties of the film in the real working conditions.

It must also be considered that hydrophylic polymers are rarely used by themselves. They are often laminated together with different polymeric films in order to obtain a multilayer structure.

MATERIALS AND METHODS

In this work a polymeric film supplied by Cryovac was investigated. It is composed by 6 layers: the inner layer is a very high barrier properties. Water permeability tests were conducted on both single layers and multilayer film.

Water permeability was determined by means of Permatran (Mocon, Model W 3/31). Samples with a surface area of 50 cm^2 were tested at 25°C. The permeation tests were conducted by keeping the relative humidity at the downstream side of the film equal to zero, and changing the relative humidity at the upstream side of the film from 0.35 to 0.90. In particular, the permeation tests were run setting the relative humidity at the upstream side of the film equal to: 0.35, 0.45, 0.55, 0.65, 0.75, and 0.90. A flow rate of 100 mL/min of a nitrogen stream was used.

RESULTS AND DISCUSSIONS

In Fig. 1 and 2 the results obtained from the water permability tests on the five layers composing the multilayer film and on the multilayer film are reported. It can be seen that water permeability of each layer (except barrier layer) is almost constant with the relative humidity at the downstream side of the film. The mean values obtained are listed in Table 1.

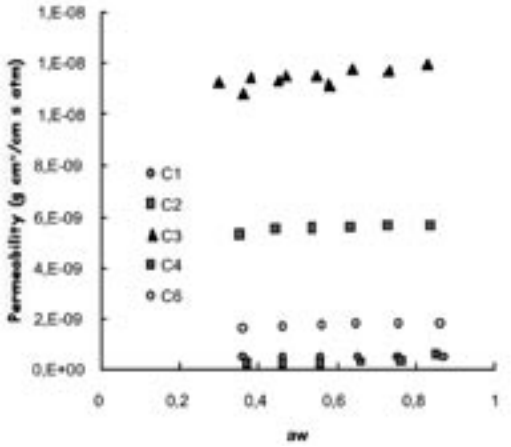


Fig. 1 - Permability of the five layers of the multilayer film as a function of water activity at the upstream side.

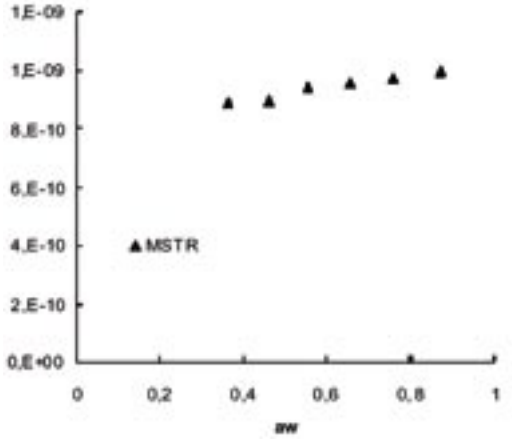


Fig. 2 - Permability of the multilayer film as a function of water activity at the upstream side.

Water permeability of multilayer film was calculated using the following expression:

$$P_{MSTR} = \frac{1}{\sum_i \frac{l_i}{l_{tot}} \cdot \frac{1}{P_i}}$$

where li is the thickness of each single layer, l_{tot} is the thickness of the multilayer film and Pi is the average permeability value of each layer. The mean value of the permeability coefficient of the multilayer film is 9.43 e-10 while the predicted value is 1.10 e-9. This can be considered a good result, considering the approximations used to derive the predicted value. But it fails when the permeability coefficient depends on the boundary conditions, on the diffusant partial pressure at the upstream and downstream side of the film. In fact, water molecules acting as plasticizers increase the macromolecular mobility of the polymer. In these cases, the permeability coefficient cannot be determined by a single measure. At the moment, a more accurate analysis of the permeation process is in progress, in order to properly determine the barrier properties of the film in the real working conditions.

Table 1 - Mean values of water permeability of each single layer.

	Water Permeability - Mean Value
C1	5,07424E-10
C2	5,52507E-09
C3	1,14426E-08
C4	3,2827E-10
C5=C1	5,07424E-10
C6	1,72713E-09

THE INFLUENCE OF USING PACKAGING FILMS WITH DIFFERENT PERMEABILITIES ON THE QUALITY DECAY KINETIC OF PLUM TOMATO (POMODORINO DATTERINO)

INFLUENZA DI FILMS A DIFFERENTE PERMEABILITÀ
SULLA CINETICA DI DECADIMENTO DEL POMODORINODATTERINO

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ABSTRACT

The aim of this research was to evaluate the shelf-life of Plum Tomato during storage in different plastic films. Plum Tomato (cultivar Iride) was packaged with three plastic films with a different gas permeability and stored in market conditions. During storage the following parameters were monitored: package weight, chemical parameters (vitamin C, carotenoids, glucose and fructose, pH and acidity, humidity, refractometric index, Brix degree), physical parameters (color, weight, dimension, hardness) and sensorial attributes. Results pointed out that using film with high barrier properties speeded up the quality decay kinetics. On the other hand, films with low gas barrier properties and high water barrier properties, can slowdown the quality decay kinetics of this specific produce.

RIASSUNTO

Recentemente è stato introdotto un ibrido di pomodoro denominato "Datterino", che riscuote notevole interesse da parte dei consumatori, ma che risulta non perfettamente idoneo ad essere commercializzato a grappoli in quanto si distacca

- Key words: food packaging, plum tomato, shelf-life -

facilmente dal rachide. Da qui l'esigenza di un imballaggio che oltre a servire da contenitore possa da un lato valorizzarne l'aspetto estetico e dall'altro allungarne la shelf-life proponendolo anche come prodotto pronto al consumo (IV gamma).

Scopo di questo lavoro è quello di valutare la shelf-life del PomodorinoDatterino durante la conservazione in differenti film plastici.

A tal fine campioni di "PomodorinoDatterino" cultivar "Iride" sono stati confezionati con tre diversi film plastici a diversa permeabilità (un imballaggio commerciale a matrice poliolefinica, attualmente utilizzato per la IV Gamma, e due imballaggi biodegradabili di nuova formulazione a matrice amidacea) e conservati alle condizioni di magazzino.

Durante il periodo di conservazione, per valutarne la qualità, sono stati determinati i principali parametri chimici (contenuto di vitamina C, licopene, rapporto acidi-zuccheri), fisici (colore e consistenza) e sensoriali del prodotto questi ultimi valutati da un panel di giudici addestrati.

I risultati hanno mostrato che l'uso di film a bassa permeabilità sia all'acqua che ai gas accelerano la cinetica di decadimento qualitativo; Mentre l'uso di film ad elevata permeabilità possono rallentare la cinetica di decadimento qualitativo dei pomodori, aumentando la shelf-life del prodotto.

INTRODUCTION

Plum tomato has recently been introduced on the market. It has the size of a cherry tomato and an oblong shape. Due to both its sensory characteristics, and the high concentration in nutraceutical constituents, such as vitamin C, carotenoids, lycopene and polyphenols, the demand for Plum Tomato has rapidly increased in the last years. Unfortunately, Plum tomatoes are not suitable to be commercialized as cluster (like "cherry tomato") because they can be easily removed from rachis. For these reasons Plum tomatoes are generally contained in a bag type package to slow down all the detrimental phenomena responsible for their quality decay during storage, and sold as "ready-to-use" food (minimally processed).

The aim of this research was to assess the influence of using packaging films with different permeability on the quality decay kinetics of Plum was tomato.

MATERIALS AND METHODS

The tests were run on samples collected at commercial maturity from a greenhouse nearby Catania (certified company ISO 9001). Plum tomatoes were selected for homogeneity, washed with distilled water and dried. Each package, containing 30 plum tomatoes, was thermally sealed by a welder machine (brand "delta" model "delta 30"). The sampling and the analysis was performed at 0, 6, 12, 18, 24, 28, 33, 38 and 42 days. The samples were packaged and stored at 15°C and at relative humidity of 75% with three plastic films with different gas permeability. In particular, a commercially available pololefinic film and two biodegradable films were used. For the sake of simplicity, the commercially available pololefinic film will henceforth be referred to as Package A, and the two biodegradable films will be referred to as Package B and Package C, whereas the samples packaged without using a film will be referred to as Reference.

To determine the quality decay kinetics of the investigated tomato the following quality sub-indices were monitored during storage: package weight, pH and acidity, humidity, refractometric index, Brix degree and dimensions of fruits; color parameters by "Handy Colorimeter Nippon NR-3000", hardness by digital penetrometer "TR 53205", sensorial analyses, vitamin C content, carotenoids content, glucose and fructose content. The above physical and chemical quantities were determined by analysing three samples from each batch. The sensory analysis (ISO 8587, 1998) was carried out by a panel of 12 judges, they were opportunely trained to evaluate the following descriptors: brightness, colour of skin, firmness determined by pressing the fruit between thumb and forefinger, juiciness, toughness of skin, sweetness, acidity and savoury. Determination of vitamin C was carried out by a liquid/liquid extraction with a solution of phosphoric acid 0.05 N on whipped and homogenised samples. Later the samples was centrifuged, purified by cartridges C18 and trigged in HPLC. Determination of carotenoids was carried out by a complex liquid/liquid extraction with acetone, up to complete decolourizing of the sample, petroleum ether and next spectrophotometric determination in hexane. Also in this case the tomato samples were first whipped and homogenised. Determination of glucose and fructose was carried out by HPLC, after a liquid/liquid extraction whit distilled water.

RESULTS AND DISCUSSION

As an example Fig. 1-4, show the evolution during storage of some of the monitored quality sub-indices (juiciness, toughness, firmness, bright-

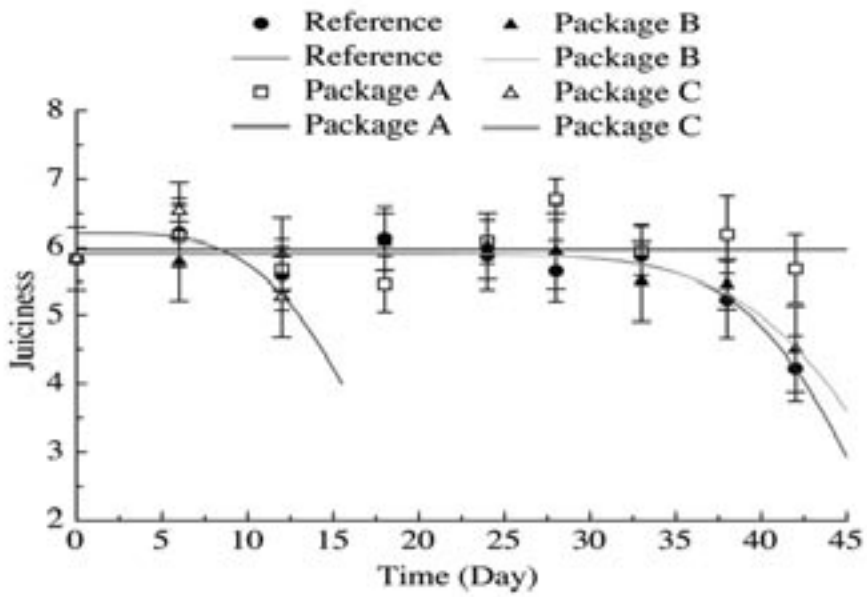


Fig. 1 - The Plum tomato Juiciness plotted as a function of storage time. The curve reported in the figure does not represent any model, it has been drawn to highlight the trend of the data.

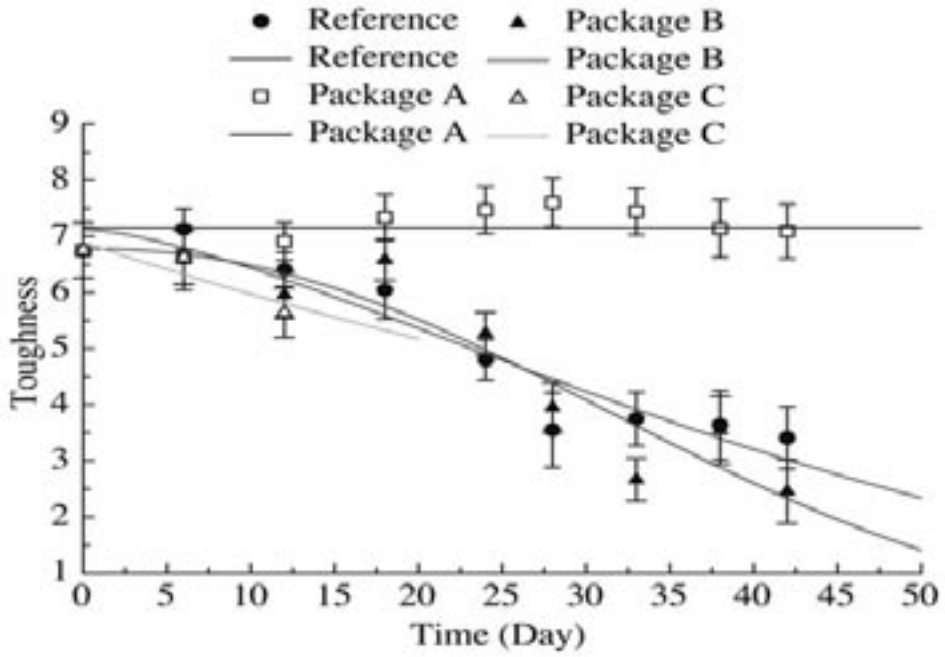


Fig. 2 - The Plum tomato Toughness plotted as a function of storage time. The curve reported in the figure does not represent any model, it has been drawn to highlight the trend of the data.

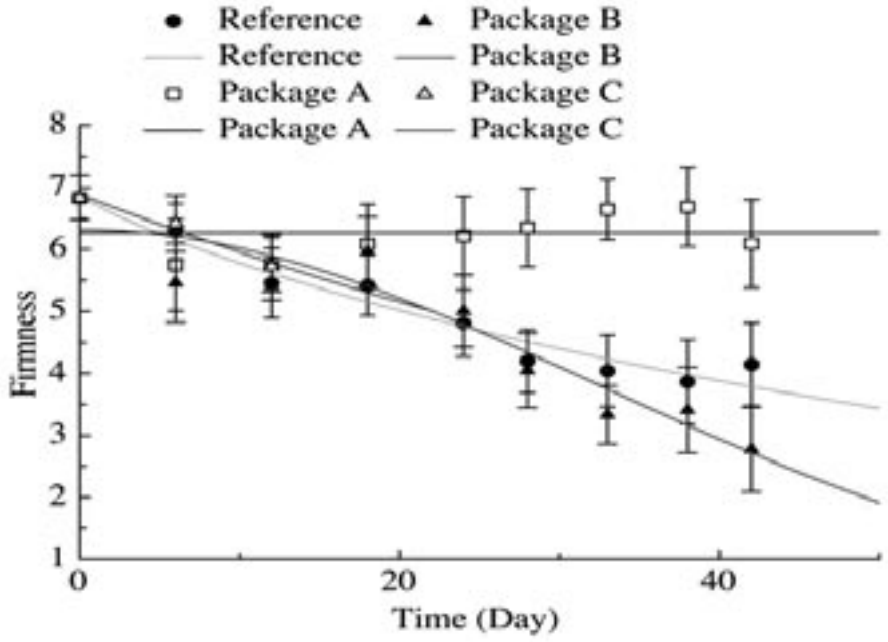


Fig. 3 - The Plum tomato Firmness plotted as a function of storage time. The curve reported in the figure does not represent any model, it has been drawn to highlight the trend of the data.

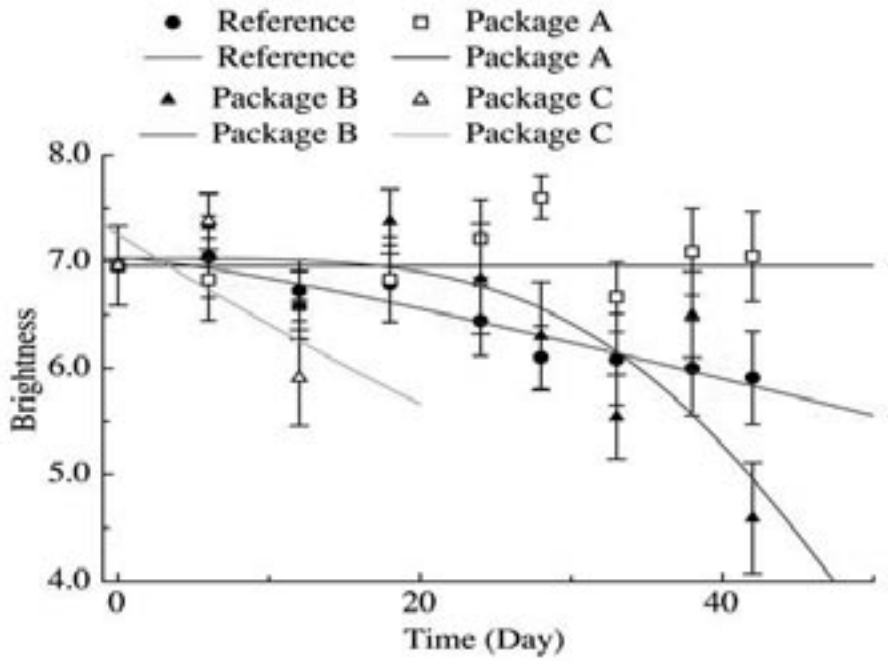


Fig. 4 - The Plum tomato Brightness plotted as a function of storage time. The curve reported in the figure does not represent any model, it has been drawn to highlight the trend of the data.

ness). The curves reported in the figures do not represent any model, they have been drawn to highlight the trend of the data. Results pointed out that using film with high barrier properties to low molecular weight compounds speed up the quality decay kinetics. On the other hand, films with low gas barrier properties and high water barrier properties can slow down the quality decay kinetics by reducing the dehydration rate and avoiding that the oxygen concentration in the package head space goes below the threshold level required for aerobic respiration.

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STUDY OF APPLE SLICE PRESERVATION BY COMBINED METHODS TECHNOLOGY

STUDIO SULLA CONSERVAZIONE DI MELE IN PEZZI ATTRAVERSO L'USO DI TECNOLOGIE COMBINATE

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ABSTRACT

The first purpose of this research was to study the effects of combined technologies, like an inhibitor treatment and the use of an alternative protective atmosphere, to extend the shelf-life of minimally processed apple slices. Four different samples, dipped or not-dipped, packed in air or in modified atmosphere (MA), were analysed for colour and texture characteristics during 12 days of storage at 4°C.

Afterwards, the combined treatment, resulting the best in maintaining the product quality from the first experiment, was chosen to test the effect of different plastic film pouches with low, medium and high gas permeability. Chemical-physical and sensory characteristics of apple slices in MAP, packed in different plastic films were evaluated after 8 days of refrigerated storage.

The dipping made and MA adopted in conjunction with packaging in a low barrier film appeared to better preserve the quality characteristics of fresh apple slices.

RIASSUNTO

In una prima fase del lavoro sono stati studiati gli effetti di tecnologie combinate, quali un trattamento di dipping e l'uso di un'atmosfera protettiva innovativa, allo scopo di estendere la shelf-life di pezzi di mela minimamente processati. Su quattro differenti campioni, sottoposti a dipping e non, confezionati in aria o in atmosfera modificata (AM), sono state effettuate analisi di colore e consistenza nel corso di 12 giorni di stoccaggio a 4°C.

- Key words: apples, dipping, modified atmosphere, packaging, quality, sensory analysis.

Successivamente, la combinazione di interventi risultata migliore in termini di mantenimento della qualità del prodotto nella prima parte della sperimentazione, è stata adottata nel testare l'effetto di confezioni a diversa permeabilità sulle caratteristiche qualitative della frutta. Dopo otto giorni di stoccaggio refrigerato sono state valutate le caratteristiche chimico-fisiche e sensoriali dei campioni di mele in AM, confezionati in tre diversi film (bassa, media ed alta barriera).

Il tipo di dipping e di AM adottati, insieme all'utilizzo di un film a bassa barriera come imballaggio sono risultati meglio conservare le caratteristiche qualitative del prodotto fresco.

INTRODUCTION

Fresh-cut or minimally processed fruits are products with attributes of convenience and fresh-like quality. Processing procedures (Bolin *et al.*, 1977; Saltveit, 1997; Wright and Kader, 1997), packaging (Cameron, *et al.*, 1995; Solomos, 1994), temperature management during shipping, handling and marketing (Brecht, 1999) and the high respiration rate cause the basic problems for the shelf-life of this kind of product: enzymatic browning (Laurila *et al.*, 1998) and texture, flavour and colour deterioration (Mencarelli and Massantini, 1994).

A combination of inhibitory treatments (Pizzocaro et al., 1993; Ponting and Joslyn 1972; Sapers and Douglas 1987) and modified atmosphere packaging (Day, 1996) have been reported to successfully retard this kind of problem in sliced apples.

Recently there has been great interest in the potential benefits of using argon (Ar) and other noble gases for MAP applications. Studies have shown that argon can effectively inhibit the growth of certain micro-organisms, suppress enzymatic activities and control degradative chemical reactions in selected perishable food products (Spencer, 1995), like fresh-cut fruit (Powrie *et al.*, 1990). Of particular relevance is the finding that noble gases can reduce the respiration rates (Spencer, 1995); while nitrous oxide (N_2O) has shown a significant anti-ethylene activity by extending the lag phase of ethylene production in fresh fruit (Gouble *et al.*, 1995).

Fruit respiration control may be achieved using packaging film that has optimum material characteristics and correct permeability to maintain or create the conditions needed for fresh products (CAMERON, 1989, KADER *et al.*, 1989). Furthermore EXAMA *et al.* (1993) found that few polymeric film MAP systems simultaneously produce the desired internal pO_2 and pCO_2 levels.

This research is aimed to study the use of combined technologies to extend the shelf-life of sliced apples. In particular modified atmosphere packaging (MAP) with novel gas mixtures (Ar and N_2O), a non sulphite dip and the use of plastic film pouches with different gas permeability were tested in order to investigate their synergistic effects on maintenance of some qualitative characteristics of fresh cut apples during refrigerated storage.

MATERIALS AND METHODS

Fresh Golden Delicious (*Malus malus*) apples from Valtellina (Italy) were purchased from a local market at commercial maturity. After washing in tap water the apples were hand-peeled, cored and reduced, with a sharp knife, to slices of 1 cm thickness.

Then, apple slices were dipped for 10 min in a solution containing 0.5% citric acid (CA), 0.5% ascorbic acid (AA) and 0.5% calcium chloride (CC) at 25°C for ten minutes in a w/w proportion of 4 to 1. After gently drying with blotting paper, 200 grams of apple slices (about fifteen slices) were put into medium permeability gas barrier plastic film pouches. Films were purchased from Hot Mould Sistem srl (Torino, Italy). The samples were packed in air or in a non conventional modified atmosphere composed of 65% nitrouse oxide (N2O), 25% argon (Ar), 5% carbon dioxide (CO_2) and 5% oxygen (O_2), using a quaternary gas mixer (CVC, Milano, Italy) and a compensated vacuum-packing, Mod. Welding Machine (Food Basic, Vigevano, Italy).

The dipping treatment and modified atmosphere used were chosen on the basis of preliminary trials.

Four different samples were realized: A (not-dipped, packed in air), B (dipped, packed in air), C (not-dipped, packed in MA), D (dipped, packed in MA). The samples were stored in a refrigerated cell at 4 (±1)°C for 12 days and analysed for colour and texture just before storage (zero time) and after four, eight and twelve days of storage.

Five sub-sample pouches per sample and storage time were prepared.

Firmness

Texture analysis was performed at room temperature (20±2°C) by measuring the force needed for a penetration of 6 mm, with a 6 mm diameter stainless steel cylinder, using a Texture analyser mod. HD500 (Stable Micro Systems, Surrey, UK) equipped with a 50 kg load cell. Test speed was performed at 0.5 mm per sec and data were expressed as kg x sec.

Colour measurements

Surface colour was measured using a tristimulus reflectance colorimeter (Chromameter-2 Reflectance, Minolta, Japan), equipped with a CR-300 measuring head. Standard C.I.E. conditions, with illuminant "C" (6774K) were used. Colour was recorded using the CIE – L* a* b* scale. Parameters were expressed as L* and hue angle (h°), where L* corresponds to lightness, h° is calculated from arctangent b*/a* (McGuire, 1992).

Afterwards, the combined technology that showed to be the best from the first experiment of work was chosen to test the effect of plastic film pouches with different gas permeability, comparing chemical-physical and sensory characteristics of apple slices in MAP after 8 days of refrigerated storage with fresh once.

Apple slices were pre-treated in the same conditions and packed in the same modified atmosphere utilised in the first part of the research.

Three different barrier plastic materials were used to pack apple slices and the following samples were realized: P1 (dipped in MA, packed in a high barrier film); P2 (dipped in MA, packed in a medium barrier film), P3 (dipped in MA, packed in a low barrier film). Colour determinations with tristimulus colorimeter and texture analysis, were made as reported above.

Image analysis

The tristimulus approach to colour measurements was compared with an image analysis in order to evaluate better the colour because the fruits do not always go brownish homogeneously (Russ, 1995).

Images of apple samples were obtained by digitalisation using a colour plane scanner (Scan Jet 6300 C - true colour - 24 bit, resolution of 300 bit x pixel) and evaluated with advance Image Analysis Software (Image Pro-Plus v. 4.1, Media Cybernetics, USA), using RGB scale, in two steps: individuation of total sample area and of different levels browning areas. On the bases of chromatic characteristics of all samples, a colour model was build up. The same colour model was applied to all apple slice images. The software, examining all pixels in the image, calculates browning area percentage in total. The measurements were obtained from the same slices used for colour determinations made with tristimulus colorimeter.

Sensory evaluation

Further the colour, flavour and texture properties of different apple samples were evaluated by 16 trained judges (QDA analysis), compared with these of fresh just cut apples sample (F). A value along a scale of nine point was assigned to each hedonic description (1 = worst, 9 = best).

Analysis of variance (ANOVA) and the test of mean comparison according to Fisher least significant difference (LSD) were applied; level of significance was 0.95.

RESULTS AND DISCUSSION

Figs. 1 and 2 report the changes in color measurements (L* and hue angle) that apple samples underwent during refrigerated storage. As samples B and D did not show substantial changes in L* and h° parameters during storage time. In

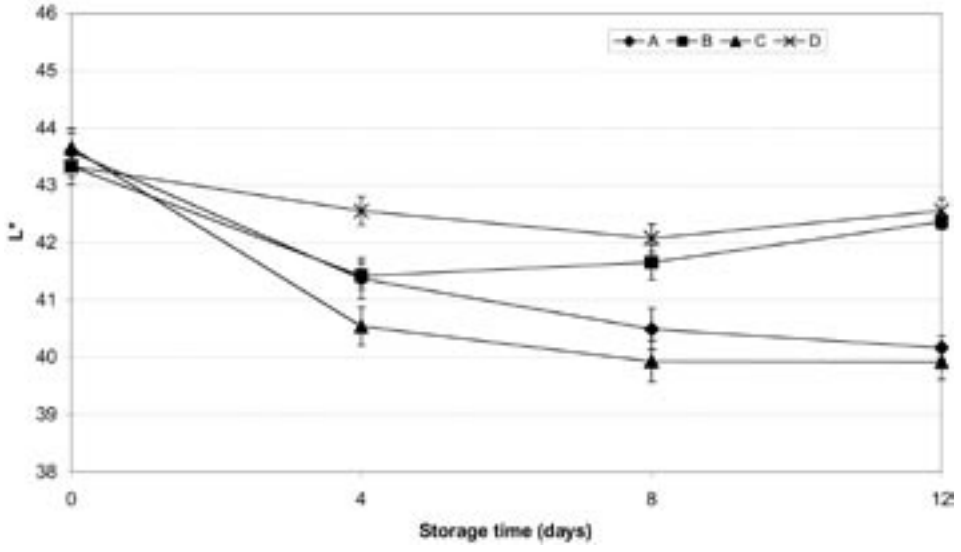


Fig. 1 - Evolution of L* parameter for minimally processed apple samples during refrigerated storage. Samples: A: not-dipped, packed in air; B: dipped, packed in air; C: not-dipped, packed in MA; D: dipped, packed in MA.

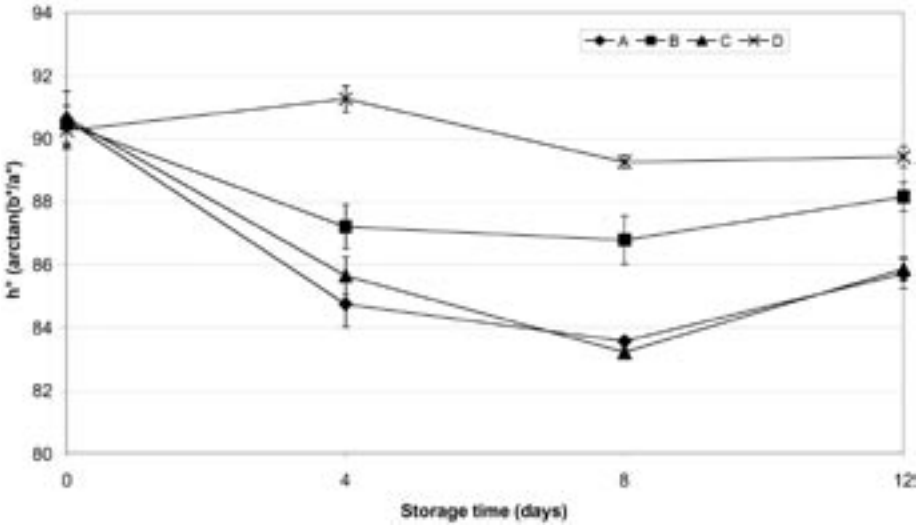


Fig. 2 - Evolution of hue angle for minimally processed apple samples during refrigerated storage. Samples: A: not-dipped, packed in air; B: dipped, packed in air; C: not-dipped, packed in MA; D: dipped, packed in MA.

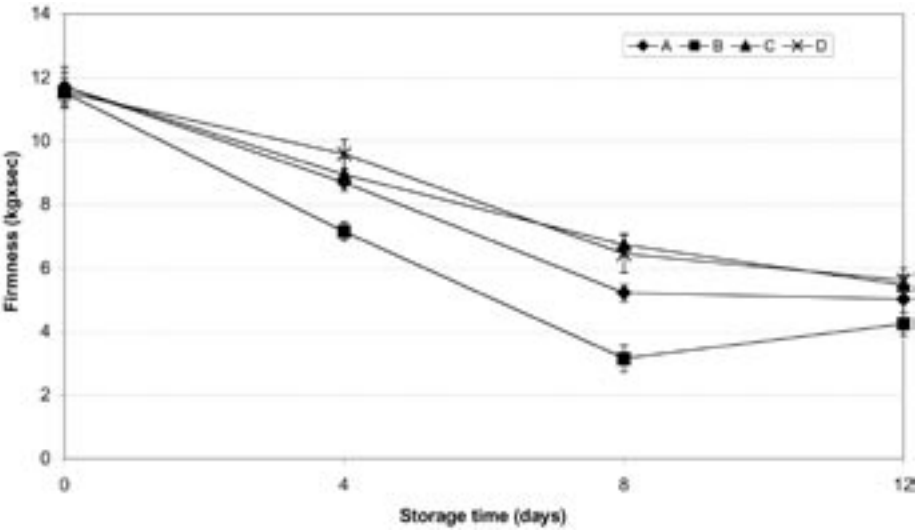


Fig. 3 - Evolution of firmness for minimally processed apple samples during refrigerated storage. Samples: A: not-dipped, packed in air; B: dipped, packed in air; C: not-dipped, packed in MA; D: dipped, packed in MA.

particular sample D packed in modified atmosphere maintained the best colour characteristics until the twelfth day of refrigerated storage. The not-dipped samples (A and C) had lower L* and h° values already at the beginning of the storage period, becoming browner than the others. From the results of tristimulus colorimeter analysis it can be seen that the dipping had an anti-browning effect.

Firmness values (Fig. 3) decreased in all samples, much more in B and A dur-

ing storage. In particular change in texture of dipped apple slices packed in air (B) was rapid and relevant (about three fold less than their initial firmness value) during the first 7 days of storage. In this case the $CaCl_2$ used in dipping solutions did not seem to have any effect on maintaining firmness of apple slices.

The greatest firmness maintenance of samples packed in MA (C and D) could probably be due to the effect of the non conventional modified atmospheres because of their potential effect on slowing down ethylene production by fruits (GOUBLE *et al.*, 1995).

Based on attributes we measured sample D, dipped and packed in MA, has preserved better their initial quality along the considered refrigerated storage period.

The same conditions of dipping in conjunction with MA were chosen to test the effect of three plastic film pouches with different air permeability on the quality of apple slices.

Influence on some chemical-physical characteristics (L*, h°, browning area percentages and firmness), is shown in Table 1.

Samples P2 and P3 packed in medium and low barrier film with alternative gas mixtures modified atmosphere preserved better their original colour, in particular in terms of lightness. These results are confirmed by those obtained from image analysis. In fact, samples P2 and P3 showed the lowest browning level; on the contrary P1 sample, after the eighth day of storage, showed the highest percentage (about more than 80%) of browning area.

Therefore image analysis could be considered as a useful tool for defining or following the quality characteristics in terms of colour and metabolic activities of this kind of product.

No difference occurred in hue angle values of apple slices among samples after 8 days of storage.

The firmness loss of P3 sample was less than that of P1 and P2 samples. P1 sample underwent the highest loss in firmness.

Results of sensory evaluation of colour (Table 2) showed that sample P3 preserved better fresh colour as well as characteristic apple odour and flavour. Sensory colour results are in agreement with image analysis data.

Dipping treatment did not influence saltiness and sourness of all samples. P3 sample presented highest judgment texture attributes and did not show significant differences compared with fresh apple sample (F).

Contrary to P1 and P2 samples, apple slices packed in low barrier film (P3)

Table 1 - Colour data, expressed as L* (lightness), hue angle and browning index, and texture values of the fresh unprocessed (F) apple sample and of the P1, P2 and P3 apple samples after 8 days of refrigerated storage at 4°C.

Samples	L*	Hue angle (h°)	Browning index (%)	Firmness (kgxsec)
F	43.59±0.32	90.65±0.59	3.44	11.91±0.46
P1	40.77±0.87	90.20±1.23	84.24	3.22±0.73
P2	41.90±0.42	90.06±1.04	12.71	5.93±0.74
P3	42.38±0.70	90.03±0.55	38.56	7.45±0.28

Mean values ± standard deviation.

Table 2 - Mean values of the sensory parameters and least significant differences of means (LSD) relative to the untreated apple sample (F) and to the P1, P2 and P3 apple samples after 8 days of refrigerated storage at 4°C. (Scale: 1 = worst to 9 = best).

	Samples			
Parameters	F	P1	P2	P3
Colour	6.13 a*	2.25 c	2.44 c	5.00 b
Visual defects	3.25 b	1.81 c	1.44 c	4.13 a
Apple odour	5.69 b	3.94 c	3.50 c	6.88 a
Apple flavour	6.50 b	4.56 c	3.19 d	7.38 a
Sweetness	6.00 a	4.81 b	4.19 b	6.56 a
Sourness	2.56 a	3.19 a	3.44 a	2.81 a
Saltiness	1.56 ab	1.94 a	1.88 a	1.06 b
Fermented	1.63 b	3.81 a	3.38 a	2.06 b
Firmness	6.56 a	4.38 b	4.69 b	6.00 a
Juiciness	6.81 a	5.25 b	5.25 b	6.31 a
Flourness	2.75 d	6.25 a	5.00 b	3.94 c
Overall defects	1.56 b	3.25 a	3.56 a	1.25 b
Overall judgment	6.56 a	3.44 b	3.31 b	6.25 a

* Means followed by the same letter are not significantly different at P≤0.05.

did not present significant differences compared with fresh samples, in terms of overall judgment attribute.

In conclusion it is possible to assess that the kind of dipping and MA adopted with a packaging in a low barrier film appeared to preserve better the initial quality characteristics of apple slices.

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QUALITY EVALUATION OF PASTRIES WITH AN ALMOND PASTE BASE

VALUTAZIONE DELLA SHELF-LIFE
DI DOLCI A BASE DI PASTA DI MANDORLE

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ABSTRACT

Almond paste has a brief shelf-life because of oxidation and hardening. The use of polymeric films having barrier properties to oxygen and moisture in combination with a low oxygen concentration in the headspace may extend shelf-life. Two flexible films, offering high barrier against oxygen and intermediate barrier against moisture, were tested. Products were packed under air, or nitrogen or in the presence of oxygen scavengers. Samples were stored at 37°C. Results showed that almond paste shelf-life was affected by the loss of moisture and the consequent hardening in the samples packed under nitrogen or in the presence of oxygen scavengers.

RIASSUNTO

I prodotti dolciari a base di pasta di mandorle hanno una breve vita commerciale a causa dell'ossidazione e dell'indurimento. L'uso di film polimerici aventi effetto barriera nei confronti dell'ossigeno e dell'umidità, in combinazione con una

- Key words: Almond paste, EVOH, flexible film, hardening, nylon, oxidation -

ridotta concentrazione di ossigeno nello spazio di testa ne possono prolungare la shelf-life.

Nel corso di questa ricerca sono stati testati due film ad alta barriera nei confronti dell'ossigeno e barriera intermedia rispetto al vapore acqueo.

I prodotti furono confezionati in aria o sotto azoto o in presenza di assorbitori di ossigeno e conservati a 37°C. I risultati mostrarono che la shelf-life della pasta di mandorle fu negativamente influenzata dalla perdita di umidità e dal conseguente indurimento che si verificò nei campioni confezionati in presenza di azoto o degli assorbitori di ossigeno.

INTRODUCTION

Almond paste, also known as "marzipan", is a mixture of ground almonds, sugar and glucose. Pastries with an almond paste base are typical products of the South of Italy. Usually stored at room temperature and sold directly from pastry cook to consumer without hermetically sealed packaging, they are characterized by a shelf-life of few days or few weeks because of oxidation of the fat fraction and product hardening.

To commercialize these products on markets far from the traditional manufacture places, it is necessary to prolong their shelf-life by decreasing the rate of oxidative degradation and hardening.

Since oxidation and hardening are induced by headspace oxygen and moisture that permeates through the packaging, respectively, the use of polymeric films having high barrier properties to low molecular weight compounds and the removal of oxygen in contact with the product may extend almond paste shelf-life.

The purposes of this research was to increase the shelf-life of almond paste pastries by packed them into two different films, containing nylon or ethylene vinyl alcohol layer as a barrier against oxygen and water vapour, under different conditions: air, nitrogen or presence of oxygen scavengers.

MATERIALS AND METHODS

Almond paste was produced according to the following recipe (quantities expressed for 1000 g of product): 500 g of almonds, 400 g of sugar, 100 g of whole eggs and aromas (cloves, vanilla). The obtained almond paste was worked in form of pastries successively cooked at 150°C for 8min in a professional oven.

Pastries were then put into aluminium vessels and hermetically packed. During this research, two multilayer flexible films were tested: the first containing nylon (PP/Nylon6,6) and the second containing ethylene vinyl alcohol (PE/EVOH//PE//PET). Both films have a high barrier property against oxygen and an intermediate barrier property against water vapour. The permeability coefficients of EVOH to oxygen and water vapour at 30°C were $1 \cdot 10^{-10} cm^3(STP) \cdot cm \cdot cm^{-2} \cdot s^{-1} \cdot atm^{-1}$ and $9 \cdot 10^{-7} cm^3(STP) \cdot cm \cdot cm^{-2} \cdot s^{-1} \cdot atm^{-1}$, respectively. The same coefficients, measured on nylon film, were $4 \cdot 1 \cdot^{-9} cm^3(STP) \cdot cm \cdot cm^{-2} \cdot s^{-1} \cdot atm^{-1}$ and $6 \cdot 10^{-7} cm^3(STP) \cdot cm \cdot cm^{-2} \cdot s^{-1} \cdot atm^{-1}$.

Products were packed alternatively under nitrogen or in the presence of oxygen scavengers. A part of them was kept as controls and packed under air.

Packets were stored at 37°C, in order to simulate a quick aging, withdrawn and analysed every 15 days up to 2.5 months in order to study the trend in oxidation of the fat fraction and the changes in firmness of the whole product.

The extracted oil was submitted to the following analyses:

- peroxide value (P.V.), expressed as milliequivalents (mEq) of active oxygen per kilogram of oil, in order to evaluate the primary oxidation (EEC Reg. 2568/91);
- p-anisidine value (p-A.V.), in order to evaluate the secondary oxidation (AOCS Official Method, 1993);
- spectrophotometric indexes (K_{232}, K_{270} and ΔK), in order to evaluate the formation of double and triple conjugate bounds (EEC Reg. 2568/91).

All these analyses were performed three times at least.

Pastries were also submitted to the following analyses:

- moisture % (AOAC method, 1994);
- firmness; compression force was taken as the maximum peak of the recorded force expressed as Newtons (N) (Maltini *et al.*, 1993) and represented the sample hardness. For each treatment, ten determinations were performed, at least.

RESULTS AND DISCUSSION

Under the operating conditions applied in this work, oxidative degradation did not affect the shelf-life of pastries with an almond paste base thanks to the presence in the almond oil of natural strong antioxidants such as tocopherols (≈25 mg/100 g of edible seed and ≈40 mg/100 g of almond oil). The primary oxidation level is negligible, considerably lower than 25 mEq O_2/kg oil, which might be taken as the acceptability threshold for nuts (Narasimhan *et al.*, 1986; Özgül Evranuz, 1983) and there are not significant difference between controls packed under air and packets in which air was removed by flushing nitrogen or addition of oxygen scavengers. Furthermore, peroxide values, after a decrease in the first step of the storage, remained rather constant. A different behaviour is shown by p-anisidine value. After an initial strong decrease (values at 1 month of storage were half of those measured on the just prepared pastries), p-A.V. showed constant intermediate values with a higher oxidation of samples packed under air. This trend, apparently not in agreement with the expected behaviour (an increase during storage), is due to the initial great content in flavour compounds developed during cooking; most of them are carbonylic compounds which react with p-anisidine in the same way of secondary oxidation products. The successive p-A.V. decrease was due to the loss of flavour compounds going in equilibrium in the packet headspace. The changes in K_{232} values during aging were not significant and no significant difference was put in evidence among samples. K_{270} and ΔK showed similar trends, a decrease within the first 15 days and a successive value constancy. At 2 months of storage, a difference between samples packed under air and samples under nitrogen or with oxygen scavengers was put in evidence: the first resulted slightly more oxidized than the others. With regards to all the oxidation indexes (P.V., p-A.V. and spectrophotometric constants), no significant differences were put in evidence among samples packed with the two different films. The true problem for almond paste quality is represented by the hardening consequent to the loss of moisture. Samples packed under air showed the best firmness values for all the considered time whereas a strong hardening after 2 months of storage happened when nitrogen

or oxygen scavengers were used. In the case of packed with oxygen scavengers, it has been supposed that they consumed and absorbed water. With regard to products packed under nitrogen, the vacuum condition realized before nitrogen flashing could have also removed a great amount of water vapour. The combinations type of film-atmosphere composition seemed more important of the same variables singularly considered. Both EVOH and nylon were able to greatly prolong the shelf-life of this kind of products and, as there were not significant differences among the results obtained with the two films, it is advisable to use the cheaper of them (nylon).

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CONTROLLED RELEASE OF ACTIVE COMPOUNDS FROM ANTIMICROBIAL FILMS INTENDED FOR FOOD PACKAGING APPLICATIONS

RILASCIO CONTROLLATO DI COMPOSTI ATTIVI
DA FILM ANTIMICROBICI INTESI PER IMBALLAGGIO ALIMENTARE

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ABSTRACT

Bacterial growth during storage of food is a problem that may be addressed with packaging materials that release antimicrobials during food contact. The controlled release of these active compounds was usually obtained by cross-linked monolayer films but recently, multilayer systems were used to slow down their release kinetics. The aim of this work was to compare monolayer and multilayer films to control the release of two different antimicrobial agents.

RIASSUNTO

La crescita batterica durante la conservazione del prodotto è un problema che può essere fronteggiato con l'uso di imballaggi che rilasciano antimicrobici durante il contatto con l'alimento. Il rilascio controllato di tali sostanze attive viene di solito realizzato con l'uso di film monostrato ma recentemente, sono stati usati sistemi multistrato per rallentare le cinetiche di rilascio dei composti. Scopo di questo lavoro è stato confrontare film monostrato e multistrato per controllare il rilascio di due diversi agenti antimicrobici.

- Key words: controlled release, lysozyme, Sodium benzoate -

INTRODUCTION

In recent years the controlled release systems have become part of a wide category known as "active packaging". This concept has involved many technologies for food preservation, one of them is the direct incorporation of preservative into the film and its consequent release from the film on the food surface to extend the shelf-life of packaged foodstuff, inhibiting the microbial growth and preserving the organoleptic and sensory properties of the packaged food.

In order to obtain controlled release systems, monolayer films were usually used but, recently, packaging researches have leaned more towards multilayer film (control layer /matrix/ barrier layer). In this paper different methods controlling the release of antimicrobial agent from polyvinilalcohol film were compared.

MATERIALS AND METHODS

One consists in a monolayer active crosslinked film; the others, instead, consist in multilayer films in which the two external layers were crosslinked while the internal layer contained the active substance. The studied films differ both for the incorporated antimicrobial agent, lysozyme and sodium benzoate respectively (they differ for their microbial target and for their molecular size: 14,000 Da and 121 g/mol for lysozyme and sodium benzoate, respectively) and for crosslinking degree (crosslinking agent A: 7.7% (w/w) of PVOH and crosslinking agent B: 0.007% (w/w) of PVOH). Samples of films were brought in contact with water and the kinetic release of active compound in the surrounding water was monitored by means of HPLC, until the equilibrium value was reached.

The investigated films are listed in following in the table:

Film	Monolayer with active compound	Multilayer Sheet 1	Multilayer Sheet 2 with active compound	Multilayer Sheet 3
1:lysozyme	crosslkA			
2:lysozyme		crosslkA	no crosslk	crosslkA
3:lysozyme		crosslkA	crosslkA	crosslkA
4:sodium benzoate	crosslkA			
5:sodium benzoate		crosslkA	no crosslk	crosslkA
6:sodium benzoate		crosslkA	crosslkA	crosslkA
7:sodium benzoate		crosslkB	no crosslk	crosslkB

RESULTS AND CONCLUSIONS

The results obtained comparing the release kinetics of the different films investigated show that the rate at which the active compound is released is affected by the crosslinking degree of the polymeric matrix, together with its molecular size.

Fig. 1 and 2 for sodium benzoate and lysozyme, respectively, show the behaviour of the kinetics release for different types of polymeric film.

It was proved that in some cases it is necessary to use multilayer crosslinked films by means of which it is possible to slow down the rate at which the antimi-

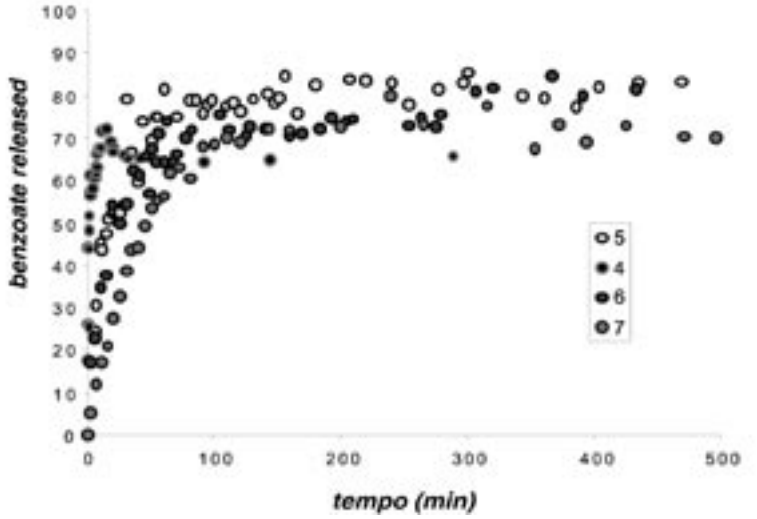


Fig. 1 - Kinetics release of sodium benzoate.

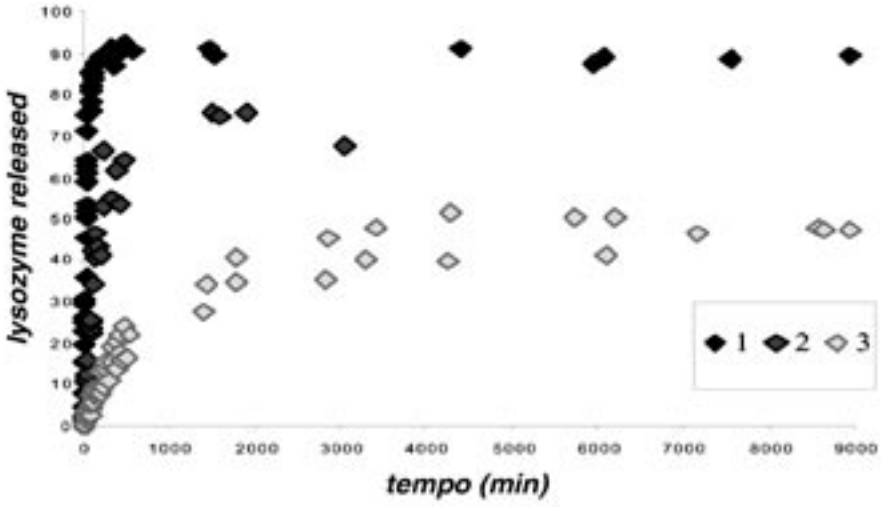


Fig. 2 - Kinetics release of lysozyme.

crobial agent is released. This result was obtained with both lysozyme and sodium benzoate. But, the most interesting and useful application of this technique is in the case of small particles, such as sodium benzoate. In this case, in fact, it's difficult to control the release kinetics when a monolayer crosslinked film is used.

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BIOLOGICAL OXYGEN SCAVENGERS FOR THE MAINTENANCE OF BRIEF MATURATION DAIRY PRODUCTS

ASSORBITORI DI OSSIGENO BIOLOGICI PER LA CONSERVAZIONE DI PRODOTTI LATTIERO-CASEARI A BREVE MATURAZIONE

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ABSTRACT

In the food sector oxygen gas on the one hand is essential for a better preservation of some products (i.e., red meats) or for the realization of some processes of transformation (i.e., multiplication of yeast), on the other it is implicated in various events, both biological and chemical, alterating the product and producing undesirable sensorial modifications. To this aim it is enough to think about fat substances that grow rancid and the enzymatic browning of vegetables, the loss of color resulting from the oxidation of natural pigments, the nutritional damage caused by the oxidation of vitamins and, finally, the ability of oxygen to promote superficial development of mould in some products (i.e., bakery and dairy products).

Therefore, generally oxygen is considered a serious danger for the hygienic, nutritional and organoleptic quality of foods, so there are several different packaging technologies to reduce the undesirable effects of this gas. The most recent and innovative packaging solutions are based on the use of oxygen scavengers able to absorb both the oxygen in the package head space and that permeating from the external environment through the wrapping.

Recent studies have been carried out with the purpose of setting up and producing films acting as oxygen scavengers, whose active elements are microorganisms. The satisfactory results obtained by these researches have led to the planning of the current study, with the aim of evaluating the effectiveness of biological oxygen scavengers in the maintenance of brief maturation dairy products. In particular, yeasts and bacteria, normally present in food, were used in combination with polymeric matrixes characterized by the following: edible, biodegradable, easily available in nature, cheap and environmental friendly. In this way an attempt is made to answer the more and more urgent demand of the consumer for fresh and slightly processed foods, with a high level of service and quality.

RIASSUNTO

Nel settore alimentare l'ossigeno gassoso se da un lato risulta indispensabile per la migliore conservazione di certi prodotti (per esempio le carni rosse), o per la realizzazione di alcuni processi di trasformazione (per esempio la moltiplicazione dei lieviti), dall'altro è implicato in numerosi eventi, sia biologici che chimici, di alterazione e d'indesiderate modificazioni sensoriali. A tal proposito basti pensare ai fenomeni d'irrancidimento della sostanza grassa e d'imbrunimento enzimatico dei vegetali, alla perdita di colore derivante dall'ossidazione di pigmenti naturali, al danno nutrizionale causato dall'ossidazione di vitamine ed infine alla capacità dell'ossigeno di promuovere in alcuni prodotti (per esempio i prodotti da forno e quelli lattiero-caseari) lo sviluppo superficiale di muffe. Quindi nella maggior parte dei casi l'ossigeno è considerato un serio pericolo per la qualità igienica, nutrizionale ed organolettica degli alimenti, per cui molte e diverse sono le tecnologie tradizionali di packaging (il riempimento a caldo, il sottovuoto, l'atmosfera modificata) messe a punto per ridurre gli effetti indesiderati di questo gas.

Le soluzioni di packaging più recenti ed innovative sono basate sull'impiego di assorbitori di ossigeno (oxygen-scavengers) in grado di sequestrare sia l'ossigeno presente nello spazio di testa della confezione, sia quello che permea dall'ambiente esterno attraverso l'imballaggio. Gli oxygen-scavengers sono sistemi basati sull'impiego di composti di varia natura: solidi ossidabili (polveri atossiche a base di ferro), sostanze antiossidanti (acido ascorbico, catecolo, fenoli) ed enzimi (glucosio ossidasi, etanolo ossidasi). Recenti studi si sono svolti con lo scopo di mettere a punto e produrre film con capacità di oxygen-scavengers, i cui elementi attivi sono microrganismi. In particolare, tale valutazione è stata effettuata determinando la vitalità e l'attività respiratoria dei microbi immobilizzati nelle matrici polimeriche, scelte per la sperimentazione.

I soddisfacenti risultati ottenuti da questo lavoro hanno condotto alla progettazione del presente studio, che ha come obiettivo la valutazione dell'efficacia di assorbitori di ossigeno biologici nella conservazione di prodotti lattiero-caseari a breve maturazione (tipo Crescenza). Nello specifico si farà ricorso all'impiego di lieviti e batteri, normalmente presenti negli alimenti, in combinazione con matrici polimeriche edibili, biodegradabili e facilmente reperibili in natura, a basso costo e a basso impatto ambientale. In questo modo si tenterà di rispondere alla sempre più stringente domanda da parte del consumatore di alimenti freschi, poco processati, ad elevato contenuto di servizio e di alta qualità.

ANTIMICROBIAL AND ANTIOXIDATIVE PACKAGING MATERIAL INCORPORATING NISIN AND α-TOCOPHEROL TO EXTEND SHELF-LIFE OF PERISHABLE FOODS

MATERIALI DI CONFEZIONAMENTO CHE INCLUDONO NISINA E α-TOCOFEROLO PER ESTENDERE LA SHELF-LIFE DI ALIMENTI DEPERIBILI

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Microbial and oxidative food spoilage usually starts on the food surface which is contaminated by microorganisms and exposed to oxygen. Controlled release of antimicrobial and antioxidative agents from food packaging materials to the food surface has been proposed to protect the food surface from microbial and oxidative spoilage of perishable foods. Nisin and α-tocopherol are from natural resources, have antimicrobial and antioxidative activities, respectively and have been reported to retain their function when incorporated in the packaging materials. However, combined incorporation of nisin and α-tocopherol has not been tried yet for food packaging applications even though there has been beneficial effect with materials individually filled or coated with the additive.

Polymer based solution coating has been suggested to be the most prospective method to attach heat-sensitive active substance on the packaging material surface with stability and adhesion. Therefore combined use of nisin and α-tocopherol in the coating was attempted in this study to give a versatile activity of inhibiting the growth of spoilage and oxidative quality changes on the foods. Nisin and/or α-tocopherol were first dissolved in 20% ethanol solution at 34% concentration, combined with vinyl acetate-ethylene copolymer solution (54.5% solid content) and then coated on the paperboard of 0.23 mm thickness to have 3% concentration based on dry basis after drying. Antimicrobial and antioxidative activities were evaluated for consideration of use situation in food packaging. The migration of nisin and α-tocopherol from the coating to model emulsion solution (66% of water and 32% of paraffin oil with 2% of emulsifier) was measured at 10°C and was related to the suppression of microbial growth and oxidative deterioration in the emulsion

inoculated with *Micrococcus flavus* ATCC 10240. The paperboard coated with nisin and α-tocopherol was finally contacted with pasteurized cream at 10°C to see its effectiveness in preserving the quality of perishable foods. During the storage total aerobic bacterial count and thiobarbituric acid value were measured as indexes for microbial spoilage and oxidative quality changes of the cream.

During the storage period of 12 days, 8.6-9.3% of nisin and 5.6-5.7% of α-tocopherol were migrated based on the incorporated amount, and the migration of each was not affected by the presence of the other.

The paper coated with nisin effectively inhibited the gram positive bacterium, *M. flavus* and the paper with α-tocopherol showed antioxidative activity, measured in thiobarbituric acid reactive substances, compared to the control paperboard coated without any additives in the binder medium. Combined inclusion of nisin and α-tocopherol in the coating gave both antimicrobial and antioxidative activities in the emulsion solution. It could also improve the microbial stability and inhibit lipid oxidation, when used for packaging a milk cream stored at 10°C. However, combined incorporation of nisin and α-tocopherol in the coating did not give any further synergistic effect in the antimicrobial and antioxidative action compared to that only with single additive. The paper with nisin and α-tocopherol showed a potential for preserving the microbial and chemical quality of perishable foods and thus extending their shelf-life.

PERFORMANCE COMPARISON OF PVC AND PE CLING FILM BY MEANS OF SHELF-LIFE EVALUATION TESTS

TEST DI SHELF LIFE COME MEZZO PER LA VALUTAZIONE DELLE PERFORMANCE DI FILM ESTENSIBILI IN PE E PVC PLASTIFICATO

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ABSTRACT

The aim of this study was to compare the performances of samples of plasticized PVC cling film (characterized by different composition) with samples of PE film by means of shelf-life evaluation test. Foodstuff used for the experimental tests included cheese, red meat, bananas and ready to eat chicory endives. During storage time chemical, physical and sensory analyses were conducted in order to evaluate the quality decay of the products. No statistically significant difference between samples packed in PVC and PE film could be found according to pH results and gas composition (% CO_2 and O_2) of the packages head space. From sensory evaluation results always emerge a clearly preference for food samples overwrapped with PVC cling film, moreover panelists always noticed water vapour presence inside PE packages due to the low WVTR that characterizes the PE film.

RIASSUNTO

Scopo del presente lavoro è stato quello di confrontare le performance di due tipologie di film estensibili, in PE e in PVC plastificato, mediante l'impiego di test di shelf life. Campioni di diverse classi di alimenti sono stati avvolti nelle due tipologie di film, e durante il tempo di conservazione, attraverso test sensoriali ed analisi chimico/fisiche, ne è stato monitorato il decadimento qualilitativo. Nessuna differenza significativa è stata evidenziata attraverso i risultati delle analisi chimiche e fisiche, mentre dalle analisi sensoriali è emersa una netta differenza a favore dei campioni confezionati nei film in PVC plastificato, soprattutto grazie all'assenza, internamente alle confezioni, di condensa e alla migliore presentazione del prodotto

- Key words: functional properties, migration of plasticizers, PE film, PVC cling film, shelf life test,

confezionato dovuta alle specifiche proprietà meccaniche che caratterizzano i film in PVC.

INTRODUCTION

PVC cling film is one of the most popular materials in food packaging applications especially in its plasticized form. The success of this polymer is due to its technical advantages related to the type and amount of additives incorporated during the manufacturing process. DEHA, di-(2-ethylexhyl) adipate, in particular it is used to impart specific mechanical characteristics and to give the exclusive property of "clingness". The use of PVC film containing this additive is widespread in retail shops and stores in Italy as well as in many other countries. For several years it was well known that the use of these cling films in contact with fatty food could lead to a substantial migration of DEHA from the polymer surface into the overwrapped product (1,3). To reduce this phenomenon plastic and packaging industries have proposed several approaches: firstly the use of alternative high grease resistance monomeric plasticizers with the consequent DEHA level reduction; secondly a partial replacement of DEHA with polymeric plasticizers (with low migration characteristics due to their high molecular weight). Another alternative that some distribution companies are considering is to replace PVC cling film with PE ones. Flexibility and general handling properties of PE film are exclusively obtained incorporating high molecular weight compounds, (typically epoxidized soybean oil), in the polymer matrix thus lowering the migration into food (2).

MATERIALS AND METHODS

Materials

Film characteristics are described in Table 1, fresh foodstuffs (bananas, "ready-to-eat" chicory endives, Emmenthal cheese and red meat) were purchased from national stores chain (GS) and stored at refrigerated temperature (5±1°C). Fruit and vegetables were placed in PP transparent trays while cheese and meat in PS ones.

Table 1 - Directions for use and main film characteristic.

Polymer Type	Directions for use as declared on label	Plasticizers Monomeric type only	
		DEHA	ATBC
PVC 1	For all categories of food	46.5± 2.3	9.5 ±0.5
PVC 2	For vegetable use only	91.5±10.8	11.50±0.68
PVC A	For vegetable use only	55.6± 8.4	57.6 ±5.8
PE 1	For all categories of food	n.r	n.r.
PE 2	For all categories of food	n.r	n.r.

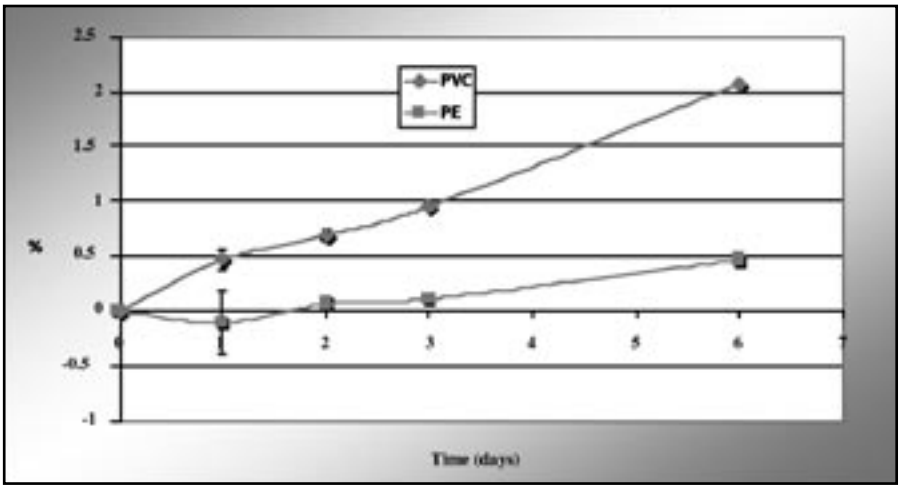


Fig. 1 - % loss of weight in red meat.

Methods

pH determinations were conducted using a pH Meter GLP 22 Crison provided with contact and immersion electrodes. The % loss of weight of packed foodstuff was monitored during storage time. Samples were weighted by means of a technical lab balance.

Turbidity (index of tissue damage) was performed for "ready-to-eat" chicory endive: 10 g of food sample were immersed for 5 min in 100 mL of distilled water and after filtration the liquid extract was analysed with a spectrophotometer (Lambda 15 UV/VIS – Perkin Elmer) focused at 660 nm wavelength.

Head space gas composition (O_2, CO_2, C_2H_4) was followed during storage time. The internal atmosphere (O_2 and CO_2) was determined with a Hewlett Packard 5890 (Palo Alto Ca USA) GC (TCD detector) while ethylene concentration was determined with a DANI 3800 GC coupled to a FID (air/H_2) detector. Sensory analyses were carried out by a trained panel using descriptive analysis with scaling. Samples were scored for specific attributes (general appearance, odour, colour, firmness, microbiological spoilage, condensed water vapour) using an interval scale with anchor points (1-little, 9-very) at each end. Data obtained from the sensory evaluation were submitted to analysis of variance.

RESULTS AND DISCUSSION

There were significant differences concerning the % loss of weight (Fig. 1); for all the categories of food tested PVC packages show higher values than PE ones due to the lower WVTR characterizing the last type of polymer. A difference between the film samples was recorded concerning the presence of ethylene in the head space of banana packages. In PE ones in particular (Fig. 2), there was a high concentration of this substance.

It is necessary to underline that it would be therefore preferable to avoid this situation in fact this compound is responsible for the ripening mechanism of climateric fruits. Concerning turbidity and % of CO_2 and O_2 no statistical difference between the films tested were found. At last from the sensory evaluation emerged that PVC samples always had better scores than PE ones, in particular in the case of "general appearance" and condensed water vapour attributes the differences were statistically significant (Fig. 3).

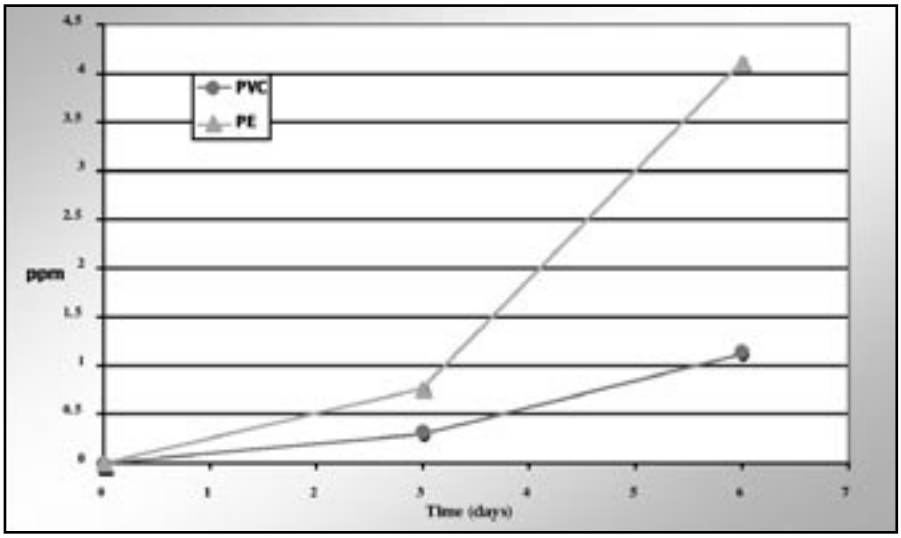


Fig. 2 - Ethylene determination in bananas.

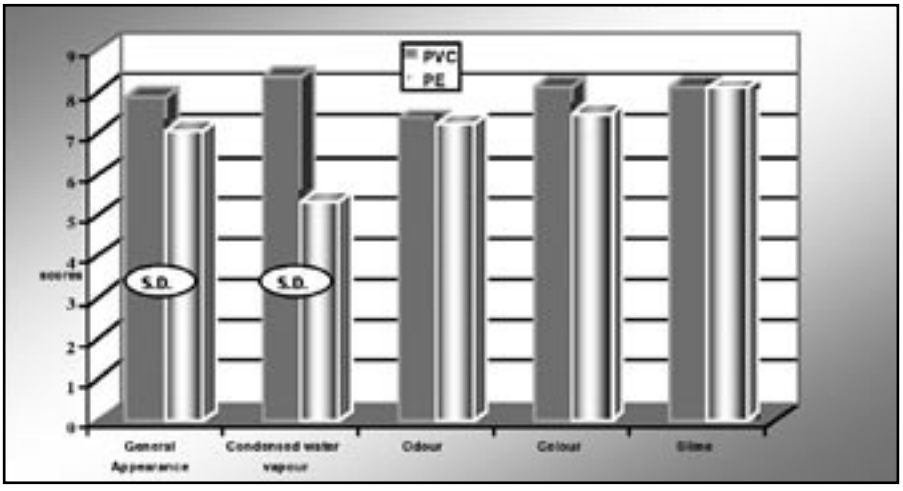


Fig. 3 - Chicory endives sensory evolution (3 days).

CONCLUSION

Both PVC and PE films show CO_2 and O_2 permeabilities suitable to preserve quality of fresh foodstuff: such diffusional properties are fit for respiring products (anaerobic conditions were not reached in fruit and vegetable packages during the whole storage time); high O_2 transmission rate, in particular, is necessary to maintain bright red colour of fresh meat.

PE films present a lower water vapour transmission rate than PVC ones. There is a low loss of weight of PE samples and a constant presence of condensed water vapour inside PE packages: this aspect causes an undesirable and unesthetic product appearance and in some cases interferes with the panelist's ability to distinguish details of food colour and texture. The presence of water on food surface could also represent a problem from the hygienic point of view.

The sensory evaluation always gives a preference to samples overwrapped in PVC film: this is related to the specific ability of this polymer to adhere to trays and food surfaces, this "cling effect" is exclusive for this material and it is due to the presence of monomeric plasticizers in its formulation.

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PERFORMANCE EVALUATION OF ACTIVE EPS TRAY FOR FRESH BASS FILLET

VALUTAZIONE DELL'EFFICACIA DI UNA VASSOIO ATTIVO
DI POLISTIRENE ESPANSO ED ESTRUSO
NEL RIMUOVERE GLI OFF-FLAVOUR DI PRODOTTI ITTICI FRESCHI

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ABSTRACT

The purpose of the research work was the evaluation of the effectiveness of a potential off-flavour scavenger system, combined into the foam of an expanded polystyrene food tray.

A large amount of odours could accumulate in the headspace of packages during the shelf life of fresh fish.

Therefore the smelling process causes the food product to be refused even if the microbial spoilage is acceptable. The active tray could be useful to improve the sensorial acceptance of the consumer.

RIASSUNTO

La rimozione di composti volatili maleodoranti dallo spazio di testa delle confezioni, può rappresentare una efficace applicazione di *active packaging* per migliorare l'accettabilità sensoriale del prodotto da parte del consumatore.

Nello spazio di testa delle confezioni possono concentrarsi sostanze aromati-

- Key words: active packaging, off-odours scavenger, polystyrene, trimethylamine -

che sgradevoli in modo da determinare il rifiuto immediato del prodotto, sebbene tale concentrazione sia risultato di un processo di accumulo e non sia correlata al decadimento qualitativo. Se il prodotto alimentare per sua natura va incontro ad una degradazione che comporta la produzione di composti maleodoranti, una certa concentrazione di sostanze volatili potrebbe portare al rifiuto di un alimento ancora perfettamente edibile dal punto di vista nutrizionale e igienico. Questo fenomeno si accentua ancor più quando si effettuano confezionamenti che prevedono l'ermeticità delle confezioni e l'impiego di materiali barriera; è il caso per esempio del condizionamento in atmosfera protettiva di prodotti alimentari "freschi".

Lo scopo di questo lavoro è stato pertanto quello di verificare l'efficacia di vaschette di polistirene (espanso ed estruso), dotate di funzioni di assorbimento di liquidi e di ammine, nel rimuovere gli off-flavours che si accumulano nella confezione.

INTRODUCTION

Many active packaging solutions have been developed in order to scavenger metabolites, gas, aldheydes, amines, coming from microorganism activities during the shelf life of packaged foods (1-3). Sometimes the accumulation process of these unpleasant compounds do not find the consumer favour. Different scavenging agents have been included in polymeric matrix of food packaging material in order to reduce the undesiderable effect (4).

The experimental work was performed to evaluate the effectiveness of an inorganic absorber to reduce the amount of off odours during the shelf life of fresh fish; in particular the TMA (trymethylamine) amount in the headspace was monitored.

MATERIALS AND METHODS

100 g of fresh fish (*Dicentrarchus labrax*) were packaged in the 3 different solutions: closed-cell EPS tray, open-cell EPS tray, open-cell ACTIVE EPS tray with the adsorbing agent. They were closed with plasticized PVC stretch film, and stored at temperature of 5°C for 1,3, 7,10 days.

40 µL of the package headspace were taken by a gas-tight syringe and analysed by MS-GC Perkin Elmer Instrument. The analysis conditions are showed in Table 1.

Table 1 - Conditions and parameters of MS-GC analyses.

Instrumental analysis (MS-GC) Instrument: Perkin Elmer mod. Autosystem XL (equipped with Perkin Elmer quadrupole Turbomass)	
Column	HP1, 50 m x 0,2 mm x 0,5 µm film thickness
Gas carrier	He 0,3 mL/min
Column temperature	40°C x 10 min
Injector temperature	220°C
Volume injected	50 µL

Table 2 - Sensorial test.

Sensorial analysis Discriminant Qualitative Triangular Test	
Panel	10
Confidence level	95% (α = 0,05)

At the same time a Triangular test was performed in the Sirap R&D laboratories in order to evaluate the consumer acceptance of the different packaging solutions.

The results obtained are showed in Table 2.

RESULTS AND DISCUSSION

Both the sensorial and instrumental results demonstrated that the open-cell structure expressed a higher capacity to remove off-flavours during the examined shelf-life, compared to the closed-cell one (Fig. 1, 2). Among open-cell trays, the "Active" solution proved to be the more performing.

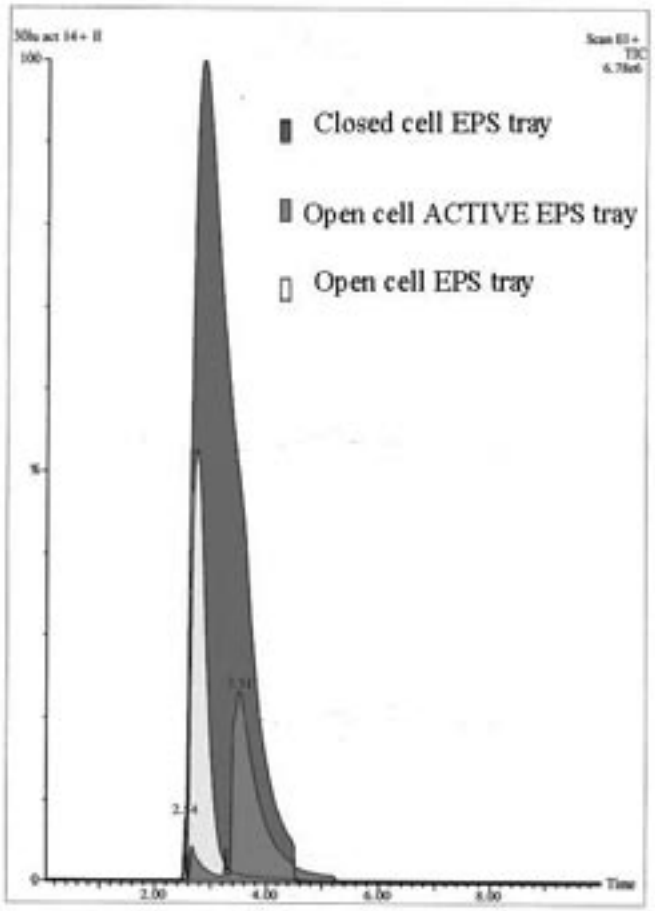


Fig. 1 - Chromatogram response of MS-GC analyse: the coloured areas correspond to the residual amount of TMA found in the headspace of the different trays.

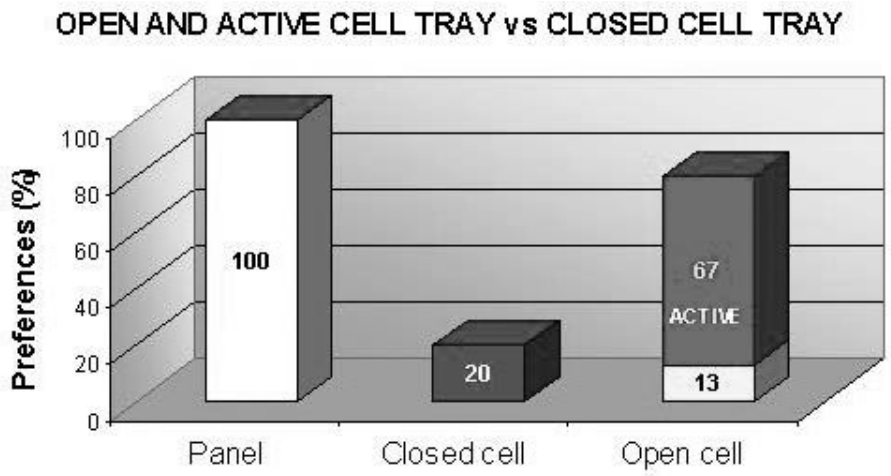


Fig. 2 - Panel evaluation results.

The positive results obtained in testing the adsorbing efficacy of the new prototype Active Tray indicate that there is the possibility to exploit this property to improve the smelling profile in the packaging and storage of fresh fish products.

Future developments will be focused on the way to increase the adsorbing capacity by operating on:

- dimension and dispersion of the adsorbing agent into the polystyrene foam;
- different types of packaging.

The application will be tested on other food categories.

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Session III

SHELF-LIFE TESTING

Main lecture: Prof. H. Hofstra
TNO Netherlands - AJ Zeist - The Netherlands

AN OVERVIEW OF METHODS AND PROCEDURES FOR SHELF-LIFE TESTING

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ABSTRACT

For assessing the shelf life of food products different options are available. They include among others the use of predictive models, storage tests, and microbial challenge testing.

Although predictive modelling has been progressed considerably during the last decade, it must be recognized that the predictions provided by the models are not exact, especially in relation to infective pathogens. In particular, growth rate, minimum growth temperature, and heat sensitivity may vary within a single species. This means that for greater precision, product storage tests and microbial challenge testing will be necessary.

Storage testing is an established means of simulating the changes that can occur in a food product after processing. It can be used to determine the microbiological consequences of processing, distribution, and subsequent handling by the consumer. Usually a storage test is only carried out in finished products and in those cases where microorganisms of concern are present in sufficient numbers. The test can provide information on the effects of both intrinsic and extrinsic factors on the microbial safety and storage life of a particular product.

Microbiological challenge testing is another established technique within the food industry and aims to simulate what can happen to a product during processing, distribution, and subsequent handling, following inoculation with one or more relevant organisms. As with storage testing, the inoculated product is processed in an appropriate manner and than subjected to a range of controlled conditions that relate to subsequent handling, storage, and preparation by the consumer. A user's guide to microbial challenge testing for ensuring safety of food products has been developed by Notermans *et al.* (1993).

Both for storage tests and microbiological challenge testing, only well established testing procedures should be used, with an emphasis on accuracy and reproducibility. For this purpose new, non invasive techniques have recently bee developed. These tests are generally based on the fact that growing bacteria produce enzymes that can breakdown substrates as a result of metabolic activity. For example, one non-ionized glucose molecule can be transformed into two molecules of the ionized metabolite lactic acid by enzymatic activity. Breakdown of a highly ionized protein will contribute to the production of may ionized metabolites

and their motility. The increased concentration of ionized products and increased motility of them can be measured as a change in electric conductivity. Electrical conductivity can increase significantly if also hydrogen ions are produced, since they are nearly seven times more effective as conductor than sodium ions. However, not all microorganisms produce metabolites that can change conductivity (Firstenberg-Eden and Eden, 1985). Conductivity can be measured in several ways. One of them makes use of external electrodes put on high-density polyethylene bottles. Non-invasive (or non-destructive) methods can successfully be used to test sterility without opening the package. This allows an increased of package units that can be tested, thus increasing the confidence in processing and resulting in safer products. This especially applies to commercial sterile aseptically packaged UHT milk. This milk can be spoiled by germination of spores surviving the heat treatment or by microorganisms that contaminate the product by post-process contamination. Experiments carried out by TNO Nutrition and Food Research demonstrated that one single *Escherichia coli* cell present in one liter of milk was detectable after 23 hours of incubation at 30°C (de Nijs *et al.*, in press).

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LECTURE

SHELF-LIFE MONITORING AND MODELLING BY E-NOSE AND IMAGE-ANALYSIS

MONITORAGGIO E MODELLAZIONE DELLA SHELF-LIFE ATTRAVERSO NASO ELETTRONICO E ANALISI DELL'IMMAGINE

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ABSTRACT

When the safety and nutritive value of a food has to be assured, sensory parameters are the best predictors of shelf-life. Among these, visual appearance and aroma are very important since they condition the choice and the consumption of food, thus involving pre-judgment or flavour anticipation. Sensory parameters are usually evaluated by means of complex and time-consuming methods (i.e. panel test), that are practically inapplicable in the shelf-life testing and monitoring studies. In the present review, the application of image analysis and electronic nose for the evaluation of shelf-life in selected type of food is presented. Results on cicorino (a minimally processed vegetable) showed a good correlation between classical microbiological parameters and both colour change measured by image digitalisation and head space analysis performed by an e-nose. In the case of cheese (e.g. PDO Taleggio was considered) image parameters (hue and hole growth) and selected e-nose sensor responses are well correlated with proteolysis indices, usually used to monitor the ripening process. In two studies, kinetic parameterisation from image analysis and e-nose data, showed the possibility to define a "stability time" and then to predict the shelf-life of the product, at different storage temperatures.

- Key words: e-nose, image analysis, modelling, MPV vegetables, PDO cheese, shelf-life -

RIASSUNTO

Viene usualmente definita shelf-life di un alimento il tempo limite entro il quale il progredire degli eventi degradativi determini modificazioni impercettibili sul piano sensoriale o comunque ancora accettabili sul piano della sicurezza d'uso. Fra le trasformazioni che procedono durante la conservazione, quelle che determinano modificazioni di aspetto (colore, forma, struttura) ed aroma sono particolarmente importanti: esse coinvolgono, all'atto della scelta o del consumo, un pregiudizio sensoriale (anticipazione del flavour). Al riguardo, image-analysis e valutazione del profilo olfattivo mediante naso elettronico sono tecniche analitiche promettenti: esse consentono la misurazione oggettiva di parametri correlabili alla percezione sensoriale in forma rapida, sovente non invasiva ed addirittura in-line. In questa rassegna viene presentata l'applicazione coordinata di queste due tecniche per la valutazione della shelf-life di due prodotti-tipo. I risultati ottenuti su un ortaggio fresco confezionato (il cicorino) evidenziano una buona correlazione fra i classici criteri micobiologici di definizione della shelf-life e la valutazione del colore (effettuata per digitalizzazione) nonché del profilo aromatico, caratterizzato mediante un e-nose. Nel caso di un formaggio (il Taleggio), i descrittori di aspetto (sviluppo di occhiature e modificazioni di tinta) insieme alla evoluzione del profilo aromatico sono risultati ben correlati alla fenomenologia proteolitica, misurata con tecniche convenzionali. In entrambi i casi la parametrizzazione cinetica degli indici ottenuti con le due innovative tecniche di indagine hanno consentito la definizione di un "tempo di stabilità" e, dunque, la previsione della shelf-life dei prodotti, anche se conservati in differenti regimi di temperatura.

INTRODUCTION

Shelf-life's prevision and control of food represents a problem of great importance for the organization of production and distribution of foodstuffs and also for what concerns the limit of storage and the possible choice of packaging. Furthermore, the shelf-life of a product doesn't correspond to its real life because the decline of certain characteristics (sensorial ones in particular) often agrees to the end of a product's marketability, but not necessarily to the loss of its commercial characteristics or of those of hygiene and security (LABUZA, 1982).

The shelf-life of a product is measurable only if all the causes of deterioration (e.g., for fresh milk, the type and concentration of microbial population) or some effect (e.g., for the same product, the acidity modification) can be quantified and kinetically modelled. It is evident that the measurement of shelf-life requires the development of rapid methods suitable to monitor any degradation of the food product. Very often, simple indices correlated to more complex events are able to describe the kinetics of overall phenomena (RIVA, 1997).

In shelf-life studies, the evolution of the qualitative characteristics must be parameterised by finding the kinetic constant of the process and its kinetic order. Furthermore, these kinetic parameters can be related to other factors, such as temperature, in order to define a suitable shelf-life forecasting (WELLS and SINGH, 1988).

Between the transformations that go on during food storage, those that determine visual modifications (colour, shape, structure) and flavour changes are

particularly important because they can influence consumer choice, thus involving pre-judgment or "flavour anticipation".

The image analysis and the evaluation of the odorous profile by means of an electronic nose are techniques that allow the objective and rapid measurement of parameters that can be easily correlated to the shelf-life of the products.

The objective of image analysis is the quantification of geometric and densitometric characteristics, acquired in a way to represent "significant elements" of the object at macro or microscopic level (DIEZAK, 1988). The evolution of these techniques and their implementation on computer as specialized hardware and software allow the use of rigorous statistic approaches, thus facilitate different kinds of application. In general terms, measures derivable from the application of the image-analysis are of two kinds, those concerning feature specific measurements and those concerning global measurements (RUSS and STEWART, 1988). Reliable software allow to draw, from a single or a series of digital pictures, several indices as descriptors of the visual appearance, that, through the application of multivariate statistical techniques, led to parameters representative of the kinetic of shelf-life, more that to the clustering and/or the classification of products (RIVA and LIVIERO, 1999).

The olfactory evaluation based on the "Electronic Nose" has the same approach of the Image Analysis. The electronic nose system parallels the human olfactory system in the following manner: each chemical sensor represents a group of olfactory receptors and produces a time-dependent electrical signal in response to an odour. Any noise and sensor drift may be reduced using signal preprocessing technique. The final stage in the human olfactory process is the cerebral cortex of the brain, which classifies and memorizes odours: the equivalent process in the electronic nose is the use of pattern recognition software (GARDNER and BARTLETT, 1999). The electronic nose is a useful tool for defining or following the shelf-life, considering that it is able to detect the changes of olfactory fingerprint due to the production of metabolites of the microbial spoilage, of the enzymatic activity or oxidative changes: phenomena like the initial decay in leafy vegetables, the beginning of proteolysis in cheese or in fish, the start of oil oxidation. These changes can be detectable by using an electronic nose in the beginning phase of the phenomena (MANNINO *et al.*, 2000).

At present Image-Analysis and E-nose are techniques that have high costs in terms of investment but they allow time and money to be saved since they are able to produce reliable and fast results when applied to on line quality control or in study of shelf-life where often many replicates and extensive sampling plans are required.

The present review will critically examine two case studies, already published (RIVA *et al.*, 2001a; RIVA *et al.*, 2002), concerning shelf-life prediction based on the simultaneous application of the two techniques: image analysis and electronic nose.

RESULTS AND DISCUSSION

First case: Minimally Processed Vegetables

Minimally processed vegetables (MPV) are fresh raw vegetables sold ready-to-use. They are usually processed by simple operations: peeling, washing, drying, sometimes cutting, and packaging in sealed pouches or in trays wrapped with an extensible cling film. Shelf-life of MPV usually reaches one week, if refrigerated

conditions are maintained during storage (KING and BOLIN, 1989). Microbial spoilage and some physico-chemical and biochemical modifications mainly affecting sensory properties, like appearance and flavour, are the main factors involved in the degradation of MPV. Degradation comes directly from the residual metabolic activity of the plant tissue, like post-harvest respiration and, above all, from the development of saprophytic microorganisms, that are responsible for the enzymatic activities occurring during storage (MARCHETTI *et al.*, 1992). To predict the shelf-life of this type of product is therefore very important to have simple and rapid methods able to quantify these modifications.

Methodology

Current practices for the evaluation of vegetable spoilage are based on total bacterial counts that requires an incubation time of at least 2 days or, for sensory properties, on the use of a sensory panel. These methods, however, are complicated, time consuming and not useful in routine analysis (GALLI and FRANZETTI, 1991). Relevant indicators of the spoilage process can be found among the colour changes and the volatile off-odour compounds produced by bacteria during spoilage.

The non-homogeneity of visual appearance of cut vegetables constitutes an obstacle to application of the classical approach to colour measurement, based on reflectance instruments. On the contrary, image analysis reveals an effective methodology, concurring to measure average chromatic parameters of also non-homogenous surface. By simple programs of photo-enhancement it is possible to measure the colour of digitalised images, expressing results in the usual chromatic coordinates L, a, b. By standardization of working parameters (conditions of lighting system, use of a black background in view to isolate the subject, insertion of a pre-printed chromatic scale in the scene of acquisition of the image), it is also possible to compare realistically the colour of images acquired at various times (PAPADAKIS *et al.*, 2000).

This methodology has shown to be very interesting in the studies of shelf-life of the raw vegetables.

Fig. 1 illustrates the evolution of the hue index (a/b) measured on images of cicorino stored at 5°C.

Images of reference samples kept in controlled storage conditions were obtained by digitalisation using a plane scanner (HP Scanjet 6100C) connected to a PENTIUM-PC platform equipped with Adobe Photoshop software version 5.5. Full colour images (200 pixel per inch resolution) were obtained by positioning on the scanner the upper surface of the packaged products held in a black box, in order to shut out the surrounding light. A reference colour scale was placed near the image in order to standardise the brightness and colour attributes. Typical R, G and B parameters were directly measured from the images using a specific feature of the software. Colour and brightness were then standardised and transformed, with a specific calculus routine, to the Lightness (L*), red (a*) and blue (b*) scale CIE 1976 (FORD and ROBERTS, 1998). Since there is no correspondence (type and geometry of lighting source, etc.) to the standard CIE procedure, the colour co-ordinates (L*, a* and b*) should be considered as relative parameters. In other words, colour indices were used to calculate only the difference between fresh and aged samples and not for measurement of their "absolute" colour. In the case of "cicorino", the "a" parameters (redness) or the ratio between the "a" and "b" parameters (hue) can be assumed as an index of browning that occurs during storage as a consequence of enzymatic reactions.

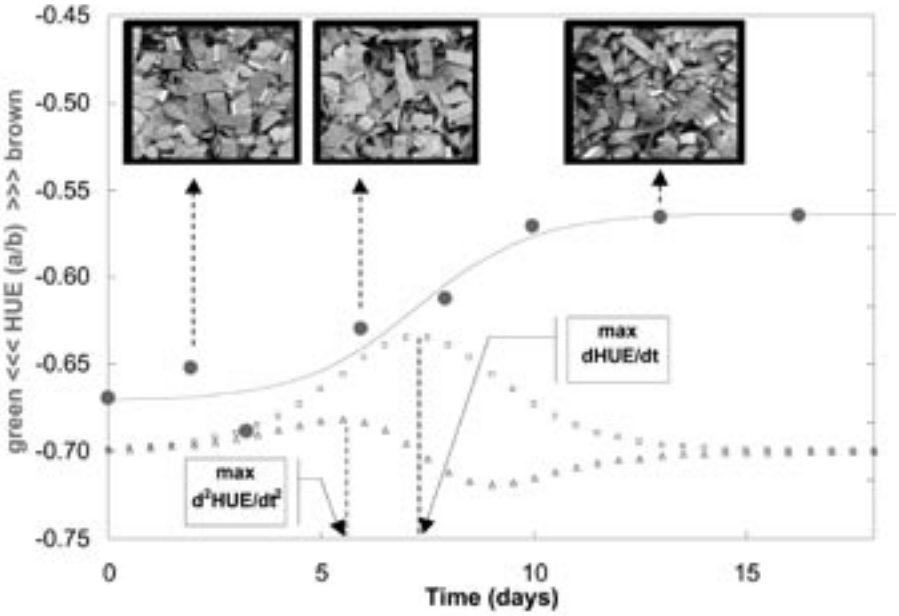


Fig. 1 - Hue kinetics during storage of fresh cut cicorino at 5°C. The dotted lines illustrates the first and second derivative of the interpolating transition function.

In order to determine the sensory quality of the headspace of "cicorino" during its shelf-life, in a parallel study (RIVA *et al.*, 2001a), an electronic nose NST 3220 Lab Emission Analyser from Nordic Sensor Technologies (Linköping, Sweden) was used. It is based on an array of Metal Oxide Semiconductor (MOS) and Metal Oxide Semiconductor Field-Effect Transistor (MOSFET) sensors. The MOS and MOSFET sensors operate at high temperatures and, with respect to other sensors, are less sensitive to moisture with consequent less carry-over from one measurement to another. Therefore, they offer the best ratio of drift and lifetime to sensitivity (SCHALLER *et al.*, 1998). All samples were analysed during their early shelf-life (SF) and at fixed times (S#t) of storage at 5°C. Fresh samples were obtained at the beginning of their commercial life and brought to our laboratory in refrigerated conditions (5±0.5°C). Two lots produced in different weeks (A and B) were also considered. The experimental design was completed by considering samples of ready-to-eat "cicorino" from five different commercial brand names, analysed at the beginning of storage (C0), at the expiration date (5 days after packaging, CE) and at different stages of their shelf-life (C#t).

Results

Fig. 2 shows the hue kinetics obtained during the storage of fresh cicorino at three different temperatures. The hue index shows a transition kinetics and a sigmoid transition function can be used to fit the experimental data (RIVA and PIERGIOVANNI, 2001). A stability time can be established considering the maximum of the first or of the second time-derivative of the relevant transition function. In Fig. 2, these kinetics are integrated with the trend of the total

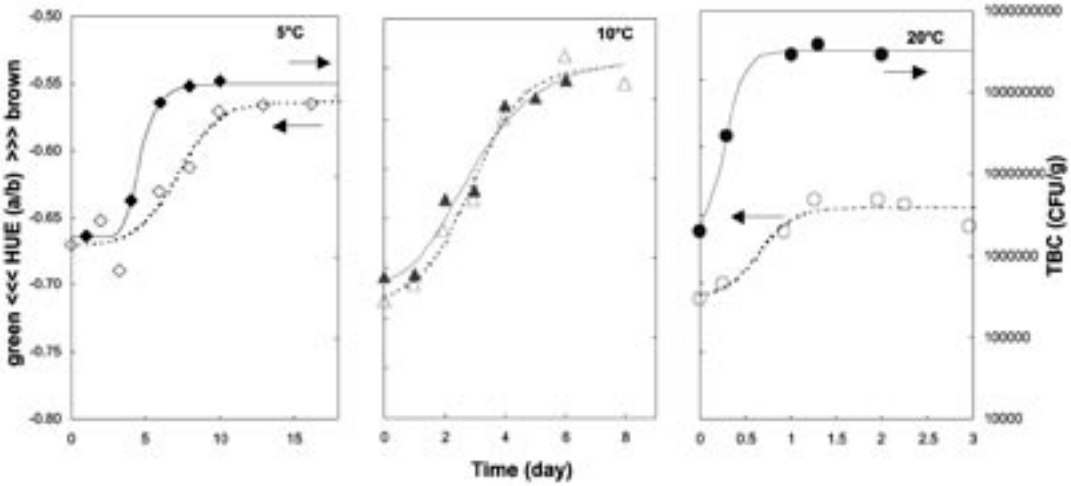


Fig. 2 - Hue kinetics (open symbols) and microbial growth kinetics (closed symbols) during storage of fresh cut cicorino at 5°, 10° and 20°C.

bacterial count modelled by using the Gompertz function (ZWIETERING et al., 1991). This last kinetic model allows the evaluation of a stability range time, namely the one to attain a TBC value of $5x10^7$ CFU/g, as recommended by French regulations (M.E.F.B. 1988).

It is also clear from Fig. 2 that a good correlation between the degradative kinetics exists. Reporting the stability time (time corresponding to the maximum browning rate – max d(HUE)/dt –, time corresponding to the maximum browning acceleration – max d^2(HUE)/dt^2 – and the one to attain a TBC value of $5x10^7$) in function of storage temperature, it is possible to define the tolerance time-temperature data shown in Table 1.

Table 1 illustrates also the existence of a sufficient superimposition between the stability times defined on microbiological base (RIVA *et al.*, 2001b) and on the hue index base. The agreement is better if the time of maximum rate of browning instead of the time of the maximum acceleration of the same phenomenon is considered.

Obviously the absolute chromatic measures are subject to great variability

Table 1 - Influence of temperature on the shelf-life (days) of fresh cut cicorino. Estimates are based on TBC (time to reach a TBC value of $5x10^7$ UFC/g) and hue (a/b, maximum values of 2nd derivative and maximum values of 1st derivative). Q_{10} values (increase of degradation rate for 10°C increase) computed for each index are also reported.

Temperature (°C)	TBC	Hue (a/b)	
	Time for $5x10^7$ CFU/g	max d(HUE)/dt	max d^2(HUE)/dt^2
5	6.22	7.25	5.45
10	3.28	2.9	1.74
20	0.83	0.66	0.41
Q_{10}	5.39	4.86	3.85

when different productions and lots are considered, as in the case of Fig. 2 where the reference samples (t=0) were different for the three storage tests. Such variability, however, doesn't really influence the kinetic of browning if measured by image analysis and expressed in normalised form, as shown in Fig. 3 for the case of 5 lots of cicorino stored at 10°C.

In a series of parallel studies (RIVA *et al.*, 2001a, RIVA *et al.*, 2001b) we have obtained analogous results in the case of carrots a la julienne and entire leaf lattughino, confirming that the image analysis can represent a simple methodology for the monitoring and the forecasting of the shelf-life, parameterising an attribute (the colour) for which the evaluation would consist in the putting in work of complex methodologies in sensory analysis.

Also E-nose approach give interesting results. PCA loadings of sensor responses showed (RIVA *et al.*, 2001a) that only 5 sensors (MOSFET 3, 8, 10 and MOS 2 and 3) contributed to the discrimination of the samples. Fig. 4 illustrates the score plot on the first and second principal components of the complete data set. Sample scores are distributed along the first component, which explains 85.8% of the variance. From the loadings it was observed that MOSFET sensors gave the major contribution to this component. The second component explains only about 7% of the variance and the MOS sensors were primarily responsible.

Fig. 4 emphasises the role of the storage time: all fresh samples (SF and C0) or retail samples before expiration date (CE) are on the same side of the plot, while aged samples are on the right side. This can be explained by the fact that, as reported in Fig. 2, during the first days of storage of ready-to-use vegetables the bacterial count is constant and their enzymatic activity is low. Hence, no degradation reactions occur and tissue integrity and equilibrium of the volatile compounds are not affected.

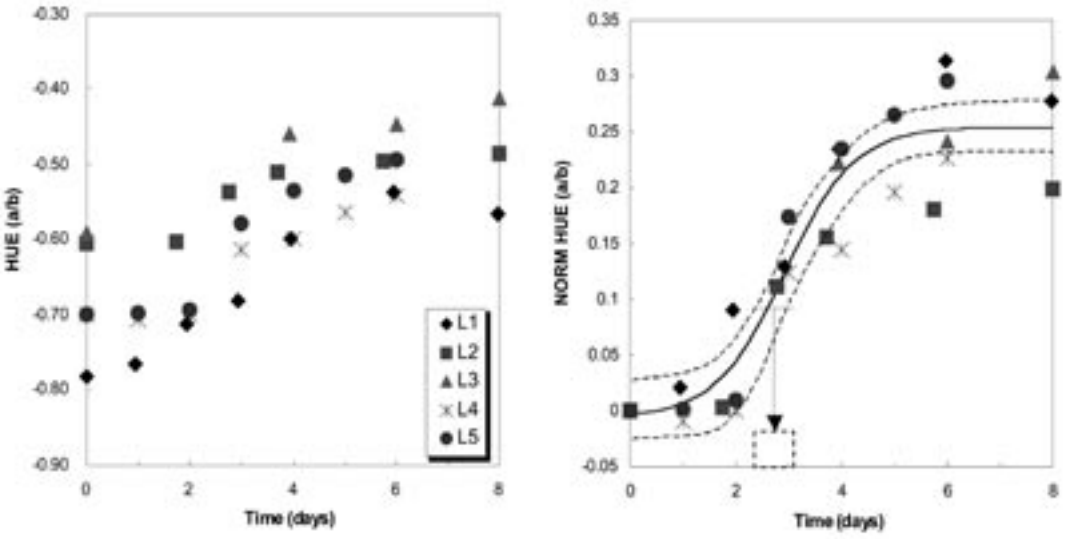


Fig. 3 - Hue kinetics (left, raw data; right normalized values) during storage of different lots of fresh cut cicorino at 10°C. The arrow indicates the time corresponding to the maximum of the first derivative of the interpolating transition function.

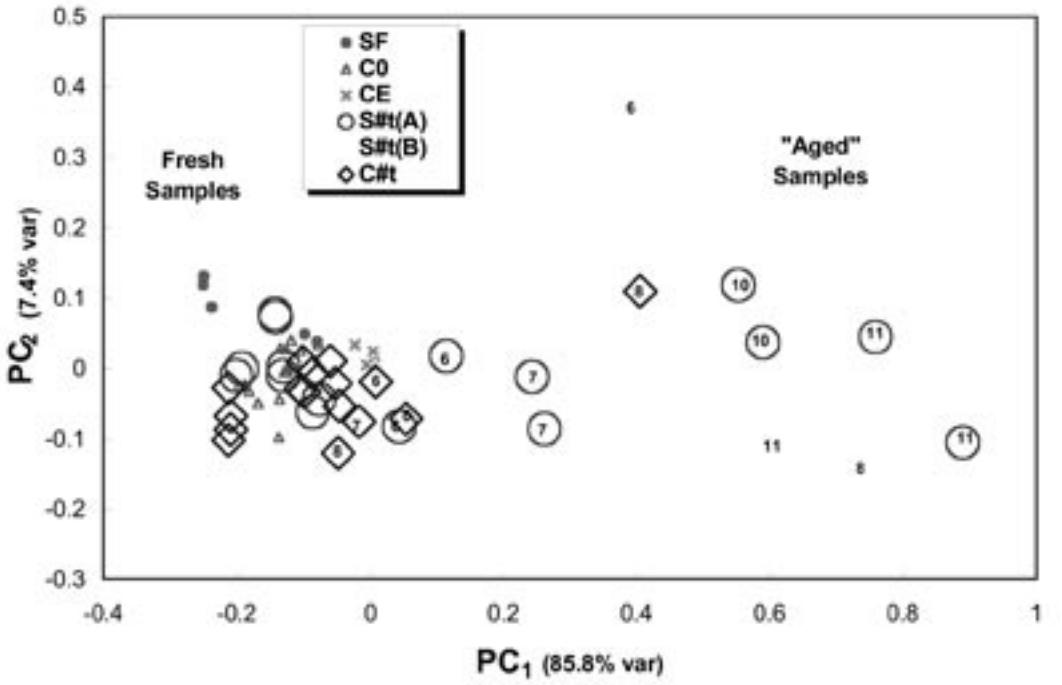


Fig. 4 - E-Nose Score plot of full data set of "cicorino" samples. Numbers in open symbols refer to storage time (days).

In Fig. 4 a strong relation between time and first component can also be observed and this is even confirmed in the case of samples of different origins (reference, producers and retail).

Finally, Fig. 5 shows hue index, microbial population and PC1 kinetic scores for S#t "cicorino" samples: this plot shows a strong correlation among the three kinetic parameters (R2 = 0.994 for TBC vs. PC1; R2 = 0.982 for Hue Index vs. PC1). Colour change, microbial spoilage and flavour decay had the same trend: an early stability phase was followed by a large and rapid change and again a new stage of equilibrium was attained, which was related to shelf-life expiration.

Considering this good set of correlations it is evident that both image analysis and electronic nose are useful tools for defining or following the shelf-life of the products under study or of similar kind, as explained elsewhere (RIVA *et al.* 2001a).

Second case: Taleggio Cheese

Image analysis and e-nose systems are valuable supports for shelf-life parameterisation of a cheese.

Consistency, paste colour, rind appearance, hole size and shape, mould extension and colour all undergo changes during the storage or commercial life, especially if the cheese is portioned and packaged.

One interesting problem concerns fresh and soft ripened cheeses since visual quality is related to conditions, especially temperature, during transport at sale points and household storage (RIVA and GIANGIACOMO, 2001, TOPPINO *et al.*,

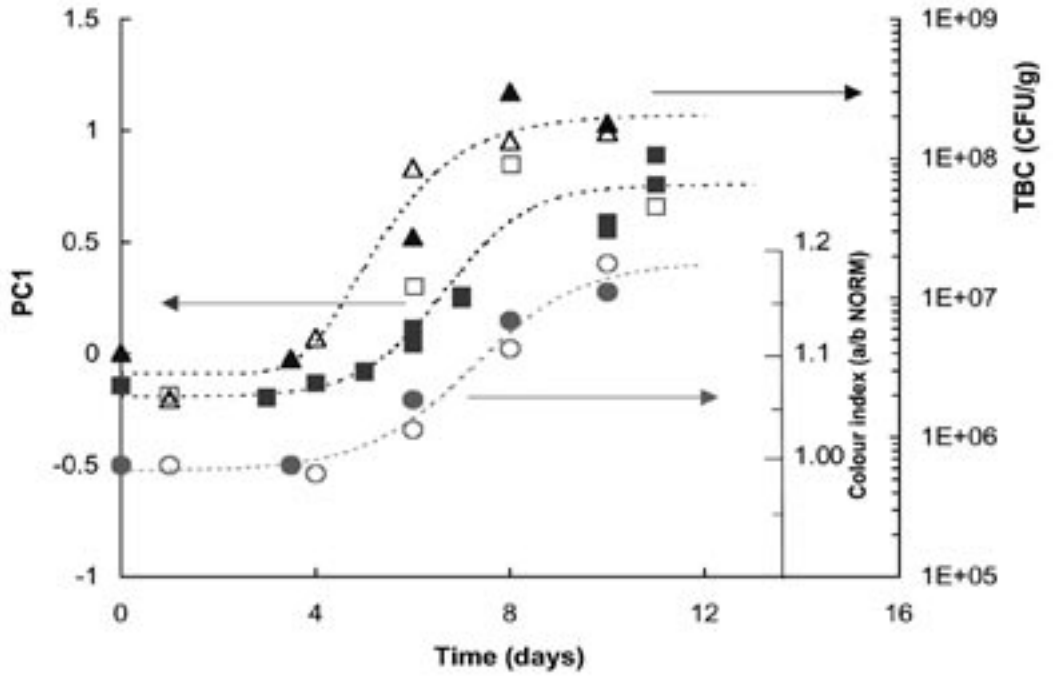


Fig. 5 - Kinetics of PC1 electronic nose scores (□), colour index (○) and total bacterial counts (Δ) for reference "cicorino" samples stored at 5°C for different times. Open and closed symbols refer two different production lots.

2002, NI and GUNASEKARAN, 1995, INNOCENTE and CORRADINI, 1998). For such products, if we consider the close relationship between proteolysis and/or lipolysis and changes in appearance and colour, parameters obtained from image analysis or E-nose can be used to define are the limit of sensory acceptability. This is particularly important for PDO cheeses, where sensorial characteristics are described in production disciplinary.

After two specific research on Gongorzola cheese (RIVA and GIANGIACOMO, 2001) and Asiago cheese (RIVA and GIANGIACOMO, 2002), a recent investigation on packaged Taleggio cheese was conducted (TOPPINO *et al.*, 2002). Samples taken from five productions lots stored at temperatures of 3°, 10° and 20°C were tested at the beginning of shelf-life and at predetermined time intervals. Investigations were carried out by using chemical analysis on the proteins fraction, measuring visual appearance attributes and the evolution of the aromatic fraction by an electronic nose (RIVA *et al.*, 2002). The visual quality was determined using two indices: hole formation and hue, defined as a ratio between a (red) and b (yellow) parameters of the CIE-L*a*b* colour values.

Methodology

The images of cheese slices, under different storage conditions, were acquired by using a simple and standardised digitalisation procedure with a flat scanner (300 dpi resolution and full-colour format). Colour and image parameters were measured as described elsewhere for Asiago cheese (RIVA and GIANGIACOMO,

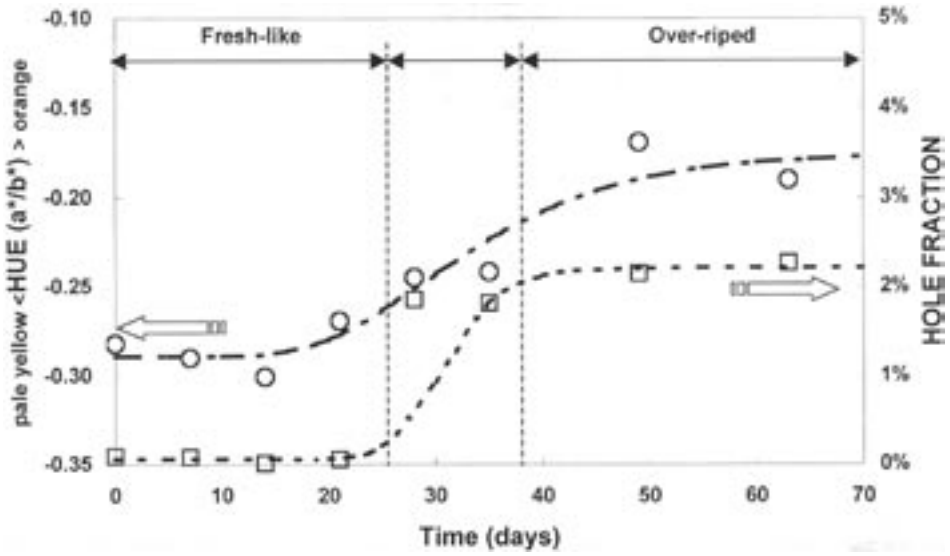


Fig. 6 - Parameterisation of visual quality of Taleggio cheese slices during storage at 10°C. The dotted lines refer to two shelf-life limits defined according to the evolution of proteolytic phoenomena.

2002). Areas with hole formation were measured with Image Pro-Plus v.4.0 software: images were converted into a grey scale and a filter was applied to eliminate local imperfections. Dark areas (holes) from light areas (paste) were isolated by an automatic threshold procedure (RIVA and GIANGIACOMO, 2001).

The shelf-life of the same samples of Taleggio cheese has been also investigated with an Electronic Nose (APPLIED SENSOR'S 3200, with an array of 10 MOSFET sensors and 12 MOS sensors) (RIVA *et al.*, 2002).

Results

Fig. 6 shows the evolution of the appearance of a Taleggio slice during the storage at 10°C. Under these conditions, the ripening process brought about evident chromatic alterations (yellowing of the paste) and formation of holes. Some of these alterations can be parameterised in order to establish acceptability limits as proposed in the bottom part of the figure.

The kinetics illustrated in figure 6 suggests (as in the case of cicorino) a simple criterion of parameterisation based on transient phenomenology (represented in a model by a sigmoidal function). The shelf-life limit represented by the maximum of the first derivative of the sigmoidal interpolating function (maximum rate of changes in the aspect parameters) can be consequently calculated. Table 2 summarises the observations at the three considered storage temperatures and includes the results of the proteolytic indices parameterisation (TOPPINO *et al.*, 2002).

Data reported confirm the relationship between the acceptability time of visual quality changes (image analysis) and the corresponding indices of proteolytic degradation. These results show the great potentiality of image analysis in the study of such phenomena.

Table 2 - Influence of temperature on the shelf-life (days) of Taleggio cheese. Estimates are based on proteolysis indices (maximum values of 2nd derivative) and on the parametrisation of the hue (a/b), and of hole surface fraction (maximum values of 1st derivative). Q_{10} values (increase of degradation rate for 10°C increase) computed for each index are also reported.

Temperature (°C)	Proteolytic Indices			Visual Quality	
	NCN/N_{tot}	NPN/N_{tot}	NH_3/N_{tot}	Hue	Hole Fraction
3	36.4	43.4	26.7	74.2	44.3
10	30.4	27.1	21.5	33.1	25.2
20	6.9	12.8	7.6	6.1	6.6
Q_{10}	2.76	2.05	2.13	2.40	3.33

The E-nose instrument allowed a univocal classification of the samples on the basis of their storage time. Six (3 MOSFET type and 3 MOS) of the 22 sensors showed a good discriminating power. Their responses are proportional to the storage time and are partially influenced by the storage temperature.

A clustering technique (Standardised Euclidean distance, Ward Method) applied to the complete data set (sensor response x samples) showed a simple classification between "fresh-like" and "over-riped" samples, as illustrated in Fig. 7.

The combined effect of the temperature and storage time is clearly illustrated in Fig. 8, where results of PCA analysis are shown. The scores of the samples (al-

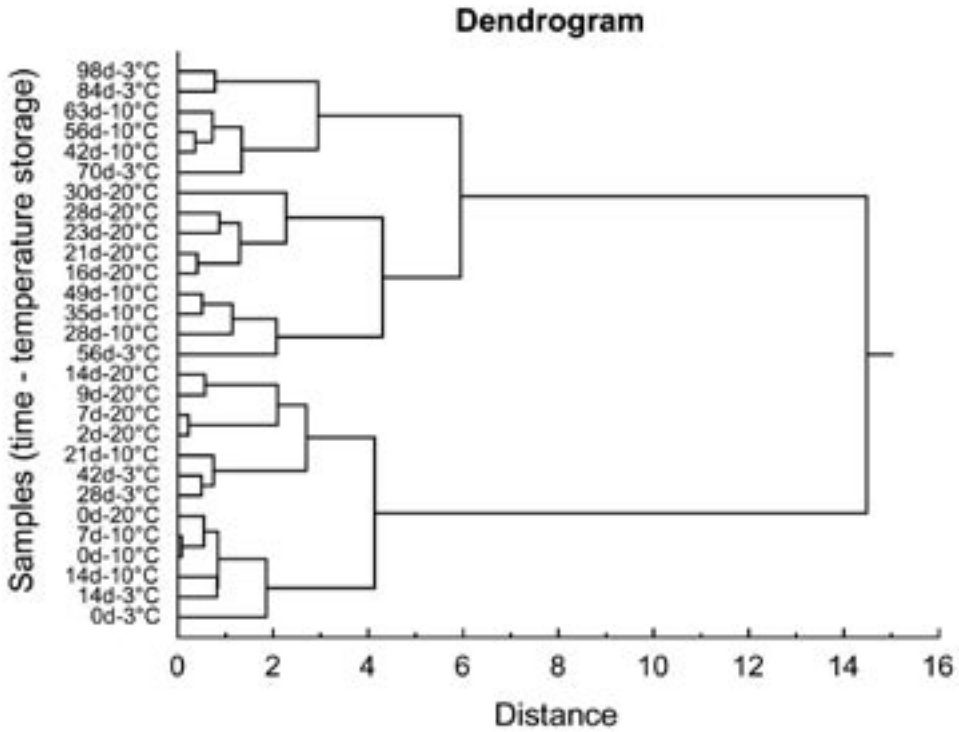


Fig. 7 - Clustering results (Standardised Euclidean distance, Ward Method) of E-Nose sensor responses for a series of Taleggio cheese samples. Sample labels refer to time-temperature storage exposure.

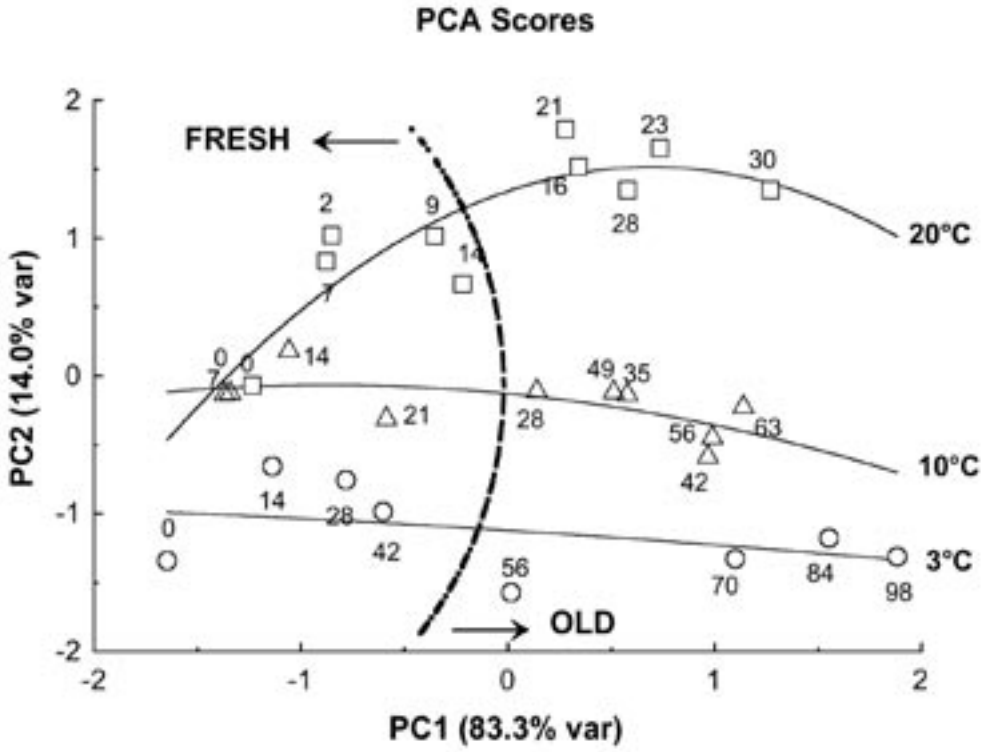


Fig. 8 - Score plot of E-Nose full data set of Taleggio cheese samples. Numbers in open symbols refer to storage time (days).

ways on the plan delineated from the first two principal components and for the 6 selected sensors) evidentiates the direction of classification (from left to the right, with the increasing of the storage time) and the effect of temperature (classification along the second PC, with increasing of the storage temperature).

The direction of the classification presumes that the sensor responses increase with the storage time. Probably this is due to the detection of the aminoacidic and ammoniacal compound (MOSFET type sensors) produced from the action of proteolytic and peptidasics enzymes of lactic and microbial origin. Carbonilic and acidic compounds due to the lypolitic activity of the microorganisms living on the crust can also have some effect and are revealed by MOS type sensors.

In Fig. 8, for each temperature, a direction of maturative evolution is illustrated. The curve overlapped to this evolution divides the plot in two zones, corresponding to the position of the scores of the samples classified as “fresh-like” and “over-ripened” in the clustering dendogram of Fig. 7. The two zones, to all temperatures, appear sufficiently correlated to the value of the first principal component.

As in the case of cicorino, the PCA analysis applied to the signal of the six selected sensors revealed that the score of the first principal component evolves during time with a sigmoidal trend and allowed to define two stability time, e.g. the time range to reach the maximum acceleration or the maximum rate of the aromatic changes. It has been demonstrated that the

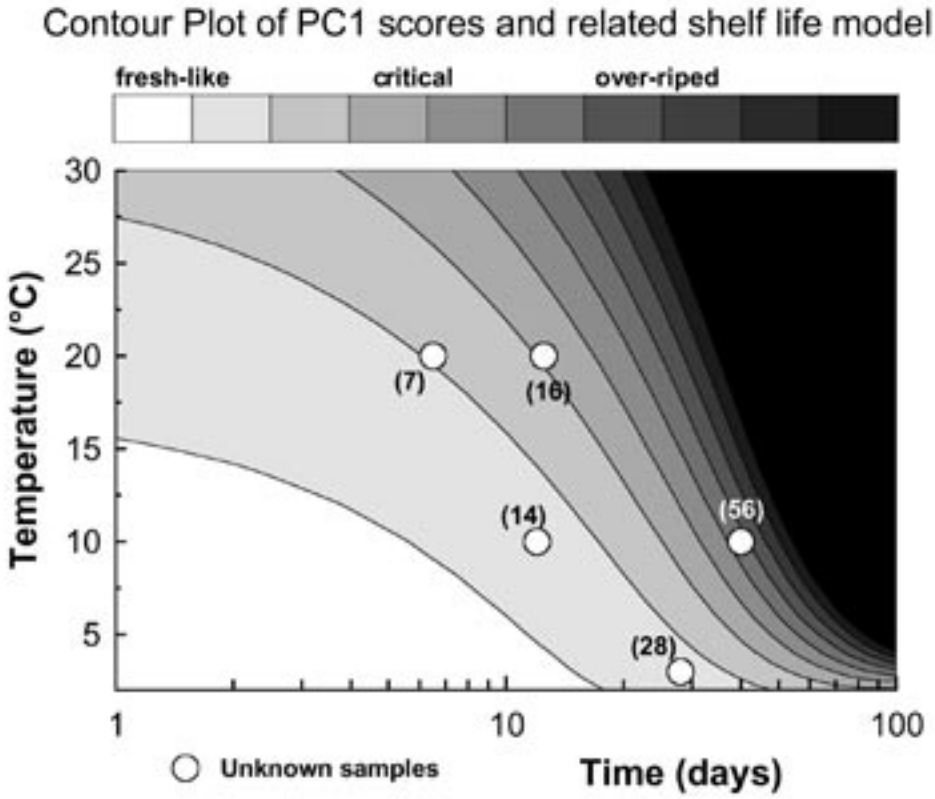


Fig. 9 - PC1-E-Nose contour plot of Taleggio cheese data set (series 1, 2, 3 and 4 are considered). White points are the predicted values for the 5th series of samples, value in brackets are its real storage time.

stability time based on maximum rate of the aromatic changes (14 days at 20°C, 22.3 days at 10°C and 44.6 days at 3°C) is in agreement with the time range corresponding to the maximum acceleration of the proteolytic degradative processes (Table 2).

Furthermore (data reported in RIVA *et al.*, 2002), with a low variability due to different samples from different productions, the Electronic Nose has demonstrated to be able to discriminate the different storage times of Taleggio cheese samples exposed to variable temperature. Figure 9 shows an example of the predictive approach, applied to our data set in view to recognise the storage time of unknown samples.

The figure illustrates the contour plot of predicted PC1 scores, modelled in terms of time-temperature dependence. It was obtained by non linear regression of PC1 scores experimental data vs storage time and corresponding temperature, considering four of the five series of original samples. PC1 scores of the 5th series and the corresponding storage temperatures are over-imposed to the plot, so that a predicted storage time can be drawn for each new sample.

Results are very interesting: the predicted storage time (6.5; 12; 12,5; 28 and 40 days) is close to the real storage time (show – in brackets – also in the plot: 7, 12, 14, 28 and 56 days) with a noticeable difference only in the case of the most severe storage condition.

CONCLUSIONS

The two case-studies above discussed show that the proposed methodologies are a useful means for shelf-life parameterisation. This is particularly true especially when applied to fresh products. Work is in progress for the shelf-life monitoring of other types of cheeses (TOPPINO *et al.*, 2003) and, with the only E-nose approach, of fresh milk.

The "core" of present study is the synergic instrumental approach to sensorial assessment of food during its commercial life. It has been demonstrated that the measurable parameters of visual appearance and aroma attributes can constitute a new and interesting perspective for the objective evaluation of the life of a food product. Furthermore, the simple mathematical models proposed in this study for shelf-life parameterisation based on transition kinetics can easily be adapted for defining current indices of food durability, e.g. the "best before" dating, the "open date" labelling or the monitoring of shelf-life with TTI devices (RIVA *et al.*, 2001c).

However, it should be pointed out that shelf-life studies require a fast and pragmatic approach: when the safety is assured, only the sensory quality decay can be monitored and parameterised. In this perspective, we believe that not only image-analysis and E-nose, but also other objective methodologies (rheology, E-tongue) can be routinely applied to shelf-life studies.

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SHELF-LIFE STUDY OF PACKED INDUSTRIAL RICOTTA CHEESE

STUDIO DELLA SHELF-LIFE DI RICOTTA INDUSTRIALE

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ABSTRACT

Ricotta cheese is a very interesting Italian dairy product, characterised by increasing consumption and very short shelf-life. Several samples of manufactured Ricotta cheese, packed in two different sizes (250 g and 10 kg), were analysed during the commercial shelf-life at three different storage temperatures (3°, 10° and 20°C). Tests were also carried out by storing samples under a fluctuating temperature regime. Storage at 3°C gave the best results. At this temperature Ricotta cheese maintained acceptable chemical-physical and microbiological characteristics for 27 days.

Under fluctuating temperature regime, storage of the product at 8°C for 3 days resulted in considerable sensory degradation, sufficient to dissuade from consumption.

RIASSUNTO

La Ricotta è un derivato lattiero-caseario interessante per le caratteristiche di conservabilità difficili, correlate a molteplici parametri, tecnologici, compositivi e microbiologi.

Sono state sottoposte ad indagine Ricotte di produzione industriale, mantenute a temperature di frigorifero industriale (3°C), di frigorifero domestico e della grande distribuzione (circa 10°C), ambiente (20°C), nonché fluttuante tra 3°, 8°, 10° e 12°C.

Sono stati determinati a tempi prestabiliti, fino a un massimo di 48 giorni: ammoniaca, acido lattico, numero di perossidi, NaCl, zuccheri, acido citrico, pH, acidità, nonché eumiceti, coliformi, *Escherichia coli*, enterococchi, microrganismi sporigeni aerobi ed anaerobi.

Sono state, inoltre, determinate la qualità e l'intensità aromatica mediante

- Key words: analytical methods, Ricotta cheese, shelf-life -

Naso Elettronico e la composizione della frazione aromatica più volatile, con la tecnica SPME-GC-MS.

La conservazione è risultata ottimale a 3°C, temperatura a cui la Ricotta ha mantenuto proprietà accettabili fino a 27 giorni, mentre a 10°C il tempo di stabilità è di circa 8 giorni. Come previsto, le Ricotte sono risultate non idonee alla conservazione a temperatura ambiente se non per poche ore.

L'influenza dei regimi termici di conservazione è risultata evidente anche nelle prove di conservazione a temperatura fluttuante: la permanenza per soli 3 giorni a 8°C determina una degradazione sensoriale tale da sconsigliarne il consumo.

INTRODUCTION

Ricotta cheese is a dairy by-product obtained by acid-heat coagulation of whey; it was already known during the Greek-Roman age and was named after the Latin word "recoctus", which means cooked twice.

About 63% of the Italian market is represented by cow's milk Ricotta cheese and about 37% by sheep's milk Ricotta cheese. Buffalo's milk Ricotta cheese is a small niche market. Almost homogeneous market distribution can be observed: 23% in northwestern, 23% in northeastern, 24% in central and 30% in southern Italy (Ottogalli *et al.*, 1981) (Fig. 1).

There are a large number of Ricotta cheese varieties; according to a CNR study, 27 Ricotta cheese types, differing in raw material origin, structure, paste texture, taste, aroma, intended use, and production and preservation technology, have been classified in southern Italy and on the Italian islands (Mucchetti *et al.*, 2002).

Some studies carried out on the average composition of industrial cow's milk Ricotta cheese by Cosseddu *et al.* (1999) and Mucchetti *et al.* (2002) showed that there was a large variability, especially resulting from a considerable difference between the wheys used depending on the rich composition of initial milk, the original milking species and the cheese type made.

With reference to microbiological characteristics, Ricotta cheese, because of its pH close to 7.0, high water content and low sodium chloride content, is an optimal substrate for growth of bacteria. Data obtained over the last twenty years (Ottogalli *et al.*, 1981; Amerio and Verme, 1992; Cosseddu *et al.*, 1997; Lodi *et al.*, 1999;

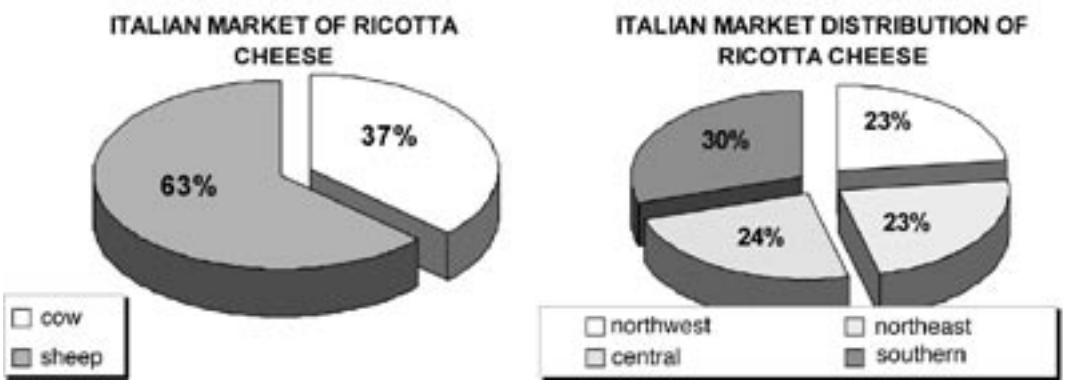


Fig. 1 - Italian market distribution of Ricotta cheese use.

Carminati *et al.*, 2002) reflected an improved microbiological quality of industrial Ricotta cheese, showing very low total microbial counts and absence of pathogens in final products. Such conditions referred to both home-style and industrial productions. This is a very important event because consumers are paying increasing attention to composition, nutritional and hygienic characteristics of foods and their freshness. Hence, it is necessary for manufacturers to market hygienically safe foods, which are able to keep constant sensory characteristics during their shelf-life and to fulfil, at the same time, market requirements.

Ricotta cheese can be subdivided into three categories, according to its shelf-life (Mucchetti *et al.*, 2002):

1. Ricotta cheese made in medium-large industrial facilities (20-40-day shelf-life);

2. Ricotta cheese made in small-medium industrial facilities (1-week shelf-life);

3. Ricotta cheese made in mountain pasture, agro-pastoral or small industrial facilities (to be consumed within 1 or 2 days).

However, Ottogalli *et al.* (1981) demonstrated that only 58% of commercially available Ricotta cheese samples in the USA showed acceptable quality on the expiration date printed on the package, although they were satisfactory from a microbiological point of view.

Hence, the shelf-life of Ricotta cheese seems to be closely linked to the technological conditions applied, especially those used to deproteinize whey. Finally, heat conditioning and packaging determine how often post-contamination hazard may occur (Mucchetti *et al.*, 2002).

In recent years changes have been made in the production technology to increase both yield and, in particular, shelf-life. Absence of both contact with the air and handling has allowed a microbiologically stable product to be obtained, which maintains its initial sensory characteristics and, when stored under suitable thermal conditions, does not undergo important physical-structural changes for up to 30 days (Ottaviani and Franceschetti, 1980; Palmas *et al.*, 1994).

Chemical-physical changes, which may occur during storage, mainly include the acidity of Ricotta cheese: pH tends to decrease and acidity, expressed as percent lactic acid, tends to increase (Hough *et al.*, 1999). This is concomitant to different phenomena: drainage and separation of the residual whey phase; sensorial modifications resulting in changes of both Ricotta cheese pigmentation from milk-white to yellow, produced by oxidative reactions, and the characteristic flavour; and changes in the paste structure, which starts to break up.

The aim of this work was to check the shelf-life of Ricotta cheese packed in both single-portion 250 g and industrial 10 kg packages. Research was intended to define evolution of packed product in order to identify objective parameters of qualitative decay and to obtain suitable parametrisation.

MATERIALS AND METHODS

Ricotta cheese was supplied by a firm where large amounts of industrial Ricotta cheese are manufactured according to Fig. 2 (flow-sheet).

Ricotta cheese samples were supplied in thermosealed 250 g plastic containers and thermosealed 10 kg plastic bags. Samples were taken directly during production, immediately after packaging at 3°C, subdivided into subgroups, stored

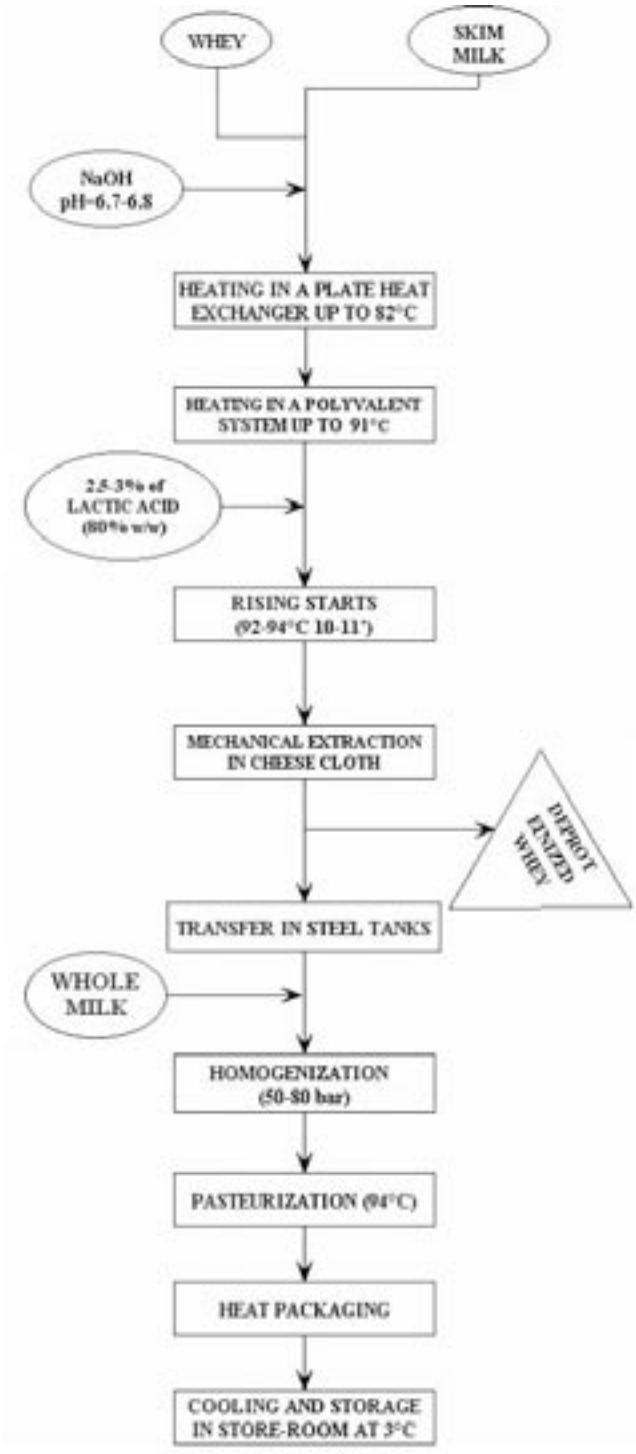


Fig. 2 - Ricotta cheese flow sheet.

at different temperatures in thermostated cells, which were checked daily, and analysed at given intervals. A first set of thermostated samples at 10±1°C (simulating the temperature of refrigerators both at home and sometimes at retailing and distribution industry) was analysed every 6 days up to 39 days. A second set was subdivided into three groups as follows: the first group was stored at 10°C and monitored every 20 days to confirm results from the previous set; the second one was stored at 3±1°C (i.e., industrial refrigerator temperature) and analysed every 10 days up to 48 days; and the third one was stored at room temperature (20±2°C) and checked after 6 and 11 days.

A trial at fluctuating temperature was also carried out to track steps of any improper distribution path (Fig. 3). This was intended to describe, by the end of the week, the occurrence of problems connected to heat conduction of storage cells or transport means.

The pH, titratable acidity (D.M., 1986), peroxide value (upon cold fat extraction by hexane cycle) [by CDR system (Campagnol, 2003)], NaCl (FIL-IDF Standard 88A, 1988), NH_3 [by CDR system (Toppino *et al.*, 2003)], citric acid (Bouzas *et al.*, 1991), lactic acid [both as L+D (Bouzas *et al.*, 1991) and as L alone by CDR system (Campagnol, 2003)], sugars (glucose, galactose and lactose) (Bouzas *et al.*, 1991), aromatic fraction by Electronic Nose (Benedetti *et al.*, 2002), and evaluation of the most volatile aromatic components by SPME-GC-MS (Contarini and Povolo, 2002) were determined on

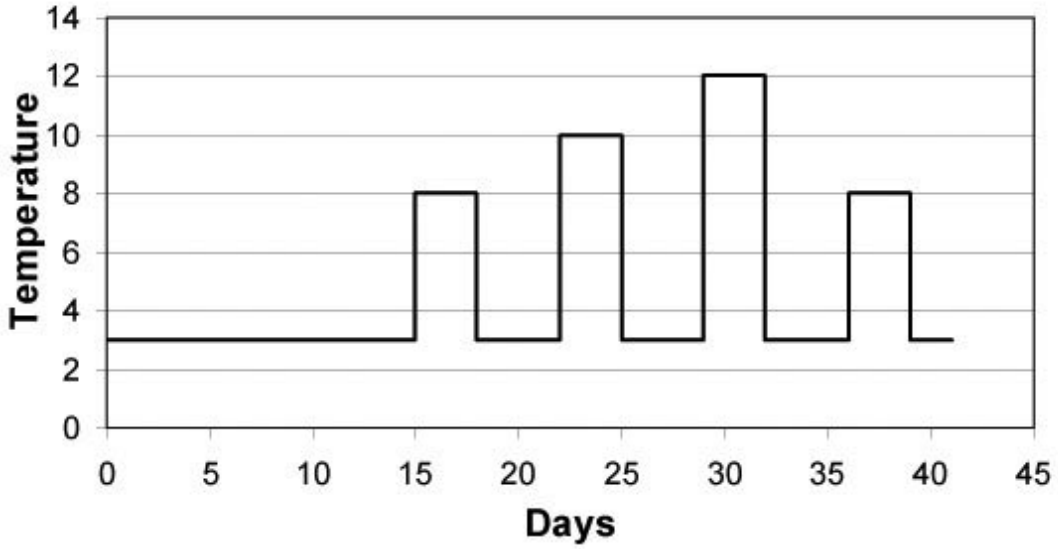


Fig. 3 - Fluctuating temperature. Samples were stored at a temperature of 3±1°C for 2 weeks, then at 3±1°C from Monday at 12:00 to Friday at 12:00 and at temperatures of 8±1°, 10±1°, 12±1° and again 8±1°C from Friday to Monday for 4 weeks.

all samples. The number of coliforms (Standard FIL-IDF 73B, 1998) and *Escherichia coli* (ISO 16649-2, 2001), moulds and yeasts (FIL-IDF Standard 94B, 1990), enterococci (Ottaviani, 1991), and both aerobic (Marshall, 1992) and anaerobic spore-forming bacteria (Ottaviani, 1991) were also evaluated. In addition, for the sample taken at time zero (t_0) on the day of packaging, parameters for the macrocomposition were also evaluated (D.M., 1986).

Analytical data, selected according to suitable discrimination criteria, were processed using mathematical models by Table Curve 2D software (Jandel Scientific, St. Raffael, CA).

RESULTS AND DISCUSSION

As expected, the centesimal macroconstituent composition showed (Table 1) a certain variability between the two sets, especially for moisture and the protein and lipid content, both on the fresh product and calculated on the dry matter.

With reference to microbial growth, coliforms, *Escherichia coli*, yeasts and enterococci were invariably not detected. Samples were found to contain both aerobic and, to a smaller extent, anaerobic spore-forming microorganisms. The number of aerobic bacterial spores increased up to 5.6×10^5 cfu/g at 10°C after 25 days and then decreased slowly in the next samplings (Fig. 4).

This microbial population did not grow at 3°C up to 40 days of storage. Within a few hours at 20°C packages were modified by swelling, unpleasant aroma and spoiled appearance.

In this context, the same parameters applied to Ricotta cheese during shelf-life were used to analyse all products at critical points of the technological process (data not shown). It was evident that microbial contamination of raw materials varied

Table 1 - Composition of two different sets of Ricotta cheese at the beginning of shelf life.

Composition	Values are expressed as	250 g		10 kg	
		First set	Second set	First set	Second set
Dry matter	g/100 g	25.03	24.12	26.12	28.47
ASHES	g/100 g	1.17	1.15	1.15	1.22
FAT	g/100 g	7.85	6.77	8.06	7.39
FAT/Dry matter	g/100 g dm	31.36	28.07	30.86	25.96
Protein	g/100 g	11.76	13.13	12.87	16.25
Protein/Dry matter	g/100 g dm	46.98	54.44	49.27	57.08
pH		6.52	6.62	6.52	6.50
Acidity	g lact.ac./100 g	0.11	0.21	0.10	0.26
Peroxide value	meqO_2/kg fat	0.30	0.30	0.22	0.22
NaCl	g/100 g	0.33	0.33	0.31	0.25
NH_3	mg/kg	4.21	3.22	4.53	4.76
Glucose	g/100 g	0.008	0.258	0.007	0.277
Lactose	g/100 g	3.306	3.118	3.200	2.535
Galactose	g/100 g	0.035	0.023	0.038	0.014
Citric acid	g/100 g	0.407	0.500	0.403	0.447
Lactic acid *L+D	g/100 g	0.137	0.084	0.133	0.101
**L	g/100 g	0.066	0.057	0.074	0.048

*Lactic acid with HPLC method (L and D); ** Lactic acid with CDR method (L).

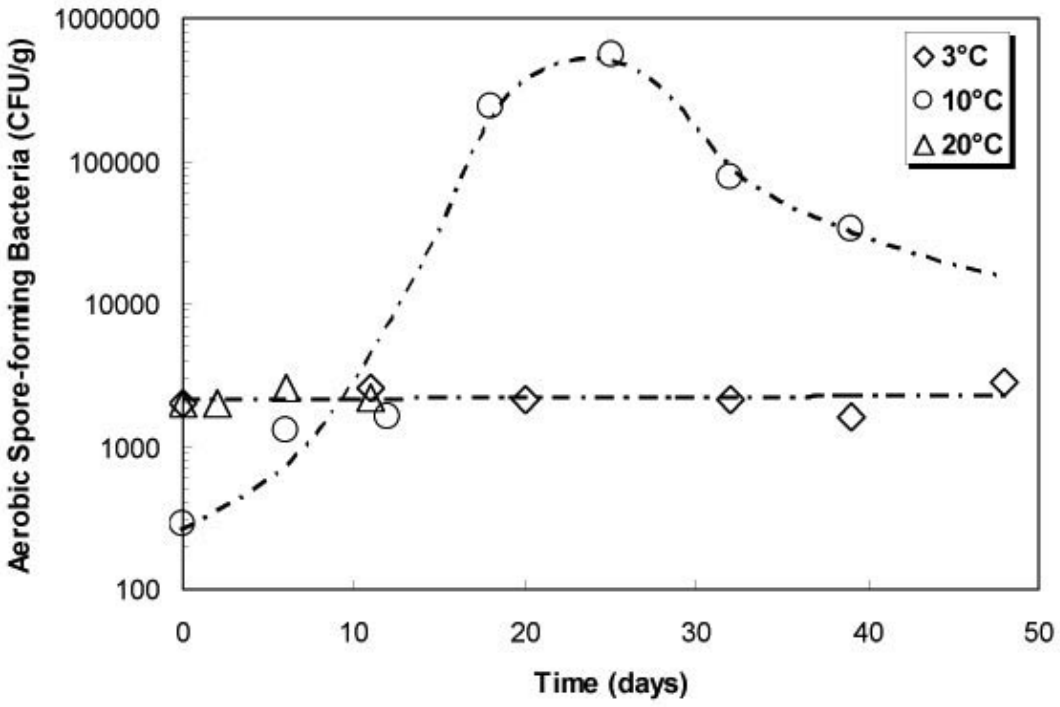


Fig. 4 - Kinetics of aerobic spore-forming bacteria during storage of Ricotta cheese.

as a function of both the origin and the storage and pre-conditioning conditions. Already after the first heat conditioning treatment, no vegetative microorganisms were detected, but only spores of anaerobic and, in particular, aerobic microorganisms having a high degree of heat resistance.

In samples of the first set of Ricotta cheese, stored at 10°C, the acidity value for total lactic acid and L-lactic acid showed a progressive increase with time up to the 25th day, followed by a tendency to decrease. This behaviour was also demonstrated by the inversely proportional trend of pH.

Ammonia was the index parameter for protein degradation beyond peptide cleavage to free amino acids. Its concentration was almost constant at 4-8 ppm and then increased around the 25th day. This partly explains changes in values for pH, acidity and lactic acid after the 25th day.

The peroxide value of the fat of Ricotta cheese stored at 10°C (Fig. 5) showed a progressive increase up to the 25th day of storage, followed by a decrease to values similar to the initial ones, thus indicating evolution of oxidation reactions towards secondary compounds such as alcohols, aldehydes and ketones.

The trend of chemical-physical indices detected in the first set of Ricotta cheese at 10°C was mostly demonstrated by analyses carried out on the second set. Slight differences found were ascribed to the different raw materials used.

Samples stored at 20°C showed such a rapid evolution of degradation indices, as a result of a series of severe changes in glucide, lipid and protein constituents, that the trial was stopped after 11 days storage because of evident product degradation. A sudden decrease in the peroxide value also reflected the above observations, indicating faster cleavage processes of peroxides (Fig. 5).

In samples stored at 3°C the reactions assigned as markers of the degree of

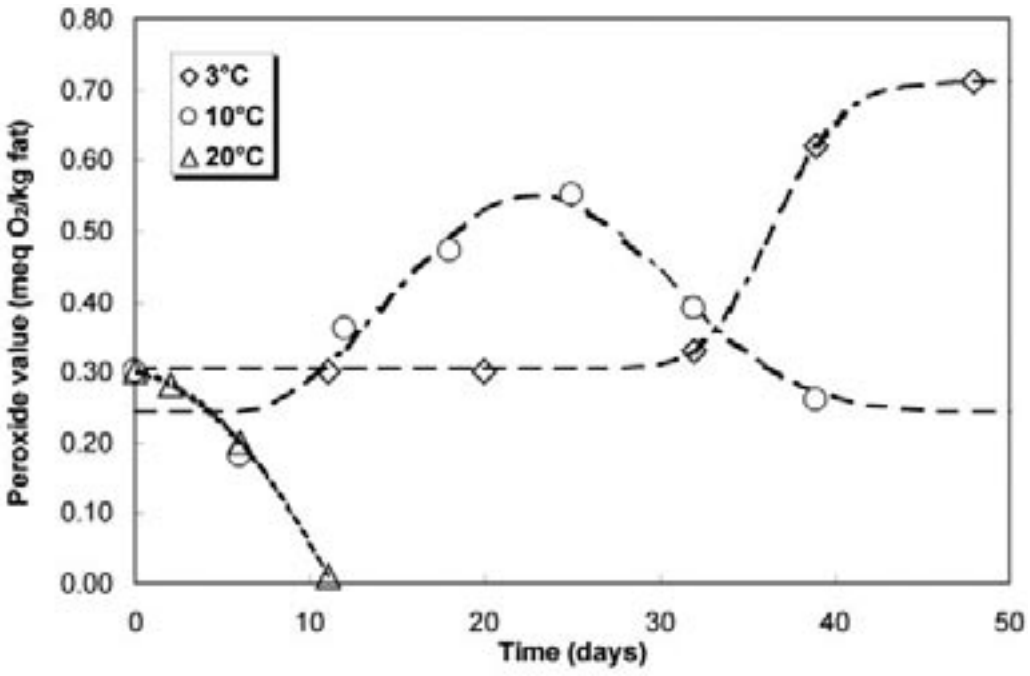


Fig. 5 - Kinetics of peroxide value during storage of Ricotta cheese.

freshness (i.e., acidity, pH, lactic acid, citric acid, sugars and free NH_3) did not show considerable changes within the first 20-30 days of storage, but they exhibited slight variations connected to inhomogeneity of industrial samples.

The peroxide value tended to increase gradually during the shelf-life. This increase, also a result of degradation reactions of the lipidic fraction, resulted in the accumulation of oxidation compounds, associated with the first steps of reaction and propagation, resulting in considerable slowing of the final cleavage step to form compounds having a lower molecular weight such as aldehydes and ketones (Dumont and Adda; Stocchi and Capella, 1972).

During result processing, discussion was simplified by using only data from 250 g Ricotta cheese packages intended for direct consumption because analysis of industrial 10 kg samples was shown to provide similar and basically repeatable results. Data processing included application of mathematical models for parametrisation of analytical data detected experimentally.

Values obtained from the first set of Ricotta cheese, conditioned at 10°C, was compared with the second set of Ricotta cheese, conditioned at 3° and 20°C.

The trend of all the indices (except NH_3) was conventionally explained by sigmoidal functions able to define a phenomenological sequence of stability, exponential evolution and attainment of further equilibrium.

The actual kinetics of changes was likely to be more complex than the one described; however, statistical processing was considerably simplified by using sigmoidal functions.

Diagrams representing sigmoidal functions for changes in pH, lactose (Fig. 6) and ammonia showed quite similar evolutionary trends consisting of both increases and decreases in components, which were notable at 20°C and progressively less

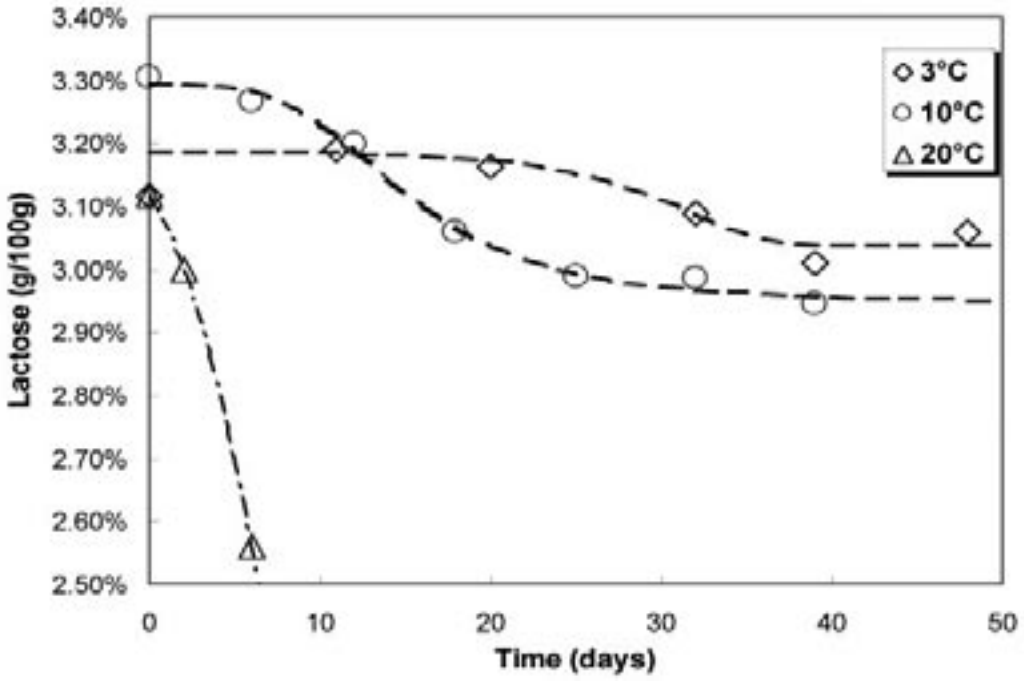


Fig. 6 - Kinetics of lactose during storage of Ricotta cheese.

important at 10° and 3°C. Storage at 3°C was shown to be optimal for the above parameters indicative of the degree of freshness of samples.

The pH, acidity, lactoses, peroxide and NH_3 values were taken into account to determine Ricotta cheese shelf-life. Maximum values for the first (maximum rate of degradation, dF/dt) and second derivative (maximum acceleration of degradation, d^2F/dt^2) of each individual index for degradation were calculated. Both criteria were calculated for all the indices at the three simulation temperatures and reported in Table 2. They showed that the parameters considered, had highly comparable values for maximum rate and maximum acceleration of degradation.

The trend of lactose was considered the most representative trend for the evolution of Ricotta cheese degradation, and the highest values for the first and second derivatives were plotted on a time vs temperature graph to obtain a time-temperature tolerance plot (Fig. 7). A rough indication of the trend of Ricotta cheese shelf-life at each temperature was provided, and a time range was identified (full lines), where the lowest limit (prudential criterion) corresponded to the occurrence of first degradation and the highest limit (tolerant criterion) corresponded to the whole development of degradation. The region between both maximum values corresponded to the time span over which Ricotta cheese may be exposed to a degradation hazard. Means were useful to predict critical times for storage of Ricotta cheese at different temperatures and, hence, to define a proper, actual expiration date. According to the same diagram, previous data were confirmed by data processing for 10 kg packages (dotted lines). Traces from the resulting time-temperature tolerance card (Fig. 7) were shown to overlap those obtained from 250 g packages (Fig. 7).

Using lactose as a marker for Ricotta cheese degradation at all times, kinetic parameters for activation energy and Q_{10} were calculated by automatic routine calculation and shelf-life prediction (Riva and Piergiovanni, 2001) (Table 2). Q_{10} values showed that an increase in storage temperature of 10°C resulted in an eightfold decrease in shelf-life with respect to the tolerant criterion and a threefold decrease with respect to the prudential criterion.

Table 2 - Time limits for stability during storage of Ricotta cheese.

Temperature (°C)	dF/dt (days) Tolerant Criterion						Q_{10}	Ea (kJ/mole)
	pH	Acidity	Lactose	Peroxide value	NH_3	Average without NH_3		
3	31.7	33.3	31.8	36.2	n.d.	33.2		
10	9.9	9.7	13.0	14.7	28.9	11.8	3.3	79.2
20	4.0	4.0	4.0	<1.0	3.0	3.0		
Temperature (°C)	d^2F/dt^2 (days) Prudential Criterion						Q_{10}	Ea (kJ/mole)
	pH	Acidity	Lactose	Peroxide value	NH_3	Average without NH_3		
3	27.9	28.2	19.7	33.1	n.d.	27.2		
10	5.7	2.7	8.0	5.5	16.2	5.5	8.2	138.3
20	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0		

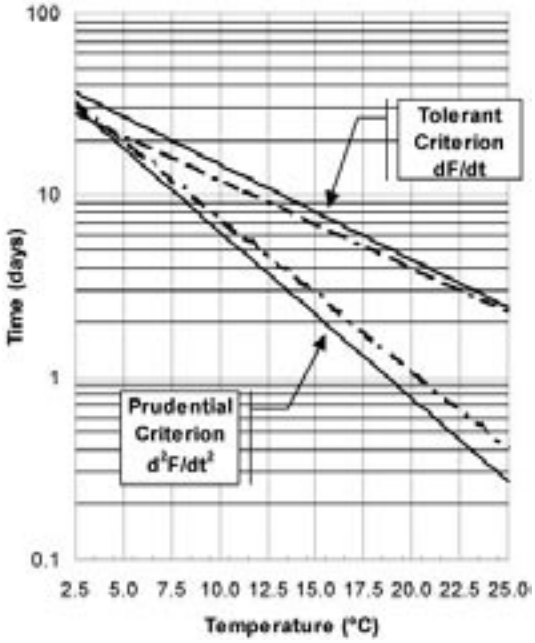


Fig. 7 - Time-Temperature Tolerance Plot.

Data obtained from microbiological and chemical-physical analyses on the samples were also subjected to multivariate statistical analysis, resulting in a loadings score plot (Fig. 8) for the first two principal components with 79% explained variance.

As expected, data processing demonstrated that the indices most involved in discriminating the evolution of Ricotta cheese degradation at 10°C were acidity, pH, lactose, peroxide value and ammonia.

Automatic routine calculation was used to predict the trend of Ricotta cheese storage under fluctuating temperature conditions. Kinetic parameters for activation energy and Q_{10} calculated previously were used to obtain the extent of "used or lost" shelf-life at given time-temperature combinations (Fig. 9). From the graph and analytical data it can be seen that,

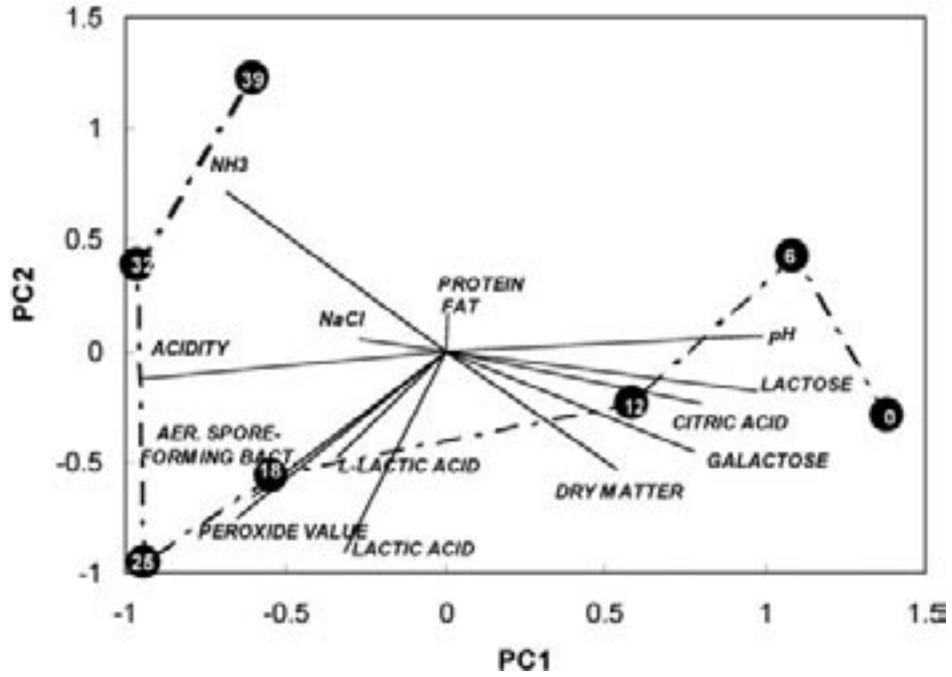


Fig. 8 - PCA biplot for chemical-physical and microbiological results of Ricotta cheese stored at 10°C.

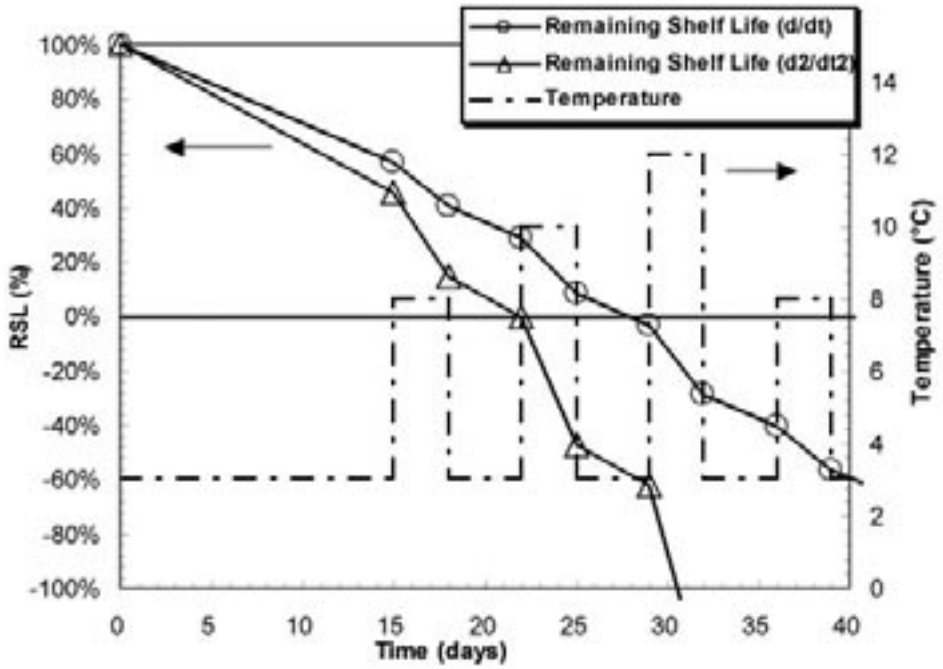


Fig. 9 - Residual shelf life (RSL %) of Ricotta cheese values calculated for lactose.

under the above conditions, the shelf-life should be "used" within 22-27 days according to the prudential and tolerant criterion, respectively. Keeping Ricotta cheese at temperatures higher than 3°C resulted in a decrease in the shelf-life by 30% from about 30 to about 20 days.

Principal component analysis (PCA) was also carried out on the volatile compounds detected by SPME-GC-MS and selected as a function of their discriminant ability.

The biplot of loadings and scores showed that most acids and alcohols were responsible for sample discrimination along the first principal component, whereas aldehydes and ketones were less important (Fig. 10). Hence, it may be supposed that fatty acids such as butyric, acetic and 4-methylpentanoic accumulated in Ricotta cheese samples at the end of storage at 10 and 20°C as a result of fermentation and lipolytic activity of enzymes, especially microbial enzymes (Bergey's Manual of Determinative Bacteriology, 1974). Similarly, primary alcohol accumulation may be correlated to a progressive decrease in peroxide value as a result of the reduction, in the absence of oxygen, of aldehydes derived from hydroperoxide cleavage (Dumont and Adda; Stocchi and Capella, 1972). Diacetyl and acetoin, characteristic compounds of milk fat aroma, were also detected.

Samples showing acceptability characteristics, i.e. those stored at 3°C for up to about 25 days and at 10°C for up to 6-7 days, were grouped on the left side of the graph. The location of these samples opposite the fatty acids, demonstrated their good characteristics.

This result confirmed data obtained from chemical-physical determinations. It also suggested that, in the case of short shelf-life cheese such as Ricotta cheese,

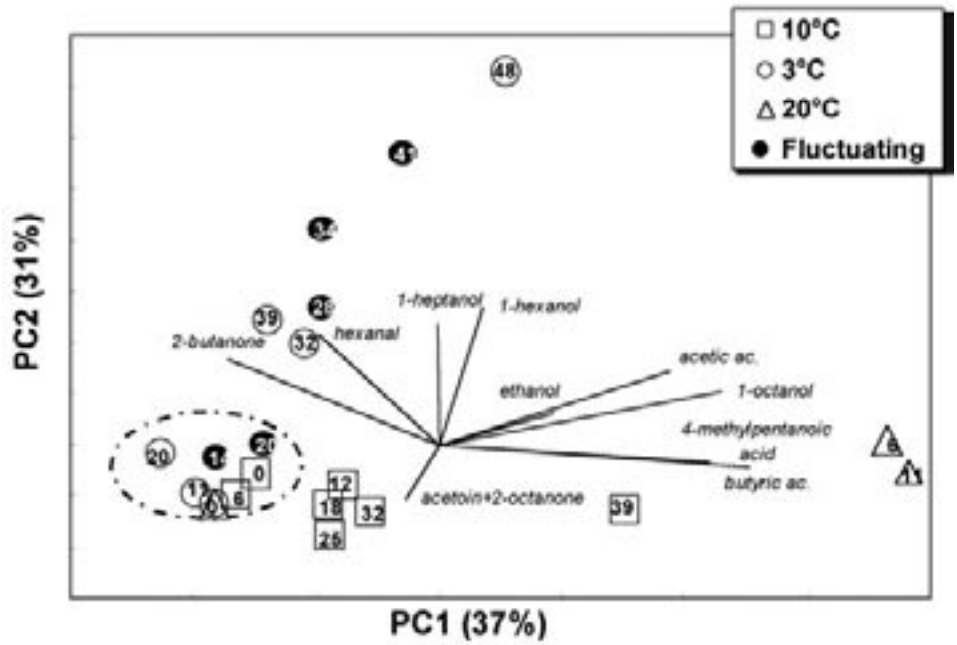


Fig. 10 - PCA biplot for 10 selected volatile compounds and Ricotta cheese samples stored at 3°-10°-20°C and at fluctuating temperature.

only the prudential criterion (Fig. 7) from the time-temperature tolerance card, should be taken into account. As a consequence, the onset of degradation caused such a loss of freshness and acceptability that Ricotta cheese could no longer be marketed.

With reference to the whole aromatic fraction determined by the Electronic Nose, responses from 6 out of 22 sensors (MOS 101, MOS 114, MOS 116, MOS 117, MOS 118 and MOSFET 103A), which were mostly involved in evaluating the aromatic evolution of Ricotta cheese, were used (upon application of principal component analysis). From the score plot for samples on the plane of the first two principal components (Fig. 11) it can be seen that the first principal component (PC1) was mainly involved in discriminating between fresh and degraded. In addition, Fig. 11 showed a localised set of samples, which was similar to that obtained from SPME-GC-MS analysis of the most volatile fraction (Fig. 10) and corresponded to samples stored at 3°C for 3-4 weeks and 10°C for about 1 week. As a result, it was verified that there was a correlation between the results obtained from both methods for the evaluation of Ricotta cheese flavour. Data derived from SPME-GC-MS and Electronic Nose were subjected to combined multivariate analysis (Fig. 12).

Selected MOS-type sensors seemed to be particularly sensitive to butyric acid and 4-methylpentanoic acid, which are indicative of fermentation and lipolytic degradation. MOSFET 103A sensor seemed to be correlated to the development of acetoin.

Fig. 12, which also represented the projection of sample scores, the confirmed classification of samples according to their ageing degree, as shown in Fig. 11.

PCA results of samples at fluctuating temperature, as for the SPME (Fig. 10)

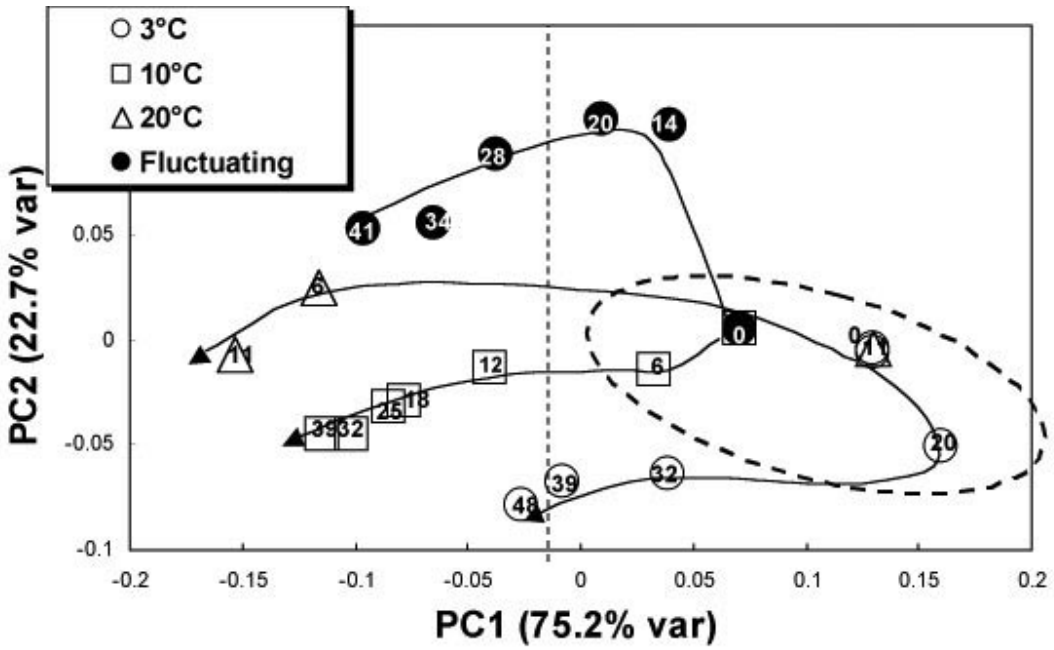


Fig. 11 - PCA biplot, after normalisation, for six selected sensors and Ricotta cheese samples at different storage times at 3°-10°-20°C and at fluctuating temperature.

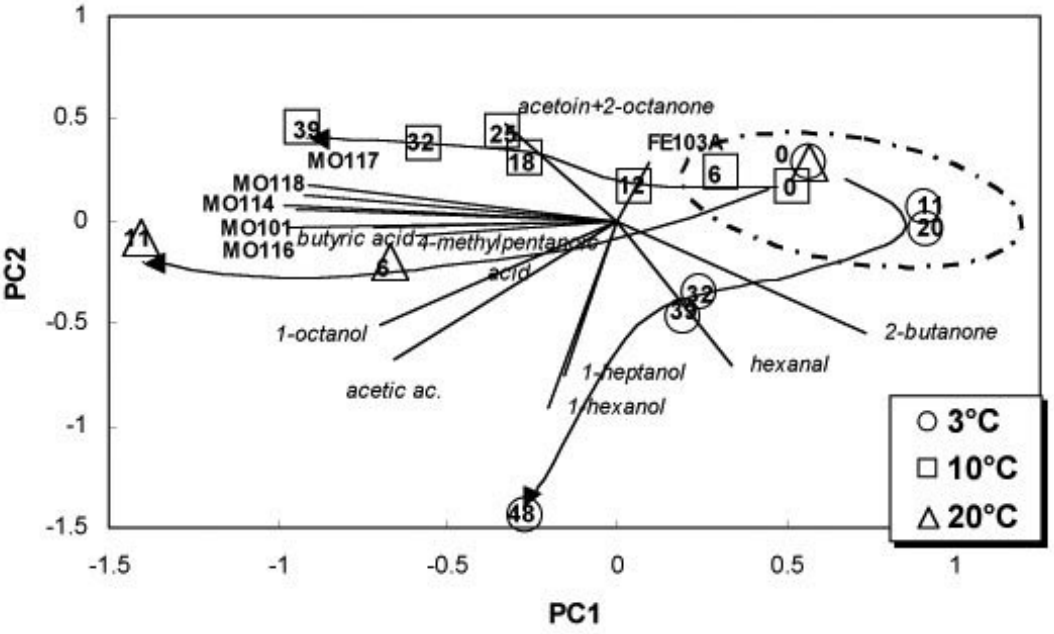


Fig. 12 - Combined PCA (69.3% of variance explained) for volatile compounds from SPME-GC-MS and sensor responses from Electronic Nose.

and Electronic Nose (Fig. 11) data, showed a progressive sample shift, along the axis of the second and first component, respectively which became notable after 28 days of storage at 3°C for 14 days.

Results derived from both compounds carried out for selected volatile aromas and sensor responses, showed that 8°-10°C can be considered the maximum temperature for improper storage.

CONCLUSIONS

Analysis of data obtained from a large number of analytical, chemical-physical, microbiological and flavour determinations allowed us to identify a maximum shelf-life as a function of the storage temperature. According to these results, Ricotta cheese stored at 3°C for 27 days and at 10°C for 7-10 days was always a marketable product. For storage of products at temperatures higher than 8°-10°C, research showed that any exposure, though restricted to 1-2 days, resulted in product unacceptability. Overall research confirmed that traditional protocols for shelf-life definition also apply to a very sensitive product like Ricotta cheese. It also provided sound indications on how to define a proper, actual expiration date of the product.

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LECTURE

VOLATILE COMPOUNDS AS INDICATORS OF MICROBIAL SPOILAGE

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ABSTRACT

Modified atmosphere packaging, in combination with low storage temperature, is one of the packaging technologies available today to prolong the commercial life of fresh products. However, a lack of tightness of the package and/or its improper storage along the distribution chain can sometimes lead to a marked quality decay, mainly due to proliferation of spoilage micro-organisms, naturally present on the product itself or selected by the particular packaging technology adopted. In absence of colour changes or discoloration on the product surface or a relevant gas production, which brings about package swelling, consumers are not able to judge the food quality. Therefore, it could be very useful to design indicators, whose colour changes by reaction with volatile compounds, produced by the micro-organisms metabolism and correlated with the micro-organisms proliferation.

In this work, a cooked ham was selected as the reference food product of the wide category of cured sliced meat products, usually sold in the supermarket and packaged under modified atmosphere. The ham was sliced in the laboratory and inoculated by dipping in a solution of NaCl 1% containing $2x10^5$ CFU/mL of a lactic bacteria suspension. Two strains of lactic acid bacteria, isolated from the same product in a preliminary experiment, were used. After the inoculation, the sliced ham was packaged in air in high barrier pouches and stored at 4°C for 1 month together with a non-inoculated reference. Every week the following controls were

performed on 4 replicates for each sample: microbial count, pH and the composition of the pouch head-space. The following growth media were used for the microbial count: PCA (Oxoid), for the growth, in presence of glucose, of the aerobic microorganisms; TSA (Oxoid), for the growth of the aerobic microflora, in absence of glucose and MRS (Oxoid), for lactic acid bacteria. The analysis of the pouch head-space was performed by means of SPME technique, using DVB/Carboxen/PDMS fibres. The analysis was carried out by first conditioning the pouches containing the inoculated and reference ham at 25°C for 1 h in a oven and then introducing the fibres into the pouches and exposing them for 40 min at the same temperature. The volatile compounds adsorbed in the fibres were desorbed directly into the gas chromatograph/mass spectrometer injector at 225°C for 3 min (splitless mode).

During storage at 4°C, both the aerobic microorganisms and the lactic bacteria increased and reached its maximum (around 10^7-10^8 CFU/g) after 3 weeks of storage. In particular, the growth curves of the lactic bacteria were very similar for both the inoculated samples and reference.

The composition of the pouch head-space varied greatly, both in those containing the inoculated ham and in the reference ones. Quite good correlation coefficients (R value around 0.90) were calculated among lactic acid bacteria growth in inoculated ham and 2-butanone 3-hydroxy, acetone and 2,3-butanedione. In the reference samples, a very good correlation (R around 0.95) was found among the microbial proliferation and 2-butanone3-hydroxy and ethanol.

In conclusion, the growth curves of lactic acid bacteria were quite the same on inoculated and reference ham, but the amount and type of volatile compounds were different. This means that lactic acid bacteria are usual contaminants of cooked ham, but different strains or species can grow, as a consequence of a selection due to different packaging conditions (modified atmosphere or air packaging). It is also possible to hypothesize that acetone and 2,3-butanedione for inoculated ham, and ethanol for the reference samples, could be useful to design possible indicators to monitor the hygienic quality of this particular food product along its distribution chain.

LECTURE

INFLUENCE OF THE OXYGEN BARRIER PROPERTIES OF THE PACKAGE ON THE SHELF-LIFE OF EXTRA VIRGIN OLIVE OIL

INFLUENZA DELLA BARRIERA ALL'OSSIGENO DELL'IMBALLAGGIO SULLA SHELF-LIFE DI OLIO DI OLIVA EXTRAVERGINE

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ABSTRACT

The influence of the oxygen barrier properties of the package on the quality decay kinetic of extra virgin olive oil was assessed. In particular, five different plastic bottles with different oxygen barrier properties were investigated: polyethyleneterephthalate (PET), PET including 1% of "oxygen scavenger" (PETA), PET including 3% of "oxygen scavenger" (PETB), PET coated with high barrier resin (PETC) and PET coated with high barrier resin including 1% "oxygen scavenger" (PETD)] and a bottle made of glass. Tests were conducted at room temperature (RT) and at 37°C for 12 months keeping the sample in the dark. The following quality indices were monitored during storage: acidity, peroxide value, K_{232}, K_{270} and organoleptic assessment. Results showed that acidity did not change during storage, except for oil packaged in glass bottles and stored at 37°C. In this case acidity increased by about 50%. K_{232} and peroxide values relative to the extra virgin olive oil packaged in glass bottles, PETC and PETD, were always lower than those of oil pack in PET, PETA and PETB. The values of K_{270} increased for all the investigated samples, whereas the sensory score decreased from 7.69, initial value, to about 6.5, for oils stored at RT, and to about 6.0, for those stored

- Key words: extra virgin olive oil, innovative packaging systems, shelf-life -

at 37°C. Results indicate that the plastic bottles having higher oxygen barrier properties slow down the quality decay kinetic more than extra virgin olive oil packaged in glass bottles, suggesting that PETC and PETD could replace traditional glass bottles.

RIASSUNTO

Sono stati studiati gli effetti di differenti contenitori plastici con differenti proprietà barriera all'ossigeno [polietileneterftalato (PET), PET con l'1% di "oxygen scavenger" inglobato nel film (PETA), PET con il 3% di "oxygen scavenger" inglobato nel film (PETB), PET rivestito con resina ad alta barriera all'ossigeno (PETC) e PET con l'1% di "oxygen scavenger" inglobato nel film e rivestito con resina ad alta barriera all'ossigeno (PETD)] e del vetro sulla stabilità di un olio extra vergine di oliva condizionato al buio, a temperatura ambiente ed a 37°C, durante 12 mesi di conservazione. Per la valutazione della shelf-life degli oli, confezionati nei differenti contenitori, sono state effettuate le analisi degli indici di qualità quali acidità, numero di perossidi, K_{232}, K_{270} e panel test. I risultati analitici e sensoriali hanno mostrato che l'acidità è rimasta costante per tutti gli oli ad eccezione di quello condizionato in vetro a 37°C il quale ha mostrato, dopo 1 anno di conservazione, un incremento del 50%. Il numero di perossidi ed il K_{232} sono risultati più bassi per gli oli conservati in contenitori con maggiori proprietà barriera all'ossigeno (vetro, PETC e PETD) rispetto a quelli conservati in contenitori caratterizzati da minori proprietà barriera all'ossigeno (PET, PETA e PETB). Il K_{270} è aumentato per tutti gli oli condizionati nei differenti contenitori ad entrambe le temperature di conservazione. Il punteggio al panel test è sceso dal valore iniziale di 7,69 a circa 6,5 per gli oli conservati a temperatura ambiente e a circa 6,0 per quelli conservati a 37°C. I nostri studi indicano che i contenitori aventi maggiore proprietà barriera all'ossigeno rallentano la cinetica di decadimento dell'olio extra vergine d'oliva in essi condizionati. Pertanto i contenitori PETC e PETD potrebbero sostituire vantaggiosamente il vetro nel confezionamento degli oli extra vergini di oliva per i loro indiscussi vantaggi soprattutto commerciali, quali una più facile movimentazione ed una riduzione dei rischi di rottura della confezione.

INTRODUCTION

Due to its peculiar organoleptic and nutritional characteristics, virgin olive oil is the fat substance at the basis of the Mediterranean diet. This kind of vegetable oil has a greater resistance to oxidation if compared to other vegetable oils because of the low content of polyunsaturated fatty acids and the presence of natural antioxidant compounds such as phenols, tocopherols and carotenoids. When virgin olive oil is subjected to oxidation, secondary products such as aldehydes, ketones and alcohols (off-flavours), which are responsible for "rancid" defect, are formed, and can lead to the rejection of the product by the consumer. Consequently, during storage the package must ensure an adequate oxygen barrier to protect the oil from oxidative processes and preserve its peculiar organoleptic characteristics. The most widely used materials for packaging of vegetable oils are glass, tinplate and plastic films. To date, the influence of these materials on oil

quality has not been adequately investigated. De Leonardis and Macciola (1998) made a comparative study on the shelf-life of clear glass-bottled virgin olive oil stored both in the dark and refrigerated condition, and stored with diffuse light at ambient temperature. These authors concluded that after 18 months only the oils stored in the dark maintained good characteristics. Gutiérrez and Fernández (2002) studied the changes of the quality indices of two glass-bottled Spanish extra virgin olive oils stored both in the dark at 2°C, and with illumination (800 lx; 12 h/day) at 30°C, over 6 months of storage. They found K_{270} and sensory evaluation to be the only quality indices that can be used to monitor the loss of the extra quality of the oil. Kiritsakis (1984) compared the oxidative stability of olive oil stored in glass and polyethylene (PE) bottles, concluding that glass ensures a better protection from oxidation. Sharma *et al.* (1990) studied the effects of contacting plastic films, including PE, polypropylene (PP) and butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) incorporated in PP, to refined sunflower and peanut oils on the storage stability at 37°C. These authors found that the changes in peroxide value were less pronounced in the presence of plastic films than in control samples. Moreover, they observed a migration for both BHT and BHA from plastic films into vegetable oils during storage. Npka *et al.* (1990, 1992) observed that crude palm oil, packaged in clear plastic bottles, sealed PE film and clear glass bottles, showed higher total oxidation values than oils packed in either lacquered metal or amber and green glass bottles. Tawfik and Huyghebaert (1999) evaluated the effect of the following on the stability and quality of olive, sunflower and palm oils: type of packaging material, oxygen permeability, storage time and temperature and antioxidants. Kaya *et al.* (1993) studied the effect of permeability and transparency of PET and glass bottles on the quality of sunflower and olive oils, evaluating their oxidative stability by means of the peroxide values. They concluded that stability during storage increased in the following order with respect to packaging materials: PET<clear glass<coloured glass.

The aim of this work is to evaluate the possibility of substituting glass bottles, traditionally used for packaging extra virgin olive oil, with innovative packaging systems. The innovative packages tested were of different types: passive (PET coated with high barrier resin), active (PET including "oxygen scavenger") and mixed (PET coated with high barrier resin including "oxygen scavenger").

MATERIALS AND METHODS

Materials

Filtered oil of "Cima di Bitonto" cultivar, which is used as base for producing the Protected Designation of Origin (PDO) extra virgin olive oil "Terra di Bari", was used. It was extracted in industrial mills by three-phase continuous system.

Plastic bottles (1.0 L and 5 dm^2) with screw cap, having different oxygen barrier properties, were provided by SIPA (SIPA S.p.A., Vittorio Veneto, TV, Italy). Green glass bottles (1.0 L and 5 dm^2) with screw cap having total oxygen barrier were used as control.

Experimental design

Oil (252 L) was transferred into the glass bottles and the plastic packages PET, PETA, PETB, PET PETC and PETD, leaving 2% headspace. For each type of

material used in this investigation, 18 bottles were stored at room temperature (RT) and 24 bottles were stored at 37°C, both were stored in the dark. The bottles stored at RT were sampled every month for 1 year, whereas those stored at 37°C were sampled every 15 days during the first 7 months, and then every month until 1 year of storage.

Analytical determination

Acidity, peroxide value (PV), specific extinction coefficients K_{232} and K_{270} and sensory analysis were carried out according to the European Official Method of Analysis (EC 1991). In particular, organoleptic assessment was performed by a panel consisting of a panel head, soundly trained expert in the various types of oil, and 8 qualified virgin oil tasters. The descriptive analysis used a six-point intensity scale, ranging from 0 (no perception) to 5 (extreme). Overall grading used a nine-point scale, ranging from 1 (the lowest quality) to 9 (maximum quality).

RESULTS AND DISCUSSION

Table 1 lists the initial characteristics of the investigated extra virgin olive oil, whereas Table 2 shows material and initial oxygen permeability of each of the investigated plastic packages.

Evolution in acidity

Fig. 1a and 1b show the evolution during storage of acidity of the samples stored at RT and at 37°C, respectively. After 1 year of storage all the oils maintained the initial values except the sample stored at 37°C in glass bottles, which exhibited an increase from the 195th day to the end of the storage period (from 0.38 to 0.49

Table 1 - Initial characteristics of oil (average value ± standard deviation, n = 2).

Acidity (g/100 g)	0.32±0.01
Peroxide value (meqO_2/kg)	8.72±0.10
K_{232}	1.77±0.00
K_{270}	0.095±0.001
Sensory evaluation	7.69±0.36

Table 2 - Initial values of oxygen permeability of the investigated packages (average value ± standard deviation, n = 10).

Material	Permeability cm^3/(package day)
PET	0.082587±0.021554
PET + 1% "Oxygen Scavenger" (PETA)	0.069357±0.071483
PET + 3% "Oxygen Scavenger" (PETB)	0.033983±0.035070
PET + "Coating" (PETC)	0.023516±0.014441
PET + 1% "Oxygen Scavenger" + "Coating" (PETD)	0.012768±0.048595

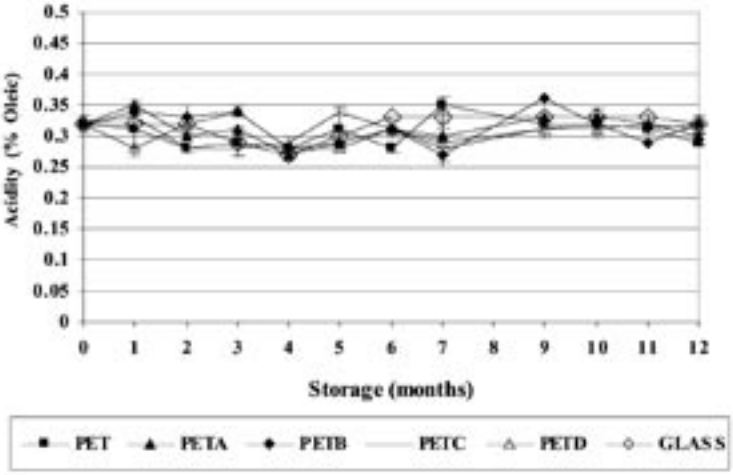


Fig. 1a - Evolution in free acidity in the oils stored at RT at different times of storage.

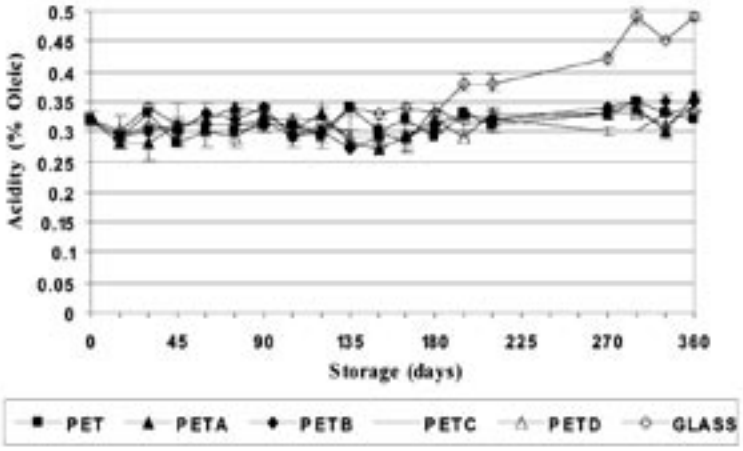


Fig. 1b - Evolution in free acidity in the oils stored at 37°C at different times of storage.

g/100 g). This finding agrees with the results reported by Paganuzzi *et al.* (1997). With respect to this quality index, all the samples preserved their extra quality (acidity less than 1%) after 1 year.

Evolution in peroxide value

The evolution in PV of oils stored at RT and at 37°C is reported in Fig. 2a and 2b. PV of oils packed in PET, PETA and PETB at RT slightly increased from the 9th month, reaching 11 meqO_2/kg at the end of storage, whereas PV of oils packed in glass, PETC and PETD did not change substantially. Concerning the oils stored at 37°C, at the end of the storage period those packed in glass, PETC and PETD showed lower values in PV if compared to the initial value (6.53-8.00 meqO_2/kg),

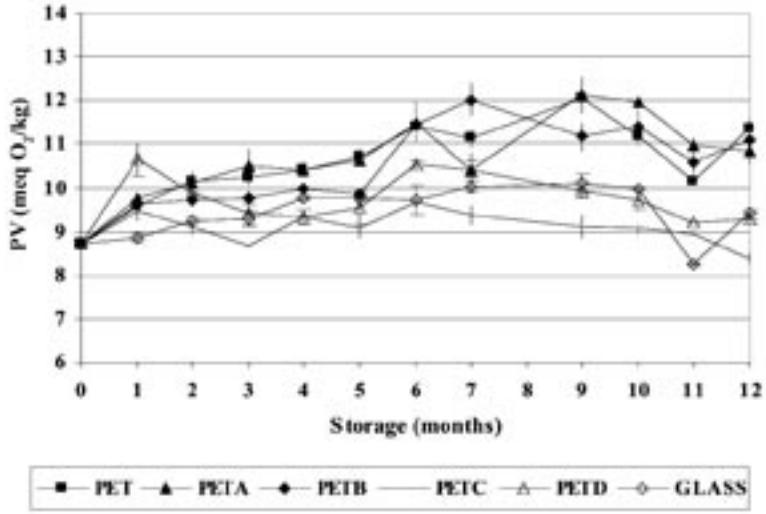


Fig. 2a - Evolution in peroxide value in the oils stored at RT at different times of storage.

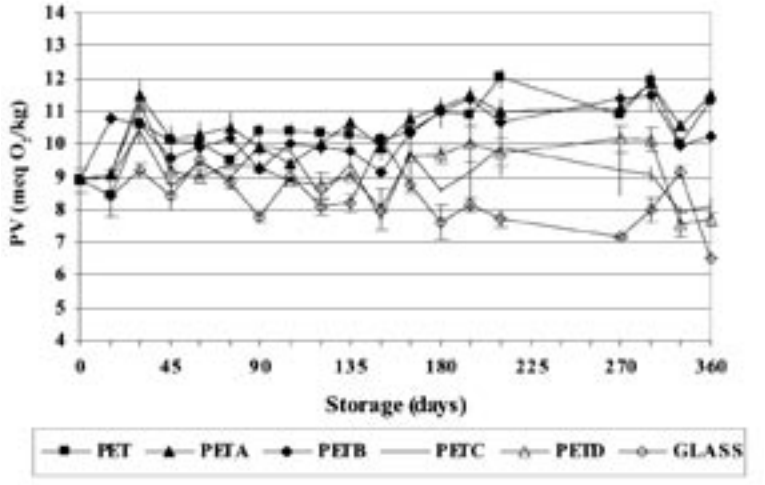


Fig. 2b - Evolution in peroxide value in the oils stored at 37°C at different times of storage.

whereas those packaged in PET, PETA and PETB showed higher values (10-11 meqO_2/kg). In particular, oil stored in glass bottles always exhibited the lowest PV values during storage time. On the whole, this quality index did not change or slightly decreased for oils in glass, PETC and PETD, stored in darkness both at RT and 37°C. These results agree with those reported by De Leonardis and Macciola (1998) and by Gutierrez e Fernandez (2002) for oils stored in similar conditions. The slight decrease in PV of the oil stored in packages having lower oxygen permeability could be attributed to the prevalence of hydroperoxide degradation with respect to their formation. In fact, the lower oxygen permeability coefficient of these materials limits oxygen diffusion into the packaged oil, reducing the hydroperoxide

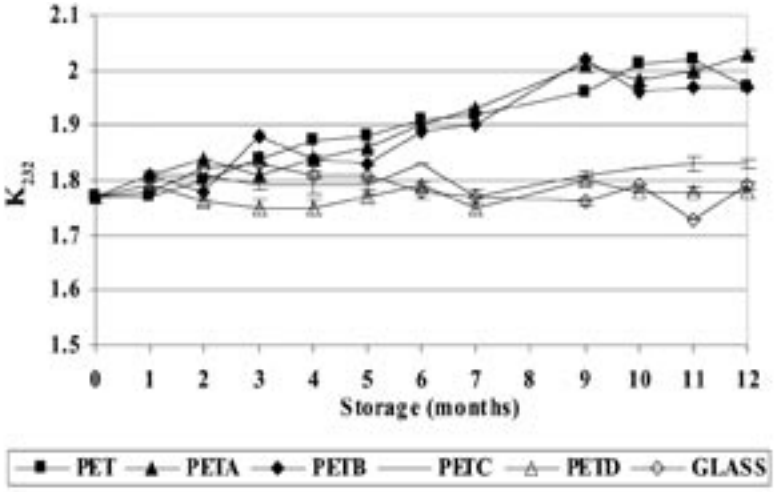


Fig. 3a - Evolution in K_{232} coefficient in the oils stored at RT at different times of storage.

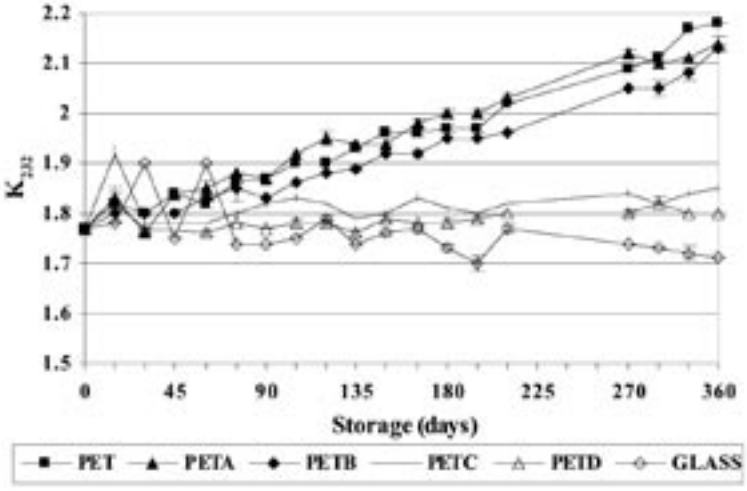


Fig. 3b - Evolution in K_{232} coefficient in the oils stored at 37°C at different times of storage.

production rate. As in the case of acidity, after 1 year of storage all samples had a PV lower than 20 meqO_2/kg, maintaining their extra virgin quality.

Evolution in specific extinction coefficients K_{232} and K_{270}

Fig. 3a and 3b show the evolution of K_{232} of the samples stored at RT and at 37°C, respectively. The K_{232} coefficient increased in oils stored in PET, PETA and PETB at both storage temperatures, ranging from 1.77 (initial value) to about 2.00 (final value), whereas in oils stored in glass, PETC and PETD, the values remained substantially unchanged. This behaviour was marked for the oils stored at 37°C, in fact, K_{232} of PET-, PETA- and PETB- bottled oils reached values higher than 2.1 after 1 year of storage,

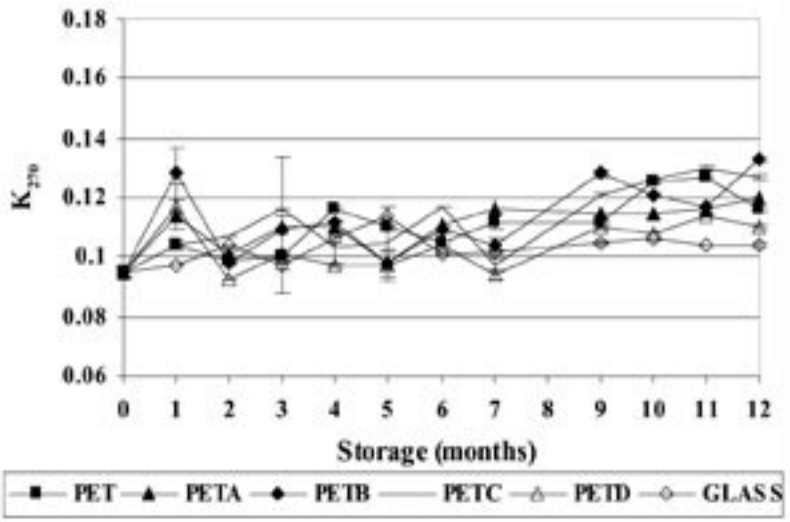


Fig. 4a - Evolution in K_{270} coefficient in the oils stored at RT at different times of storage.

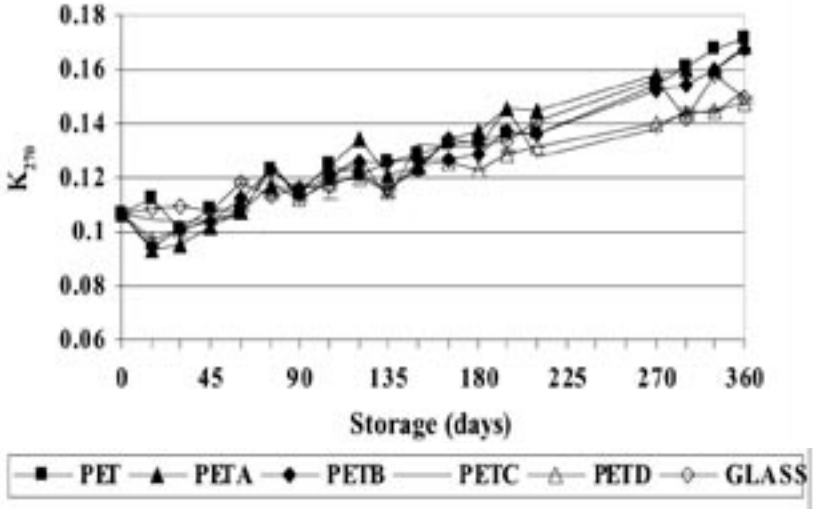


Fig. 4b - Evolution in K_{270} coefficient in the oils stored at 37°C at different times of storage.

whereas for glass, PETC and PETD, no significant change was observed. The above results suggest that the low oxygen permeability of these latter materials allows the K_{232} and PV values to remain unchanged. As far as K_{270} evolution of the oils stored at RT is concerned, a slight increase was observed for all packages tested (Fig. 4a) and oil stored in glass bottles showed the lowest values. For the samples stored at 37°C, the increase was higher than that observed for the oils stored at RT, ranging from 0.095 (initial value) to 0.148 (oil in glass) and to 0.171 (oil in PET) (Fig. 4b). Similar results have been reported by Olias *et al.* (1981) and by Gutierrez *et al.* (1988) for oils stored in different packages at 30°C. Considering the influence of the material, for

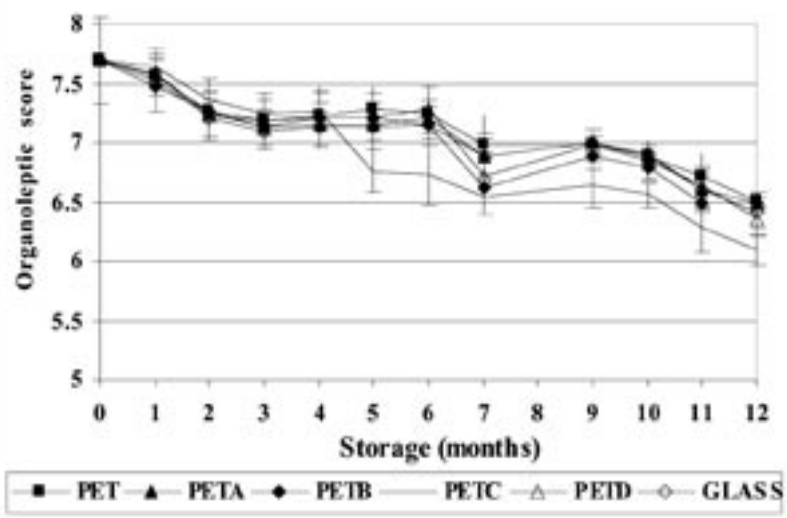


Fig. 5a - Evolution in sensory score in the oils stored at RT at different times of storage.

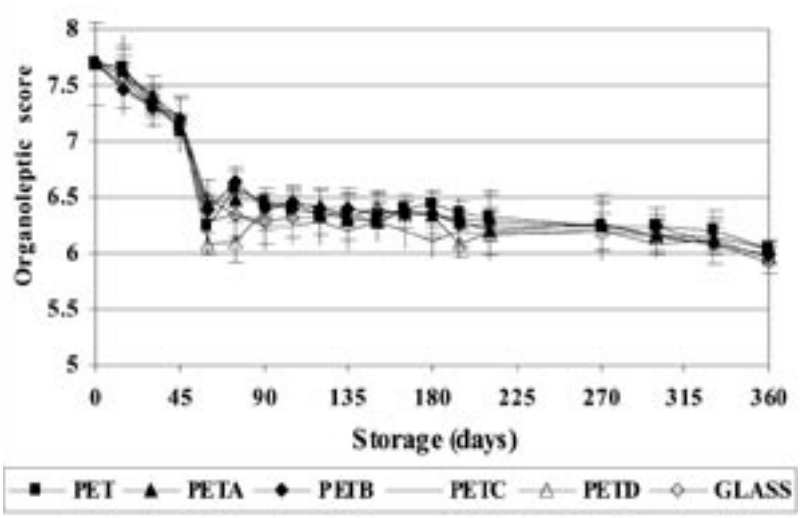


Fig. 5b - Evolution in sensory score in the oils stored at 37°C at different times of storage.

the oils stored in glass, PETC and PETD the K_{270} increase was lower than the other packages. Also in this case such behaviour has to be attributed to the lower amount of oxygen that penetrated into the package because of the high barrier properties of these materials. Nevertheless, for all packages tested and for both the temperatures of storage, the extra virgin quality was preserved after 1 year (<2.5 and <0.2 for K_{232} and K_{270}, respectively).

Evolution in sensory score

Fig. 5a and 5b show the evolution of sensory score of the samples stored at RT and at 37°C. All oils stored at RT had similar sensorial characteristics, which, as one

would expect, tended to decrease with time. After the 5th month of storage, the PETB had slightly lower scores. After 1 year of storage, all samples obtained scores higher than 6.0. For oils stored at 37°C, already after 2 months the scores decreased by about 1 (6.08-6.48) with respect to the initial values. In the successive stages of storage the sensory score did not significantly change, showing only a slight decrease. The decrease observed at 2 months was mainly due to the loss of the fruity characteristics. A similar behaviour was observed from the 40th day of storage by Gutierrez and Fernandez (2002) for Spanish oils stored at 30°C with illumination. On the whole, after 1 year of storage all oils stored at RT obtained the minimum for extra virgin quality (6.5), except the sample stored in PETB (about 6.1, virgin quality), whereas the samples stored at 37°C were all classified as virgin olive oil (scores below 6.0).

CONCLUSIONS

The results of the investigation indicate that among the packages tested, those having high oxygen barrier properties (glass, PET coated with high barrier resin and PET coated with high barrier resin including "oxygen scavenger") maintained almost unchanged the values of the oxidation primary compounds (PV and K_{232}) of the oils, both at RT and 37°C. Moreover, these packages retard formation of the oxidation secondary compounds (K_{270}). No significant differences in the organoleptic properties were observed by the tasters among the oils stored in the different packages. Our experimentation indicate that PET coated with high barrier resin and PET coated with high barrier resin including "oxygen scavenger" could replace glass as packaging for extra virgin olive oils, due to their similar properties, also considering other advantages, such as easy handling and low risk of package breakage.

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WATER VAPOUR BARRIER PROPERTIES OF BIODEGRADABLE FILMS

PROPRIETÀ BARRIERA AL VAPOR ACQUEO DI FILM BIODEGRADABILI

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ABSTRACT

Biodegradable polymers are rarely used in food packaging applications due to their low moisture barrier properties. In order to improve this point, it is important to understand the influence of chemical structure and processing conditions on their barrier properties. Biodegradable polyesters, were investigated by means of water permeability, crystallinity level, thermal and mechanical tests. When the chemical structure changes, despite a crystallinity level decrease, water vapour permeability values diminish because the amorphous phase characteristics improve. Two different process conditions were investigated: aluminium deposition and co-extrusion; both lead to permeability enhancement.

RIASSUNTO

I materiali biodegradabili sono attualmente poco utilizzati come packaging alimentare a causa delle loro scarse proprietà barriera al vapor acqueo. Quest'ultime dipendono principalmente dalla natura chimico-fisica della matrice polimerica che costituisce l'imballaggio. Il presente lavoro ha lo scopo di valutare come le proprietà barriera di film biodegradabili possano essere migliorate agendo sia sulla struttura chimica della matrice polimerica sia sul processo di produzione. Il primo obiettivo è stato perseguito analizzando film a base di poliesteri, sia campioni omopolimerici sia blend. Di tutti i campioni sono state determinate le proprietà termiche e meccaniche, quelle barriera al vapor acqueo ed il livello di cristallinità. I risultati ottenuti hanno evidenziato che, nonostante la diminuzione di cristallinità, la proprietà barriera delle blend aumenta grazie al miglioramento delle caratteristiche della componente amorfa. L'effetto del processo è stato invece valutato analizzando i medesimi materiali ma metallizzati, nel caso degli omopolimeri, o coestrusi, per le blend. È stato evidenziato in entrambi i casi un miglioramento delle proprietà barriera dei materiali testati.

- Key words: biodegradable, permeability, water vapour -

INTRODUCTION

Traditional polymeric materials are obtained from petrochemical industry, and when discarded as waste, they are not naturally decomposing; so they are considered to cause environmental problems. For this reason, many scientists have developed various alternative materials, with particular attention given to biodegradable polymers (1).

Biodegradable plastics are a newly emerging class of polymers; the interest for these materials is growing because of their usefulness and suitability in many applications (1). At the present, biodegradable materials have rarely been used in food packaging applications due to their low barrier properties to low molecular weight compounds, especially to moisture. In fact, one of the most important characteristics of polymeric materials, intended for food packaging applications, is the permeability coefficient, which is strictly related to packaged foodstuff shelf-life. In fact, packaging materials controlling the rate at which small molecular weight compounds permeate into or outside the package, are able to extend the shelf-life of packaged food. In order to improve biodegradable polymer barrier properties, it is important to understand the relationships between the physical and chemical characteristics of these materials and their mass transport properties of low molecular weight compounds (2).

Therefore, it is critical to know what kind of polymer characteristics influence and control the permeability coefficient: polymer preparation methods and processing conditions, as well as crystallinity level and chemical and physical properties (3). It has been shown that an increase of crystallinity, density, molecular weight or crosslinking reduces polymer permeability (4) (5).

The aim of this paper is to evaluate the effect of the chemical structures and processing conditions on the barrier and mechanical properties of biodegradable polymers.

MATERIALS AND METHODS

Materials

The different biodegradable polymers were supplied by Novamont S.p.a. and for the sake of simplicity, they will be referred to as follows:

Sample A and Sample B: two homopolymeric biodegradable polyesters;

Sample C and Sample D: the polymeric blends obtained mixing Sample A and Sample B with an aliphatic biodegradable polymer G, respectively;

Sample E: obtained by aluminium deposition on Sample A;

Sample F: obtained by means of co-extrusion of Sample A and Sample G.

Measurements

Thermalgravimetric analysis was carried out with TGA 2950 (TA Instruments) by increasing the temperature from 25° to 600°C at the heating rate of 20°C/min. Analyses were performed in nitrogen atmosphere and under air. Degradation temperatures were calculated as the inflection point of the curve.

Main thermal transitions of the samples were assessed by a differential scanning calorimeter DSC 2920 (TA Instruments) cooled with the RCS cooling system.

Samples were cut from the film and placed in sealed aluminium pans. The pan was hermetic to prevent water evaporation during scanning. A first scan was performed between -40°C to 250°C, the sample was cooled to -40°C and then rescanned to 250°C. All the heating and cooling measurements were carried out at a rate of 10°C/min. An empty pan was used as reference and the glass transition was computed using the inflection point as the midpoint of the step transition. Crystallization and melting temperatures were determined as the temperature at the maximum value of crystallization and melting peaks.

X-ray diffraction tests were performed with a Philips X-ray generator and a Philips diffractometer. The X-ray beam was nickel-filtered Cu-Kα radiation with a wavelength of 1.54 Å. Diffraction intensity data were collected in $2\theta = 5°\text{-}60°$ range; for each 2θ value the intensity determination was repeated six times; hence, each diffraction pattern consists of six different sets of data. Samples crystallinity percent was quantitatively estimated from relative areas of crystalline and amorphous regions: six sets of data were simultaneously fitted with a series of Gaussian functions.

Young's modulus films were determined by means of a Universal Testing Machine (Instron 4204), at room temperature and with a crosshead speed of 1mm/min. Five replicates of each sample investigated were performed (ASTM D638 – 02).

Permeability measurements were performed with a Permatran Mocon (Model W 3/31); samples with a surface area of 5 cm^2 were tested at 25°C. Permeation tests were conducted by keeping the relative humidity at the downstream side of the film equal to zero, and changing the relative humidity at the upstream side of the film from 0.35 to 0.90. In particular, the permeation tests were run setting the relative humidity at the upstream side of the film equal to: 0.35, 0.45, 0.55, 0.65, 0.75, and 0.90. A flow rate of 100 ml/min of a nitrogen stream was used.

RESULTS AND DISCUSSION

Chemical and physical properties

Thermal stability of polyesters A and B were investigated by means of TGA. Results, reported in Table 1, show that polymers A and B degrade at 410° and 445°C, respectively. Main thermal transitions, determined by DSC, are listed in Table 2. Melting temperature of polymers A and B are 66° and 74°C, respectively; Sample B enthalpy (83 J/g) is less than the polymer A one (95 J/g). The results indicate the presence of a different crystallinity level, as confirmed by X-ray diffraction pattern. Fig. 1 presents the X-ray diffraction scan for homopolymers A and B. Both present two crystallinity peaks at the same angles (21.5° and 24.5°) but the crystallinity percent is different: 52% for Sample A and 36% for Sample B (Table 3). Data agree with values of Young's modulus: 230 MPa for polyester A and 180 MPa for the other polymer (Table 3). As foreseen, an increase of crystallinity gave an improved Young's modulus.

When homopolymers A and B were blended with the polyester G, samples C and D were obtained, respectively. Thermal stability analysis showed two different degradation temperatures, one for each element of the blend; TGA data are listed in Table 1. When Sample C and D were investigated by means of DSC (Table 2), curves obtained showed different contributions from the two polymers. Melting

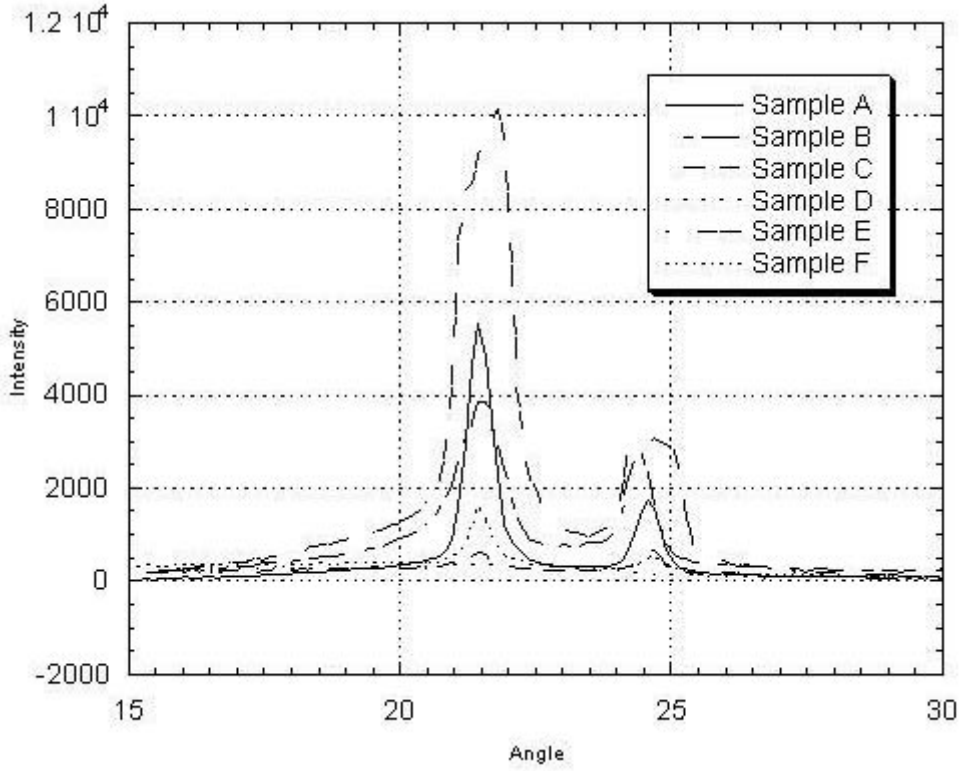


Fig. 1 - X-Ray Diffraction Patterns.

Table 1 - Thermogravimetric Data.

Sample	Nitrogen		Air	
	T deg (°C)	T deg (°C)	T deg (°C)	T deg (°C)
Sample A	-	426	-	410
Sample B	-	460	-	445
Sample C	335	420	335	420
Sample D	330	455	330	400-440
Sample E	-	430	-	420
Sample F	350	420	370	-

temperatures of elements A and B did not change with respect to the value found in homopolymeric material heating scan. Polymer G was characterized by a melting temperature of 154°C and a cold crystallization of 99°C, that shifted to 123°C in the second heating scan. X-ray diffraction patterns (Fig. 1 and Table 3) show a dramatic decrease of crystallinity level when polymers A and B were blended with polyester G. Crystallinity percent was calculated: 12.5% for blend C and <10%

Table 2 - Main Thermal Transition Data.

	First heating scan						
Sample	Tg (°C)	Tf_1 (°C)	ΔHf_1 (J/g)	Tf_2 (°C)	ΔHf_2 (J/g)	Tc (°C)	ΔHc (J/g)
Sample A	0,45	66	95	-	-	-	-
Sample B	-74	83	-	-	-	-	
Sample C	-1,8	65	52	154	12	99	5
Sample D	-73	52	157	12	-	-	
Sample E	-68	100	-	-	-	-	
Sample F	-2,2	67	40	150	3	-	-
	Second heating scan						
Sample	Tg (°C)	Tf_1 (°C)	ΔHf_1 (J/g)	Tf_2 (°C)	ΔHf_2 (J/g)	Tc (°C)	ΔHc (J/g)
Sample A	-	65	85	-	-	-	-
Sample B	-	71	76	-	-	-	-
Sample C	-	64	40	151	10	123	7
Sample D	-	72	33	155	15	120	14
Sample E	-	63	92	-	-	-	-
Sample F	-	64	29	-	-	-	-

for Sample D. In spite of these results, Young's modulus values (Table 3) showed a better behaviour of the material. The Young's modulus of a semi-crystalline polymer gave characteristics of amorphous phase, crystallinity percent and distribution in terms of crystal dimensions. If the distribution is thought to be similar in homopolymeric as well as blend materials, Young's modulus enhancement is related to better amorphous phase properties, because of mixing of homopolymer with Sample G (6).

Biodegradable aliphatic and semi-crystalline polyester A was covered by aluminium coating and the film obtained, sample E, was analysed. Main thermal transitions did not change with respect to data found for Sample A (Table 2). Degradation temperature shifted to values higher than Sample A. (Table 1). X-ray diffraction scan (Fig. 1) showed an increase of crystallinity percent that moved from 52% (Sample A) to 62% (Sample E) (Table 3). Surface treatment and deposition of aluminium vapour modified morphology and properties of Sample E. This behaviour agreed with Young's modulus data: Sample E modulus is higher than polymer A one as shown in Table 3.

Table 3 - Crystallinity Level and Young's Modulus Data.

Sample	Crystallinity %	Young's Modulus (MPa)
Sample A	52	230
Sample B	36	180
Sample C	12.5	1,070
Sample D	<10	270
Sample E	62	320
Sample F	20.3	820

Sample F was a film with the same composition as Sample C, but obtained by means of co-extrusion of Sample A and Sample G. When thermal stability tests

were performed in nitrogen atmosphere, the sample showed two degradation temperatures higher than those for blend C; analysis carried out in air showed a single degradation step (Table 1). The thermal properties also differed from Sample C data; component G melting transition, for instance, disappeared in the second heating scan (Table 2). The X-ray diffraction pattern for Sample F is reported in Fig. 1: it shows the same crystallinity peaks as sample C and a higher crystallinity level value (Table 3). Co-extrusion process led to a decrease of Young's modulus (Table 3) with respect to blend C.

Water Vapour Barrier Properties

Semi-crystalline polymer permeability depends on the crystallinity level of material, in particular, if crystallinity percent increases, permeability value decreases and barrier properties of materials improve (2).

In the present study, permeability tests were performed, by changing the water activity at upstream side of the film from 0.35 to 0.85. For each water activity value, the water vapor transmission rate was measured, then permeability was calculated and the curves obtained are shown in Fig. 2. Permeability is almost constant for Sample A and Sample B:

$$P = P_A \alpha 2 \qquad \text{(Eq. 1)}$$

allows amorphous phase permeability (PA) to be calculated with semicrystalline polymer permeability (P) and amorphous phase volumetric fraction (α); data are listed in Table 4. When homopolymer A and B were blended with polyester G, samples C and D were obtained. Permeability values increased slowly as water activity improved. With respect to Sample A and B, blends C and D showed enhanced barrier properties, in spite of a decreased crystallinity level, as X-ray diffraction patterns have already shown. To explain this result, it is important to analyse the amorphous phase permeability data (Table 4): each blend had a lower amorphous phase permeability value with respect to the homopolymer sample. Notwithstanding the minor crystallinity percent of blends, amorphous phase characteristics seem to get better because of mixing homopolymeric materials with polyester G. The results agree with the mechanical behaviour, discussed above (6).

In Fig. 2, the permeability curve of Sample E is shown and, as forseen, the values are higher than Sample A. Deposition treatment modifies material morphology and the physical-chemical properties: the crystallinity level and Young's modulus improved. It is evident that aluminium coating improved Sample E barrier property, without changing the amorphous phase characteristics (Table 4).

Table 4 - Amorphous Phase Permeability Value.

Sample	Permeability (g cm/cm^2 atm sec)
Sample A	1.57 E-7
Sample B	1.14 E-7
Sample C	1.21 E-8
Sample D	6.46 E-9
Sample E	7.67 E-8
Sample F	2.61 E-8

Regarding Sample F, it can be seen that the mean value of its permeability values is lower than that of Sample A but higher than the Sample C data. The co-extrusion process enhanced barrier properties with respect to homopolymer A, because of improved amorphous phase properties (Table 4). On the other hand, Sample F behaved differently from Sample C: notwithstanding

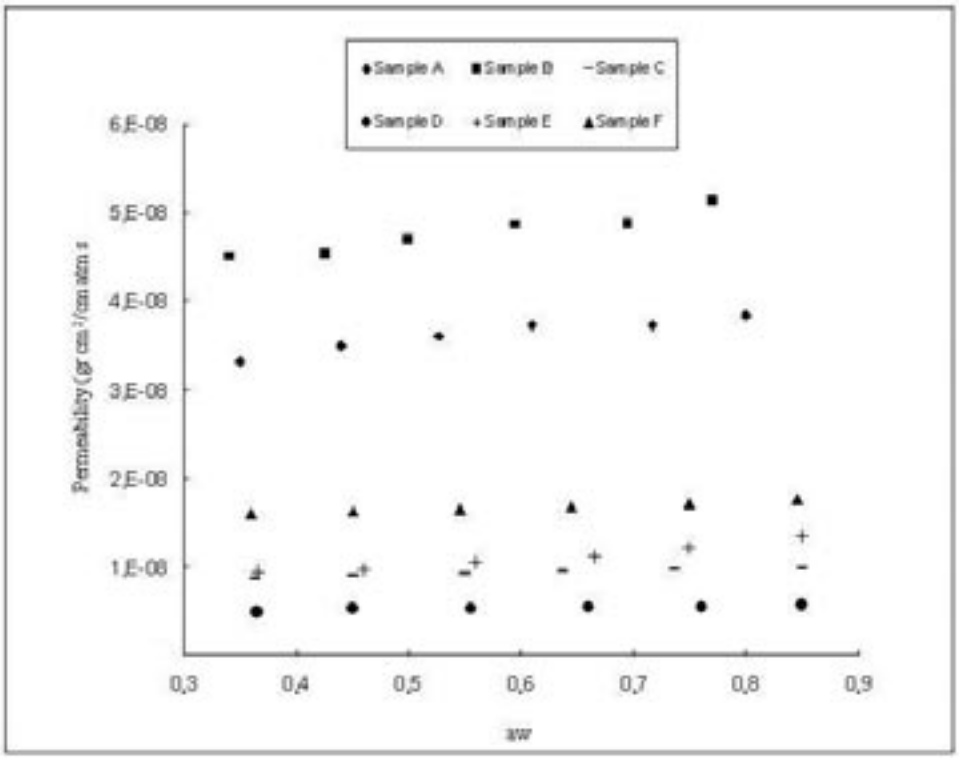


Fig. 2 - Permeability Curves.

the increased crystallinity level, permeability decreased. This was probably due to the fact that Sample F did not have the effect present in Sample C due to the mixing of the two polyesters A and G.

CONCLUSIONS

Biodegradable aliphatic and semi-crystalline polyesters were investigated in order to determine the relationships between their structures and mass transport properties. For each sample water barrier properties, crystallinity level, main thermal transitions and mechanical properties were determined.

In order to understand the influence of chemical composition on transport properties, Samples A, B, C and D were characterized. For Sample A and B a decrease of permeability coefficient was due to an increased crystallinity level. Results for samples C and D show that notwithstanding a decrease of crystallinity level, water permeability values decreased due to the improvement of amorphous phase characteristics, as shown by the Young's modulus.

In order to understand the influence of processing parameters on barrier properties, Sample E and Sample F were studied and compared with Samples A and C, respectively. Results obtained for Sample E show that aluminium coating

improved barrier properties and increased the degree of crystallinity with respect to Sample A, without changing the amorphous phase properties. The results obtained for Sample F pointed out that a co-extrusion process decreased barrier properties and improved mechanical properties, according to Young's modulus.

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LECTURE

SHELF-LIFE OF *BRASSICA RAPA* L. VAR. *SILVESTRIS* IN PROTECTIVE ATMOSPHERE PACKAGING

SHELF-LIFE DI *BRASSICA RAPA* L. VAR. *SILVESTRIS* CONFEZIONATO IN ATMOSFERA PROTETTIVA

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ABSTRACT

The aim of this paper was to describe a methodological approach for the development of a ready-to-eat product: cooked broccoli (*Brassica Rapa* L. var. *silvestris*). Samples of broccoli were husked, washed, stewed, packaged with 6 different mixtures of gas, and then stored at 4±1°C for 14 days. HACCP procedure was performed to identify critical control points during the process. Colour, gravimetric analysis and sensorial profile evaluation were performed to monitor qualitative losses of packaged samples during shelf-life. The volatile head-space of samples was analyzed by GC/MS.

RIASSUNTO

L'applicazione del confezionamento in atmosfera protettiva consente di estendere la shelf-life dei prodotti alimentari, per limitazione di processi chimici e biologici che ne causano il deperimento. Nel presente lavoro è stato scelto come prodotto il broccolo (*Brassica Rapa* L. var. *silvestris*). Obiettivo è stato quello

- Key words: broccoli, protective atmosphere packaging, ready-to-eat, shelf-life -

di realizzare un modello di studio multidisciplinare, individuando una miscela gassosa idonea alla conservazione dell'ortaggio per un tempo tale da ottenere un alimento dotato di un elevato livello di convenience e di caratteristiche chimico-fisiche, organolettiche e funzionali analoghe al prodotto appena preparato. I campioni di broccolo sono stati stufati con olio extra-vergine di oliva per 15 min, confezionati in presenza di sei differenti miscele di gas e conservati alla temperatura di 4±1°C per diversi tempi. Analisi microbiologiche hanno garantito la sicurezza d'uso del prodotto ed hanno consentito di individuare e superare, mediante l'applicazione di un piano HACCP, i punti critici del processo. Il rilievo dei fenomeni di decadimento qualitativo è stato condotto mediante analisi colorimetrica, gravimetrica e sul profilo sensoriale. I campioni sono stati sottoposti inoltre ad analisi gas cromatografica di tipo quali-quantitativo al fine di determinare la natura e l'incidenza dei componenti volatili che caratterizzano l'aroma e l'odore del prodotto.

INTRODUCTION

In comparison to other foods, packaging of fresh vegetables has different prerogatives. Fruits and vegetables are living-organisms with an elevated metabolic activity which involve oxygen and backup metabolite consumption. Respiration and an elevated transpiration lead to CO_2 and heat release that cause weight and turgidity losses. Moreover metabolic reactions lead to alteration of aspect (colour change due to chlorophyll degradation), physical properties (tissue softening and weight loss due to transpiration and cell wall alteration) and chemical composition (sugar and pectin transformation, or acidity reduction) (Kader *et al.*, 1989). Shelf-life of packaged products depends on different variables tied to food composition and on productive technology, to barrier performances of packaging films and to storage conditions. Protective atmosphere packaging (P.A.P.) technology extends the shelf-life of foods, inhibiting chemical and biological factors responsible for food deterioration. Packaging of these products is usually done in the presence of gas mixtures with N_2, CO_2 and O_2 concentrations different from the air. A suitable choice of gas mixture introduced into the package extends shelf-life and reduces vegetable degradation. That is why different gases, used in packaging technology, act in various ways on food components slowing down degradation reactions. CO_2 inhibits vegetable respiration activity, acidifies tissutalis liquids, inhibits vegetable growth hormones, reduces vegetable cold-damage, can denature some proteins, and inhibits pectin hydrolysis. Nitrogen inhibits some proteases, some lipases and some decarboxylases. Oxygen activates enzymatic and chemical oxidations, activates β-carotene degradation and is a substratum for vegetable and microbial cell respiration (Piergiovanni, 1995). Kader (1985a) defined optimal O_2 and CO_2 concentrations in protective atmosphere packaging in order to preserve fruit and vegetable quality. Moreover Kader and Morris (1977) gave information about tolerance levels for fruit and vegetables stored in protective atmosphere at low O_2 and elevated CO_2 concentrations. Tolerance limits depend on the vegetable, cultivar and storage temperature. If the head-space atmosphere oxygen level falls below the tolerance value or CO_2 surpasses established limits, physiological

and qualitative damage may occur. In the presence of an oxygen level less than 1-2%, aerobic respiration gives way to anaerobic respiration that evolves toward glycolysis and piruvic acid decarboxylation into acetaldehyde, from which it is possible to produce ethanol. For broccoli 1% is the lowest level of tolerance for oxygen, while 10% is the maximum value of tolerance for CO_2 (Kader, 1989). Some fruits and vegetables are not acceptable because of intense off-flavour development due to volatile compounds formed under anaerobic conditions (Kader, 1985b; Kader, 1989). Off-flavours originate from the accumulation of ethanol and acetaldehyde in the package head-space, and from the synthesis of compounds, for example, ethylesters, that have a direct influence on food aroma. Excessive sulphide compound production, in broccoli stored under anaerobic conditions, causes the development of off-flavours (Forney *et al.*, 1991). Moreover, if vegetables were stored in atmospheres with oxygen levels equal (or less than) to 0.5% and CO_2 greater than 15%, it was possible to note a strong odour of methylsulfide and H_2S after a few days of conservation at 5°C. Characteristic compounds of broccoli aroma are hexanal and dimethylsulfide, which confer typical prickly odour to cooked and preserved product. According to Lim (1989), cystine lyase is the enzyme responsible for off-flavour production in cooked broccoli. Barrett (2000) evaluated the concentration of these volatile substances in broccoli, raw and cooked for different times, using GC/MS. In comparison to cooked products, raw broccoli and samples boiled for 45 sec did not show appreciable levels of dimethylsulfide. In cooked samples, hexanal concentrations decreased, if blanched for less than 45 sec, while it was constant if blanched for more than 3 min. Barrett *et al.* (2000) studied colour variations in the samples of broccoli cooked for different times and at different temperatures. Samples blanched for 45 seconds had a less intense coloration than raw samples for the brightness (L*) parameter. Cooking treatments, prolonged for more than 45 sec, reduced brightness and developed a darker green colour. Colour variations in green vegetables, during post-harvest, are related to lipoxygenase activity and oxidation of unsaturated fatty acids. Chlorophyll and carotenoid decolouration is often used as an indicator of lipoxygenase activity (Buckle and Edwards, 1970). Thermal treatments produce further variations in food chromatic profile and are usually responsible for chlorophyll degradation and its conversion to pheophytins, substances that produce the typical grey-green cooked vegetable colour. The aim of the present work was to describe an integrated approach methodology which helps in developing minimal processed vegetables. Stewed broccoli was used as a ready-to-use food in response to customer demand. This choice was based on the importance of ready-to-eat food and its role in fast-food and catering. Commercial success of ready-to-eat products depends on their ability to not appear manipulated and have a high level of convenience.

MATERIALS AND METHODS

Broccoli (*Brassica rapa L.* var. *silvestris*) were selected by inspection of visual characteristics, washed and husked. Broccoli (900 g) were stewed for 15 min in 50 mL extra-virgin olive oil. Packages used, belong to Aerpack line mod. B5.70, had volume equal to 1,500 cc and O_2 permeability at 23°C - 50% U.R. equal to 0.070 $ccatm^{-1}wrap^{-1}\ atm^{-1}$. Packages were welded by laminate film (mod. F410 ANTIFOG) supplied by Coopbox. O_2 permeability of the film, at 23°C

and 0% U.R., is equal to 1.3 $cm^3m^{-2}d^{-1}atm^{-1}$, while CO_2 permeability under the same conditions, is equal to 4.5 $cm^3m^{-2}d^{-1}atm^{-1}$. Nitrogen permeability is less than 1 $cm^3m^{-2}d^{-1}atm^{-1}$ and vapour permeability at 38°C and 98% UR is equal to 5.1 $gm^{-2}d^{-1}$. Packages with 70 g stewed broccoli, filled with gaseous mixtures, were stored at 4±1°C for at maximum of 10 days. Microbial investigations were made to identify and eventually eliminate critical points in process technology (HACCP). In order to study micro-organism evolution in broccoli, investigations on just-cooked samples (t_0) and stored for a week (t_1) in protective atmosphere were performed. The following microrganisms were evaluated: Aerobe Mesophile Total Microflora (MAMT), Aerobes Psychrotrophic Total Microflora (MAPT), Lactic bacteria, Yeasts and Moulds, *Enterobacteriaceae* and *Pseudomonas* spp. To count Aerobe Mesophile Total Microrganism, *Pseudomonas* spp. and Aerobe total Psychrotrophic Microrganism PCA (plate count agar) plates, were used incubated at 30°C for two days and for 10 days. Violet red bile glucose agar (VRBGA) plates was used to determine E. coli, in particular Enterobacteriaceae, and stored at 37°C for 24 h. Lactic bacteria were counted on MRS Agar plates incubated at 37°C for 48 h, Yeast and Moulds, instead, were evaluated on DRBC-Agar incubated at 25°C and examined after 3, 4 and 5 days. Besides appraising microbial evolution in the product during storage, a system, based on HACCP principles, allowed Contamination Points (CP) and Critical Control Points (CCP) to be identified. To reach the objective and to guarantee hygienic-sanitary safety of the product packaged in protective atmosphere, the following samples were analysed:

- whole sample;
- fresh sample, washed and cut, before cooking;
- cooked sample in wrap;
- sample stored in air;
- samples stored in different atmospheres and preserved at 4°C.

Broccoli were then analyzed performing sensory and instrumental tests to evaluate colour, weight loss and head-space composition in order to monitor quality losses. Sensory tests were performed to study quality time-dependence in stewed broccoli. Eight trained judges assessed QDA sensory properties of broccoli packaged in protective atmosphere. Quantitative Descriptive Analysis (QDA) allows food sensory profile to be evaluate by using non-structured scales, in graphic representation arranged in the form of a "spider web" with a branch or spoke from a central point for each attribute. Each scale reports the average attribute value assigned by the panel. Just-cooked broccoli were used as the control. Samples stored in protective atmosphere, and the control, were evaluated by the panel three times. In order to define the qualitative level of broccoli stewed and stored in protective atmosphere, the panel defined attributes concerning appearance (Colour), odour (Odour of "cooked broccoli"), taste (Acidity, Salty, Bitter, "cooked broccoli" aroma) and texture (Juiciness). Sensory data were statistically analysed by SPSS 11.0 with ANOVA and Duncan test ($p<0.05$) in order to identify significant differences among samples. Colour evaluations were performed by a colorimeter MINOLTA Chroma Meter (mod. CR-300, Minolta, Osaka, Japan) provided with an 8 mm diameter head, D65 lamp and CR-A43 standard calibration. L* (brightness), a* (+a red; -a green) and b* (+b yellow; -b blue) defined in CIE (1976) system, and colour difference ΔE^* (1), evaluated by Hunter-Judd between stored and "just cooked broccoli", used as control, were measured.

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)2]^{1/2} \quad (1)$$

Weight losses in broccoli samples stored in protective atmosphere were evaluated by means of analytical scales, Sartorius BP 61. Samples were dried in an oven (Continental Equipment) for 24 h at 105°C, then weighed to evaluate water evaporation. For headspace analysis 1 g of broccoli was sealed in a 20 mL headspace vial (Chemtek Analitica, Bologna, Italy). Samples stored at 4±1°C were analyzed after 0, 4, 7 and 10 days. Volatile analysis was performed by an Agilent 7694 headspace auto sampler attached to a 6890 Hewlett-Packard gas chromatograph (Agilent Technologies, USA) equipped with a 30 m x 0.25 mm ID, film thickness 0.25 µm capillary column (HP-5MS, Agilent Technologies, USA) and a 5973 HP mass spectrometer (Agilent Technologies, USA). Gas carrier was helium (flow 1,2 mL/min) and split injector ratio was 1/5. Oven temperature was kept at 40°C for 4 min, programmed to 120° at 7°C/min and maintained at 120°C for 5 min, from 120° to 220° at 10°C/min and maintained at final temperature of 220°C for 10 min. Injector temperature was 270°C. Mass spectrometer operated in scan mode over mass range from 35 to 350 amu (2 s/scan) at an ionization potential of 70 eV. Mass spectral matches were made by comparison of mass spectra and retention time with those of MS database (NIST 98 e WILEY 275). Broccoli samples in headspace vial were equilibrated in headspace auto sampler at a plateau temperature of 80°C. After thermal equilibration, samples were mixed for 1 min by operation in mix mode pre-programmed in auto sampler. The vial was shaken during this mode, which may reduce mean diffusion path length of solutes as they migrate to gas/sample interface within the vial. Samples were then stabilized for 1 min, pressurized for 1 min, and equilibrated for 1 min in the auto sampler. After the loop was filled for 1 min and equilibrated for half a minute, carrier gas (helium) back flushed loop and carried volatiles through the heated transfer line (100°C) into GC. Released volatiles were automatically injected and trapped on GC column. Blank vials were run between each sample to clean the column and carry-over between samples.

RESULTS

The first step of the design procedure was to find a suitable composition of a protective atmosphere which preserved broccoli appearance for at least 10 days. To make a first screening, stewed broccoli were packaged in different gas-mixtures of N_2, O_2 and CO_2. Further broccoli samples were stored in air (PA 1) to study qualitative losses in non-protective conditions. These samples were taken as control in the screening phase. Gas-mixture percent composition (Table 1) was chosen by tolerance limits (2% <[O_2]< 8% e 1% <[CO_2]< 10%), reported in the literature (Myers, 1989). As a starting step, the qualitative profile of broccoli, cooked and preserved up to 10 days in suitable gaseous mixtures was evaluated in order to choose the atmosphere which guaranteed hygienic-

Table 1 - Gaseous mixtures analyzed - % composition.

P.A.	N_2	CO_2	O_2
1	78	1	21
2	93	5	2
3	88	10	2
4	83	15	2
5	100	0	0
6	0	0	100

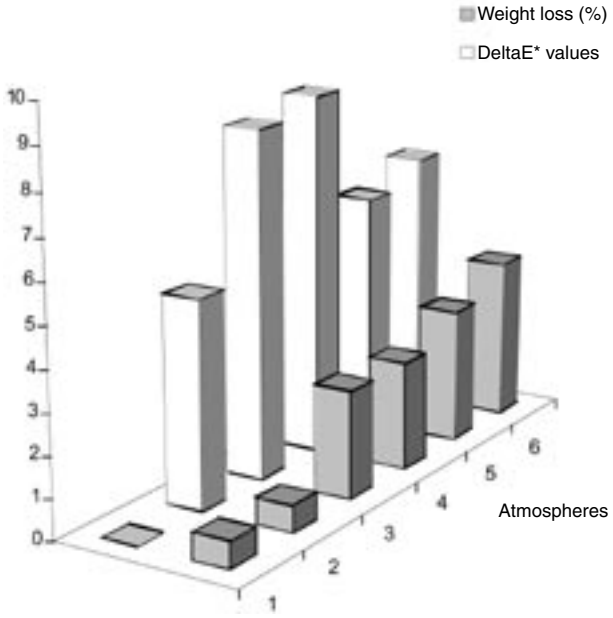


Fig. 1 - ΔE* and weight loss in broccoli stored 14 days in different PA.

sanitary safety at a determined qualitative standard. Package screening was performed after 4, 7 and 10 days of storage. Sample appearance (colour, mould or superficial alterations), odour (off-flavours) development and texture proprieties were evaluated. In addition colour and weight loss were evaluated. Colour of broccoli was found to be a suitable parameter to discriminate among different samples. ΔE* values and weight loss of broccoli stored in air and under different PA are reported in Fig. 1. Broccoli stored in PA 3 and 4 lost brightness and revealed a dark green colour, close to brown and was totally different from the sample stored in air. Browning was probably due to phenolic compound oxidation but could be caused by chlorophyll conversion to pheophytins during the cooking treatment. Samples stored in PA 2 were more similar in color to the control than samples stored in other atmospheres. Broccoli stored in PA 6 (100% oxygen) lost more weight than the control. Only samples stored in 100% N2 (P.A. 5) and in 93% N_2 – 2% O_2 – 5% CO_2 (PA 2) showed better results after 2 weeks, hence these compositions were chosen for the remaining analyses.

Microbial analysis

To assess packaged product safety, the count of the total aerobic micro-organisms (TMA) and the Enterobacters and Sporigen growth during the storage period was monitored. Cooking stage assured product microbiology safety; however air-cooling of product prior to packaging was a critical phase. To overcome this problem stewed broccoli were packaged while still warm. Microbial data relative to stewed broccoli stored for 2 weeks in presence of the protective atmospheres 1, 2 and 5 are reported in Table 2. During storage, microbial populations increased until product was not acceptable. Only the PA composed of 93% N_2 – 2% O_2 – 5%

Table 2 - Microbial data on broccoli stored in protective atmosphere for 2 weeks.

Stewed broccoli	TMA	Enterobacters	Sporigens
Atm 1	$2.0x10^5$	$1.75x10^4$	<10
Atm 2	$5.0x10^3$	<10	<10
Atm 5	$5.0x10^4$	$1.6x10^4$	<10

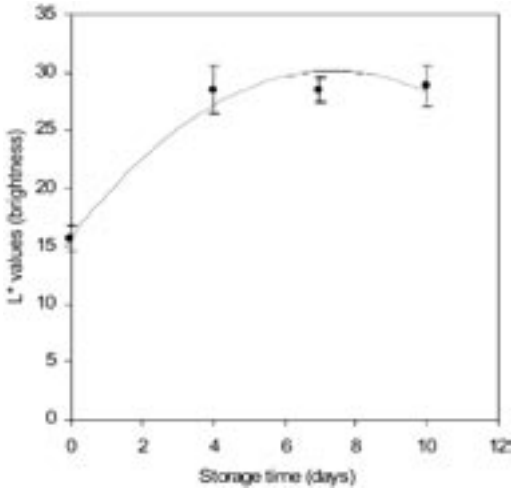


Fig. 2 - L* values (brightness) of broccoli stored under protective atmosphere until 10 days.

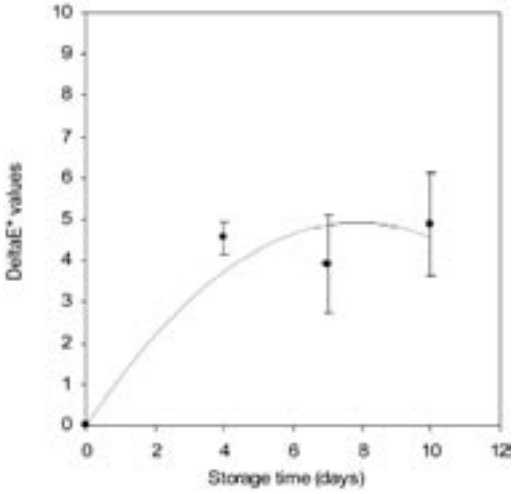


Fig. 3- ΔE* values of broccoli stored under protective atmosphere until 10 days

CO_2 (PA 2) guaranteed hygienic safety during two weeks of storage. This atmosphere was selected for the next investigations.

Colour analysis

Colorimetric analysis suggested that protective atmosphere packaging keeps broccoli appearance at a good level during storage time. Brightness (L*) of broccoli stored in protective atmosphere is reported in Fig. 2. There is a significant difference ($p \leq 0.05$) between broccoli samples stored in protective atmosphere and the control, however there is little difference between samples stored for 4, 7 and 10 days. The parameter a*(-) decreased slightly during storage time (data not shown), while b*(+) remained constant. The parameter a*(-) is a measure of the "blue-green" intensity. The fact that it diminishes indicates that green colour is lost in part because of chlorophyll degradation subsequent to cooking. The colour difference ΔE* of "just cooked" broccoli and samples stored in protective atmosphere were significantly different (Fig. 3), although ΔE* remained constant during storage time.

Moisture content

Weight loss (%) in broccoli stored up to 10 days under protective atmosphere is shown in Fig. 4. Storage in PA caused a partial dehydratation of broccoli. Stored samples differed from the control, but there were no differences ($p<0.05$) at different storage times (data non shown).

Gas Chromatographic Head-Space Analysis

In the analysis of volatile components present in the different samples, a total of 5 volatile compounds were repeatedly detected and quantified including 1 sulfide (R-Sn-R), 2 thionitriles (R-S-CN), 1 nitrile (R-CN) and 1 isothiocyanate (R-CNS)

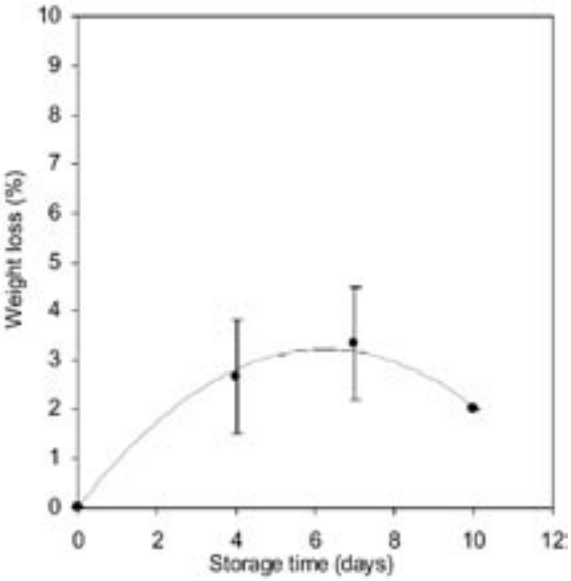


Fig. 4 - Weight loss (%) in broccoli stored under protective atmosphere.

(Table 3). The identified volatiles were reported previously as constituents of broccoli (Di Cesare *et al.*, 2001). The sulfur species impart the characteristic aroma of broccoli and are considered to be the most important volatile compounds (Barrett *et al.*, 2000). Azanza *et al.* (1994) determined that grassy flavour and aroma were logarithmically correlated with dimethyl sulphide (DMS) concentration. DMS is a product of the thermal degradation of S-methylmethionine (Bills and Keenan, 1968), produced during the cooking process. "Just" - packaged sample analysis showed a total of four compounds including sulphide dimethyl (21%), 4-methyilthiobutil-1-nitrile (12%), 5-methyilthiopentil-1-nitrile (51%) and propyl-benzylnitrile (14%) (Fig. 5). The two thionitriles make up the greatest concentration. Data given in Table 3 show that the major volatile content decreased during storage period. Propyl-benzylnitrile was the only volatile nitrile present in broccoli. From the 7th day of storage, up to 10 days, 1-butyl 4-isothiocyanate was the most abundant volatile in head-space (Fig. 6); DMS concentration remained constant in the investigated range. After 10 days of storage, the percentage of dimethyl sulphide (16.7%) in the total volatile mass was almost at the same level as 5-methyilthiopentil-1-nitrile (15.3%) and propyl-benzylnitrile (14.0%). At this time, head-space also contained hexanal, pentanal, heptanal and nonanal (data not shown).

Sensory analysis

Sensory quality of stewed broccoli packaged in protective atmosphere for 4, 7 and 10 days was investigated in order to define qualitative losses perceived by consumers. Broccoli stewed and stored in presence of 93% N_2 – 2% O_2 – 5% CO_2 gas mixture, were evaluated by a panel which expressed a judgement on aspect

Table 3 - Composition (%) of volatile organic compounds by GC/MS.

Compounds	Storage time (days)			
	0	4	7	10
1 dimethylsulfide	20.7	35.8	17.7	16.7
2 4-methyilthiobutil-1-nitrile	12.4	9.9	8.6	6.0
3 5-methyilthiopentil-1-nitrile	51.1	33.0	30.1	15.3
4 1-butyl 4-isothiocyanate			32.7	28.5
5 propyl-benzylnitrile	13.9	10.1	8.7	14.0

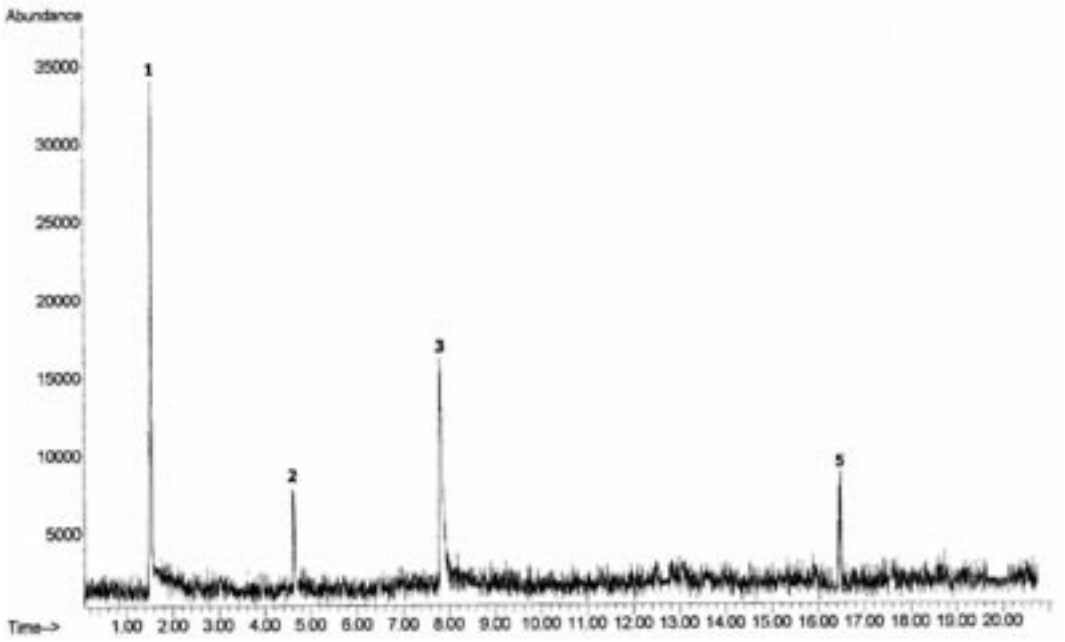


Fig. 5 - Gas chromatogram of "just cooked" broccoli, taken as control.

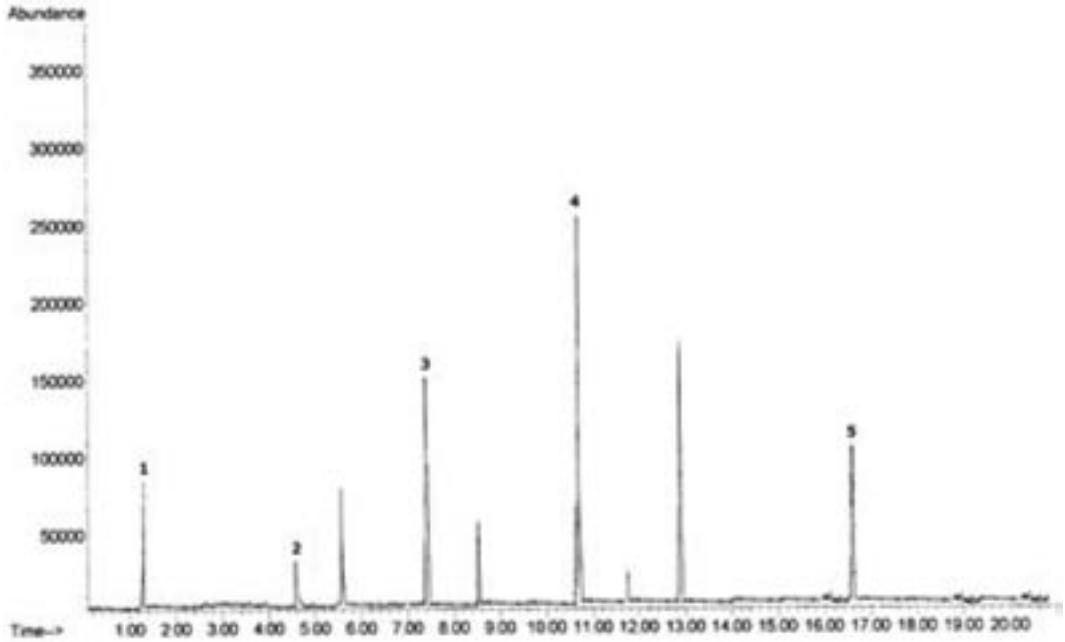


Fig. 6 - Gas chromatogram of broccoli stored for 10 days under protective atmosphere.

(colour), odour (odour of cooked broccoli, odour "at opening" of package), aroma (aroma of cooked broccoli), taste (sweetness, acidity, salty, bitterness) and texture (juiciness). Judges, were assisted by a data acquisition and elaboration system (FIZZ Win). Mean values derived from three separate repetitions were elaborated through one-way Anova and Duncan's Test. Samples stored in protective atmosphere at different storage times differed in aroma, acidity, bitterness and juiciness attributes. To analyze and confirm statistical elaboration of the data the sensory data is shown by means of QDA profiles. In Fig. 7 QDA of "just cooked" broccoli (control) and of samples stored in protective atmosphere for 4, 7 and 10 days are shown. Differences among samples are observed for texture (juiciness) and taste (bitterness, sweetness, acidity). Juiciness of samples, increased during storage period probably because of phenomenon of dehydration that caused a loss of tissutalis liquid. Bitter taste, typical of this kind of vegetable, had a low intensity during storage. "Sweetness" increased. "Odour" and "aroma of broccoli" were nearly constant during storage time. Even if sensory analysis suggested that the quality of ready-to-eat broccoli decreased during storage time, qualitative decay was restricted to aroma and odour characteristics.

CONCLUSION

Protective atmosphere packaging preserves and extends shelf-life of stewed broccoli for a time well-suited to market demands. PA technology reduces quality decay of broccoli and slows kinetics reactions giving a product with acceptable quality characteristics after 10 days of storage. Microbial safety was guaranteed by means of HACCP: air-cooling was defined as a critical point (CP) in the process as a pollution source, so it was necessary to adopt control actions. Sensory quality of the stored vegetable was slightly different than the control quality profile. After

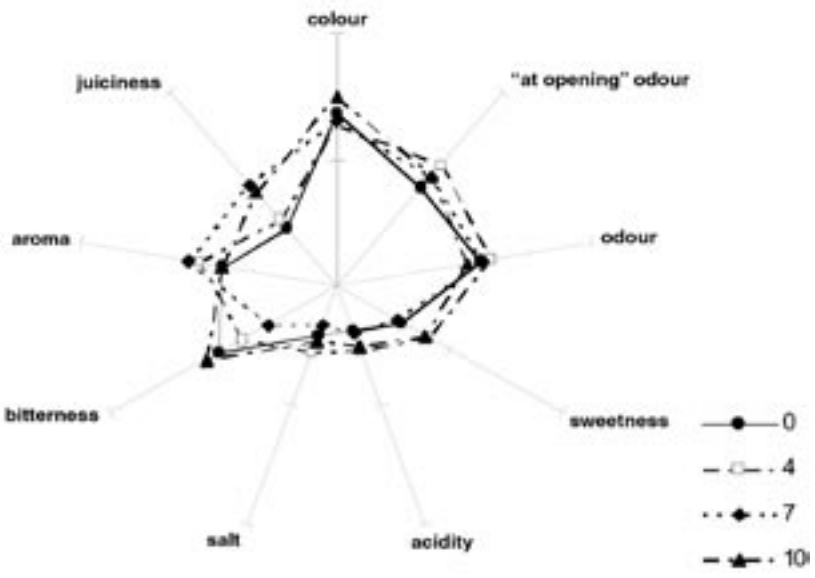


Fig. 7 - QDA profiles of broccoli samples stored in protective atmosphere.

10 days of storage the taste attribute (aroma, bitterness, sweetness) increased, while odour of broccoli became more intense and prickly than the control. Same results were obtained from GC-MS head-space analysis. Some volatile compounds, characteristic of broccoli odour profile (sulphides) were isolated in head-space of stored samples and also perceived through sensory analysis. GC-MS quantitative analysis revealed that levels of these substances increased during storage and produced more intense prickly flavour. From an instrumental point of view, there were no differences among samples stored for different times. The colour profile of stored samples differed from the control ("just cooked" broccoli) but was the same at each time analyzed (L* and ΔE^* values remained constant during storage time), weight losses were also constant in samples stored and differed significantly from the control. Data analysis showed protective atmosphere could be effectively used to preserve cooked and ready-to-eat vegetable from alterative disease occurring in air. Sensory analysis was, more useful than instrumental analysis to define and monitor quality losses of ready-to-eat broccoli. Protective atmosphere packaging technology applied to broccoli (*Brassica rapa* L. var. *silvestris*) gave qualitatively acceptable products up to 10 days.

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BLOOD ORANGE SLICES MINIMALLY TRANSFORMED: CHEMICAL, MICROBIOLOGICAL AND SENSORY STUDIES

SHELF-LIFE DI ARANCE PIGMENTATE DI IV GAMMA: ASPETTI CHIMICI MICROBIOLOGICI E SENSORIALI

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ABSTRACT

In the present work chemical, microbiological and sensory changes of blood orange slices of different cultivars, packaged under different conditions and with different permeability films were analyzed, in order, firstly, to find out the most suitable cultivar to be processed and, secondly, the best packaging conditions to extend the shelf-life and marketability of this kind of product. The chemical analyses show significant changes, for some clones studied, during storage at 4°C, only for firmness, total acidity, colour and anthocyanin content. Although all clones show loss of vitamin C, the antioxidant defences of orange slices were maintained in the orange cultivar analyzed. All the samples had Colony Forming Units (CFU) per gram values, for all microbial groups researched, lower than the two microbiological criteria generally used, until the 12th storage day. The best clone of orange, from chemical and sensorial points of view, was the tarocco "Gallo". For the different permeability films tested no correspondence was found between microbial growth and changes in sensory aspects.

- Key words: blood orange slices, packaging, shelf-life -

In this study the isolation of false positive *Listeria monocytogenes*, pointed out the need to set up a suitable method to isolate the pathogen from these products.

RIASSUNTO

Nel presente lavoro i cambiamenti chimici, microbiologici e sensoriali di arance pigmentate di IV gamma appartenenti a diverse cultivar, confezionate in aria e sotto vuoto, con tre film a diversa permeabilità sono stati studiati, allo scopo di individuare sia la cultivar più idonea alla trasformazione in IV gamma sia le condizioni di confezionamento in grado di allungare la shelf-life e quindi la valorizzazione di tali prodotti. I risultati delle analisi chimiche hanno evidenziato cambiamenti significativi, durante la conservazione, solo per alcuni cloni studiati, limitatamente a pochi parametri considerati (compattezza, acidità, colore e antociani). Inoltre, sebbene tutti i cloni analizzati abbiano mostrato perdita di vitamina C, il potere antiossidante riconosciuto a tali prodotti non viene compromesso, essendo elevato il contenuto iniziale nelle cultivar di arance analizzate. Per quanto riguarda i risultati delle analisi microbiologiche, tutti i campioni analizzati hanno mostrato, per tutti i gruppi microbici ricercati, valori di CFU/g (Unità Formanti Colonia/g) inferiori ai relativi limiti microbiologici fissati per i vegetali di IV gamma, fino al 12° giorno di conservazione. Per quanto riguarda la ricerca di Listeria monocytogenes l'isolamento di falsi positivi ha fatto emergere l'esigenza di mettere a punto un sistema di isolamento adeguato per tale matrice alimentare.

INTRODUCTION

The development of Minimally Transformed Vegetables (MTV) can allow diversification and promotion of the processed fruit product industry, which is very important for the economy of the Mediterranean regions and for Sicily, where the production of oranges is very widespread. The market of MTV is increasing because of important consumer satisfaction in different areas: the need for convenience food, due to increase of out meal occasions, women's work, and demand for fresh, untreated and healthy food products (JACXSENS *et al.*, 1999; SENESI *et al.*, 2000). The MTV products are already popular in catering, such as airplane meals, but, like all unpreserved food products, they have a short shelf-life, between 5 and 7 days (ALBENZIO *et al.*, 1998; CREMONESI *et al.*, 1999). Current trends are to extend the shelf-life of MTV products by finding the most suitable cultivar, reducing the microbial load (washing or sanitizing procedures, modified-atmosphere packaging, refrigeration and others) and the enzymatic degradation through short heating treatment (WATADA *et al.*, 1996; WATADA *et al.*, 1999; QUAGLIA *et al.*, 2001). Studies have been done on different MTV: fennel (ALBENZIO *et al.*, 1998), vegetables (TOMASONI *et al.*, 2000), apricots (MASSANTINI *et al.*, 2000) and apples (ANNESE *et al.*, 1997; BUTA *et al.*, 1999). Although many of these have concerned the quality of MTV, the industrial production of Minimally Transformed Fruit (MTF) is still considered difficult, for the great assortment of fruits and for the faster enzymatic spoilage, the only exception is represented by fruit salads (SENESI and DE REGIBUS, 2002). The MTV and MTF, are consumed without cooking, making the presence of pathogens a

concern (JAY, 1995). The usual sources of pathogen contamination come from irrigation or wash water, fertilizers of animal waste, infected operators and operation of facilities with poor sanitation (FDA *et al.*, 1998). In Europe there is still no microbiological policy for MTF and MTV, but two criteria are widely used: the DGCCRF (1988) and the CNERNA-CNRS (1996) criteria. The first correlates the shelf-life of MTV with $5x10^7$ CFU/g of aerobic total count, and advises testing for *Listeria monocytogenes* and *Yersinia enterocolitica*, fixing their absence in 25 g of sample. The CNRNA-CNRS fixed the limit for aerobic total count as 10^8 CFU/g and the absence of *L. monocytogenes* in 0.01 g of sample, at consuming time. Contamination of MTV with *L. monocytogenes* is a major concern that needs to be addressed in order to increase the safety of these products, since incidences of *L. monocytogenes* infections have been associated with fruits and vegetables (SCHLECH *et al.*, 1983; MARRANZANO *et al.*, 1996; HO *et al.*, 1986; HEISICK *et al.*, 1989). In 1998, the National Advisory Committees on Microbiological Criteria for Foods suggested, as regards juices, that *L. monocytogenes* has to be consider as a target organism in the absence of known specific pathogen product associations (FOOD and DRUG ADMINISTRATION, 1998).

Oranges, being a climacteric fruit, and thanks to their morphological and physiological characteristics show good potentiality to be minimally transformed (PRETEL *et al.*, 1998). Studies on minimally transformed blond oranges have been done regarding: enzymatic peeling and modified atmosphere packaging (PRETEL *et al.*, 1998); sensory changes during storage at low temperatures (ROCHA *et al.*, 1995); peeling of fruit by citric acid treatment (PAO and PETRACEK, 1997); microbiological stability and development of bitter substances (PAO *et al.*, 1997). To our knowledge no studies have been done on blood orange cultivars, like tarocco, moro and sanguinello, that - showing excellent sensory characteristics as red flash, distinctive aroma a balanced ratio between sugars and acids (RAPISARDA, 2000; RAPISARDA *et al.*, 2001); presence of anthocyanins, higher vitamin C and hydroxycinnamic acid content (RAPISARDA *et al.*, 1996) - have potential for being minimally transformed.

The aim of the present work was to find the best blood orange clone and the best packaging conditions to extend the shelf-life of orange slices minimally transformed under chemical, microbiological and sensory aspects. For this purpose orange slices were packaged in two different conditions (air and under vacuum) and with three different permeability films (OPA-PET, low permeability, LP; PET, medium permeability, MP; PET- PP, high permeability, HP). In the first time, the chemical changes observed, during storage at 4°C, of orange slices, in LP film, were exploited to choose the most suitable clones to be tested with MP and HP films to determine the microbiological and sensory parameters.

MATERIALS AND METHODS

Preparation of the sample

Fruits from 2 consecutive years (2002-2003) belonging to different clones of cultivar tarocco ("Arcimusa", "Scirè", "Gallo", "Rosso", "Vecchio Clone" and "Meli"), cv. sanguinello ("Nucellara") and cv. moro ("Nucellara"), cultivated in the experimental field of the "Istituto Sperimentale per l'Agrumicoltura" were used. Selected oranges were washed with a solution containing 200 ppm of Cl_2, rinsed

with distilled water, dried and peeled manually. Then they were sliced, using an electric slicer, and packaged under normal atmospheric conditions and under vacuum, in white CPET plastic boxes, heat sealed with: LP film (permeability to oxygen at 23°C, R.H. 0%: 35 cc/m^2/24h; permeability to water vapour at 38°C, U.R. 90%: 35 cc/m^2/24h); MP film (permeability to oxygen at 23°C, R.H. 70%: 56 cc/m^2/24h; permeability to CO_2 at 23°C, R.H. 79%: 217 cc/m^2/24h; permeability to water at 38°C, U.R. 90%: 13 cc/m^2/24h) and HP film (permeability to oxygen at 23°C, R.H. 70%: 110 cc/m^2/24h; permeability to water vapour at 38°C, U.R. 90%: 45 cc/m^2/24h), supplied by Tecnofoodpack. A TecnoFoodpack, mod. Easypack, sealing machine was used for the packaging. Each box, containing 4 orange slices, was kept at 4±1°C for 15 days. During the storage period the boxes were sampled, every three days, to determine the main chemical-physical, microbiological and sensory parameters.

Chemical-physical analyses

For chemical-physical analyses the samples were prepared under a suitably equipped and hygienically controlled environment to reduce microbial contamination. The determination of the firmness of the orange slices was done using a TR-Italy digital penetrometer, while the colour evaluation (CIE L* a* b*) was carried out with a Minolta CR200 colorimeter. Chemical analyses were made on the juice obtained by squeezing the orange slices, from each box, using a kitchen blender. The parameters determined were the pH, total acidity (TA) and total soluble solids (TSS) following classic analysis methods (OFFICIAL ANALYSIS METHODS FOR PLANT PRESERVES, MAF, 1989; KIMBAL, 1999). The anthocyanins, expressed as mg/L of cyanidine-3-glucoside, were determined using the RAPISARDA *et al.* method (1994). The determination of the ascorbic acid concentration was done by HPLC (RAPISARDA *et al.*, 1996), using a Waters mod. 600-E liquid chromatograph, interfaced to a PDA Waters 996 detector and run by Millenium 32 Waters software. The column was a C-18 Hypersil ODS-5µ (Phenomenex, Torrence, CA) maintained at 35°C and the elution was performed with a buffer solution constituted of KH_2PO_4/H_3PO_4 at pH 2,3, at a flow rate of 1 ml/min. The analysis wavelength was set at 260 nm. The sugars (sucrose, fructose, glucose), were determined by HPLC, using the same liquid chromatograph described above, interfaced in this case, to a refraction index detector (W-410) and fitted with an aminic column IB-SIL5-NH_2 (Phenomenex). The mobile phase used consisted of a 80:20 mixture of acetonitrile-water and the flow in the column was 1.8 mL/min. The statistical elaboration of the results was carried out using the MSTAT WIN 10 program. The program performed the analysis variance (ANOVA) and the separated means with the Tukey test. Lastly, the trends of each parameter evaluated during storage were traced using the aid of Windows EXCEL OFFICE XP professional.

Microbiological analyses

Immediately after processing, samples were transported, in ice, into the laboratory and stored at 4±1°C until analysis time, that were performed, in triplicate, at 0, 3, 6, 9, 12 and 15 days of storage. All slices of each box (about 100 g) were aseptically weighed and put in a sterile plastic bag to be blended in a Stomacher (Lab-Blender 400, Steward Medical, London, UK) for 3 min. The sam-

ples so obtained were used for the following analysis. Mesophilic viable counts (MVC) were performed in Plate Count Agar (Oxoid, CM325), at 32°C for 24 h: psychrotrophic viable counts (PVC) were done in the same media at 15°C for a week. *Enterobacteriaceae* count was determined in Violet Red Bile Glucose Agar (Oxoid, CM485) at 37°C for 24 h; lactic acid bacteria count (LAB) was determined using Man-Rogosa-Sharp (Oxoid, CM359). The pH of media was adjusted to 5.4 with lactic acid and the Petri dishes were incubated at 37°C for 48 h, in anaerobic conditions. Mould and yeast count was determined in Sabouraud Dextrose Agar (Oxoid, CM41) at 25°C for 4 days; acetic acid bacteria in GYCA medium (g/L: 50 g glucose; 10 g yeast extract; 30 g calcium carbonate; 25 g agar l^{-1}) at 32°C for 48h; acidophilic bacteria count was performed using Orange Serum Agar (Oxoid, CM657) at 32°C for 48 h.

Since MTF are consumed without cooking, the presence of pathogens is a concern, being all vegetables are susceptible to different sources of contamination. In this study, 24 boxes of orange slices packaged in LP film, were randomly analysed, from the 6^{th} up to the 15^{th} day of storage, for the isolation of *Listeria* according to the USDA method (McCLAIN and LEE, 1988). Twenty five g of sample were added to 225 mL of Listeria Enrichment Broth Base (Oxoid, CM863) plus supplement (Oxoid, SR141E) The sample, blended in the stomacher for 2 min, was incubated at 30°C. After 4, 24, 48 hours and 7 days, aliquots of 0,1 mL were picked and used to plate enumeration in *Listeria* Selective Agar Base (Oxoid, CM856), added of supplement (Oxoid, SR140E). Black colonies on this media were considered as presumptive *Listeria* colonies. In this study 2 colonies, from each plate, were randomly chosen, recovered on Triptone Soya Agar (TSA) (Oxoid, CM 129) plus 0.6% of Yeast Extract and the morphology was determined by optic microscopy and the isolates were tested for Gram stain, catalase activity, tumbling motility, β-haemolysis, fermentation of sugars and growth in Nutrient Agar (Oxoid) supplemented with NaCl (up to a final concentration of 20%).

Sensory analysis

The sensory test most suitable for the aim is a descriptive test which involves the discrimination and description of both the qualitative and quantitative sensory components of a sample by a trained panel (ISO/ FDIS 13299, 2002). This sensory test is used for quality control, comparison of product prototypes and may also be used to track product changes over time with respect to understanding shelf-life and packaging effects.

The training phase begins with the development of a common language which accurately describes the product attributes. Selecting the descriptors for inclusion in the final language is generally a consensus procedure. Once the descriptors have been selected, the next step is to choose an appropriate response scale to define the intensity of rated attributes. In this study the sensory test was carried out by a panel consisting of 14 judges, recruited from the students of University of Catania, aged between 24 and 28 years.

The trained panel, in a laboratory consistent with standards (UNI ISO 8589,1990), evaluated the intensity of the chosen descriptors (UNI ISO/WD 4121, 2001) by assigning a score between 1 (minimum) and 9 (maximum) to each attribute (card 1). The scores obtained were submitted to ANOVA to verify significant differences among attributes during storage of the different samples studied. The spider diagrams are used to graphically represent the data.

CARD 1

UNIVERSITÀ DEGLI STUDI DI CATANIA
FACOLTA' DI AGRARIA

Sezione Tecnologie Agro Alimentari
DOFATA Via S.Sofia 98 Catania
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E-mail cmlanza @ unict.it

Set N° _____ Judge: _____ Date _____

Evaluate the attribute of following samples by drawing a vertical dash on the graduated scale (1-9). The score assesses the attribute intensity (1= absence and 9=maximum).

Sample.....

Freshness



Aroma



Acidity



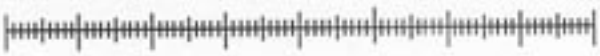
Juiciness



Sweetness



Firmness



Off-flavour



Overall



RESULTS AND DISCUSSION

Chemical and physical analyses

All cultivars, previously described, were tested for chemical parameters to select the best clone, among the different cultivars, to be transformed and to evaluate the chemical and physical changes during storage.

Fig. 1 reports the firmness values of the slices of the tarocco clones studied. Unlike the tarocco "Gallo", which showed a slight increase in this parameter during storage, there was a decreasing trend in the other clones, with statistically significant differences only in "Meli" and in some samples of "Arcimusa". This behaviour may be attributed to the result of two conflicting effects: the first due to the loss of humidity which produces a stiffening of the slice and the second due to the action of the pectin esterase enzyme which, activated with

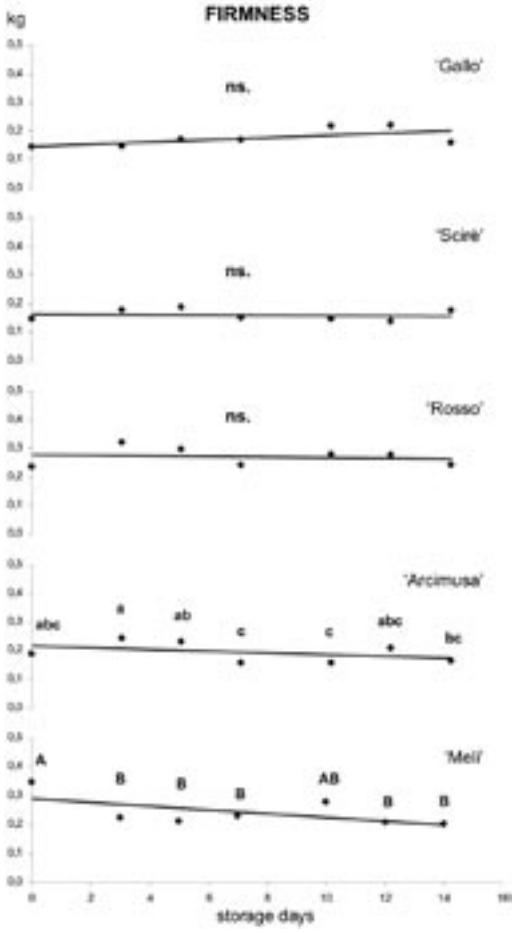


Fig. 1 - Orange slice firmness variation of five clones of cv. tarocco during storage days at 4°C.

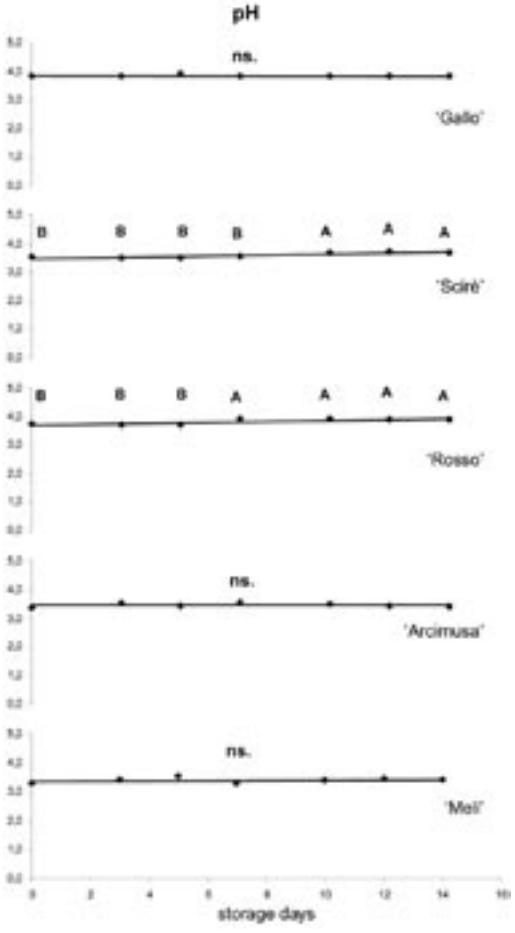


Fig. 2 - Orange slice pH variation of five clones of cv. tarocco during storage days at 4°C.

the cutting of the slices, the cell wall destroys and causes a loss of firmness (ALZAMORA *et al.*, 2000). The fruits of the tarocco clones had two different pH levels: the lowest in "Meli" and "Arcimusa" (3.31- 3.37) and the highest in the other clones (3.60-3.83). These differences are also reflected in the total acidity content (TA) which was above 1% in the first two clones and between 0.6% and 0.8% in the other clones (Fig. 3). During the storage period, there was a significant increase observed ($p<0.01$) in pH only in the "Scirè" and "Rosso" clones, manifested after roughly 8 days of storage. Regarding the total acidity, the data highlighted a constant trend in all clones, with the exception of the "Arcimusa" tarocco clone, where there was a significant drop ($p<0.01$) after the 7th day of storage. The lack of any correlation between TA and pH is probably due to the buffer capacity of the orange juice that tends to annul small variations in acidity. Also the TSS values (Fig. 4), in all the examined clones, did not show variations in storage. Such a trend was confirmed by the evolution of the glucose, fructose

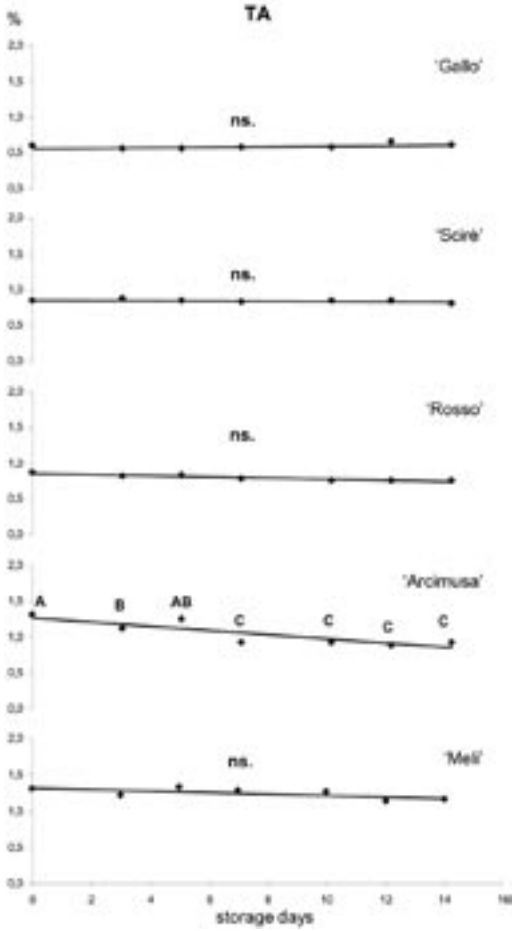


Fig. 3 - Orange slice TA variation of five clones of cv. tarocco during storage days at 4°C.

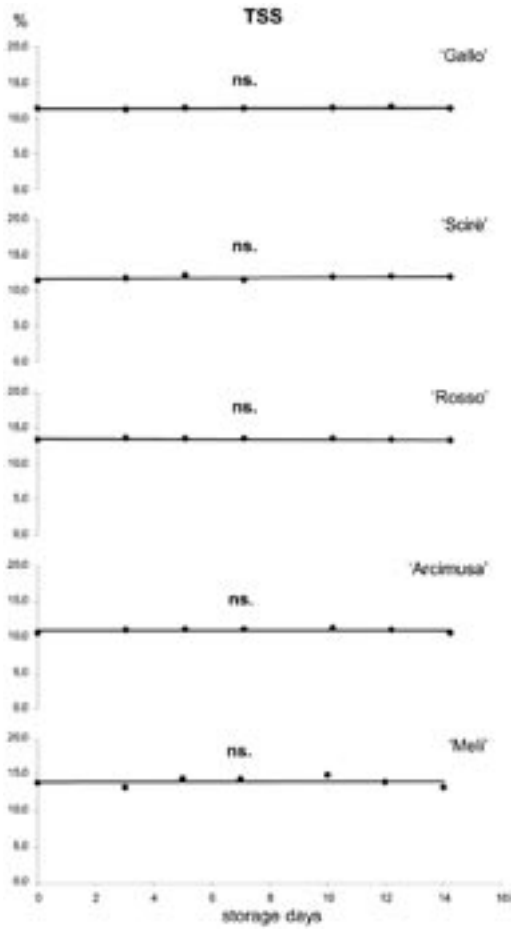


Fig. 4 - Orange slice TSS variation of five clones of cv. tarocco during storage days at 4°C.

and sucrose, which, except in the case of the tarocco "Gallo", remained almost constant during the entire conservation period. With regard to this last clone, there was a decrease in sugars from the first days of storage on. The lowering of the glucose and fructose concentrations may be caused by the respiration of the fruits in the period immediately after cutting, when the monosaccharides are used as substrates in this metabolic process (ROCHA *et al.*, 1995). One of the more important objectives for minimally processed fruits and vegetables is the maintenance of the original colour (DRANTES-ALVAREZ *et al.*, 2000). In the case of the blood oranges, the substances responsible for the characteristic red colour of the flesh are anthocyanins. Although these pigments prove stable inside the fruit, when they are sliced and kept at low temperatures, they may undergo deterioration processes by light, oxygen or endogenous enzymes. The high acidity of the product helps to balance out these negative effects, which, on one hand, stabilise the anthocyanins by shifting the equilibrium of dissociation towards the form of flavilium salts (BROUILLARD, 1982) and on the other, prevent peroxidase (LO SCALZO *et al.*, 2003) and polyphenoloxidase enzyme activity (GONZALEZ *et al.*, 2000), responsible for the browning of the slices. The experimental evidence, emerging from the storage tests on the slices, has highlighted a decrease in the anthocyanin content in all the tarocco clones examined. Only in the "Rosso" tarocco, the clone with the greatest initial pigment content (229.8 mg/L), was the decrease statistically significant ($p<0.05$). These results

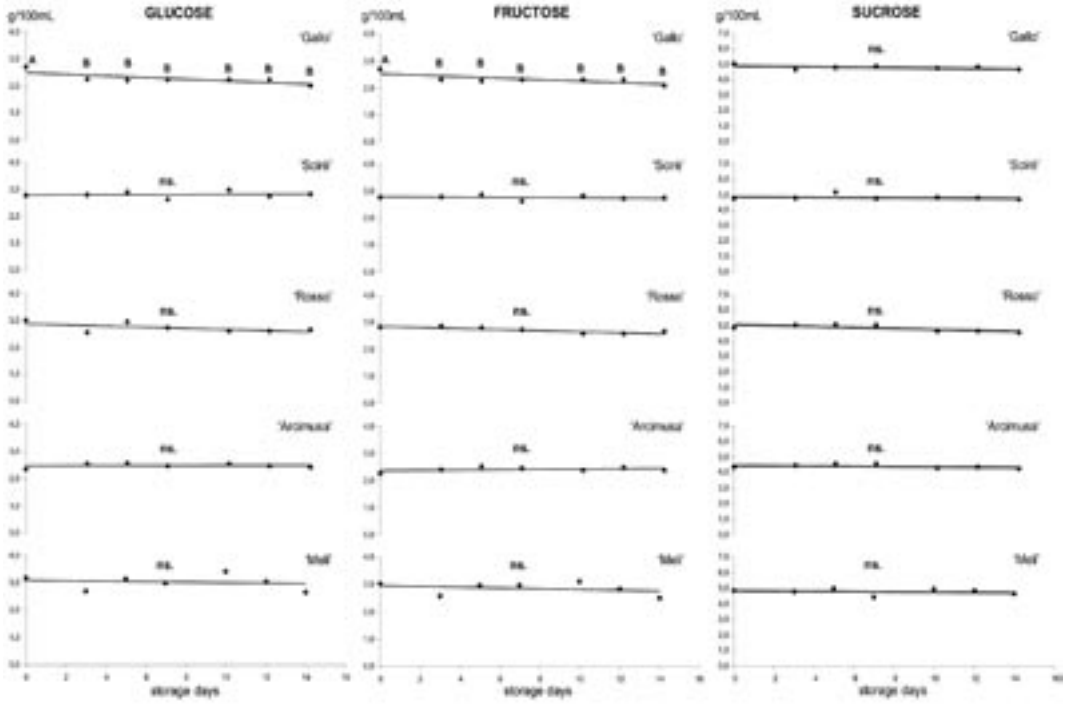


Fig. 5 - Orange slice sugar (glucose, fructose and sucrose) variation of five clones of cv. tarocco during storage days at 4°C.

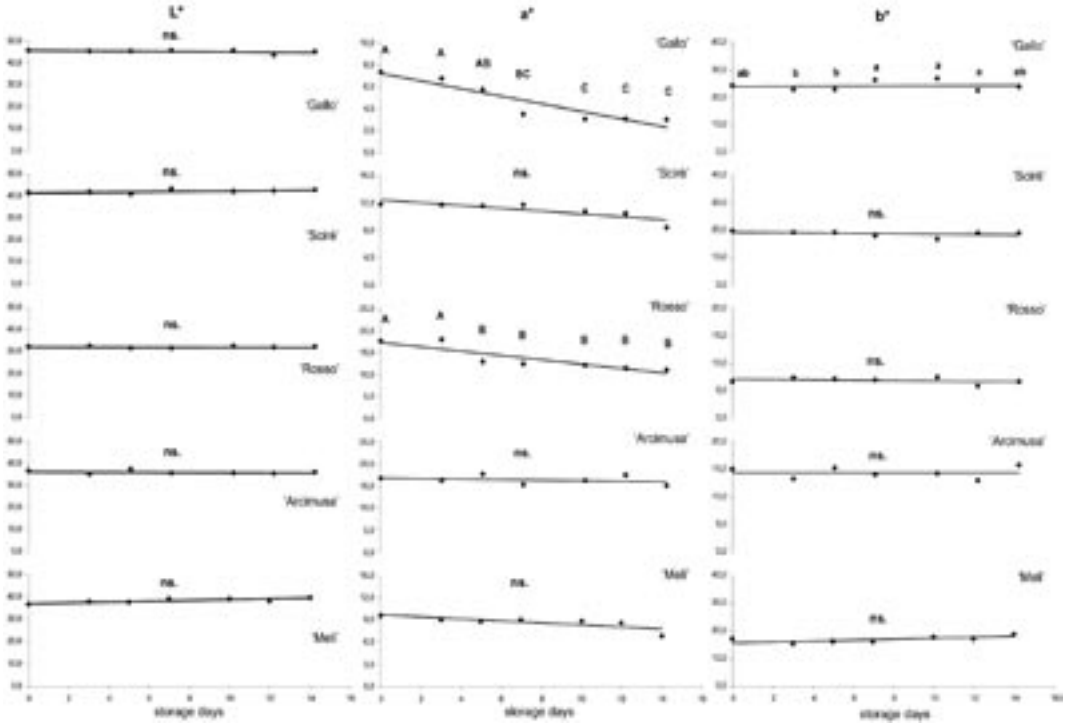


Fig. 6 - Orange slice colour parameter (CIE L* a* b*) variation of five clones of cv. tarocco during storage days at 4°C.

were also confirmed by the measurements of the colour coordinates, CIE L* a* b*, from which a decreasing trend of the red index a* emerged in all clones, with statistically significant differences with respect to only the "Gallo" and "Rosso" clones. Regarding the yellow index b* and luminosity L*, no important variations were observed in these parameters, in all the examined clones. These data constitute further confirmation of the absence of browning phenomena of the slices during storage which would have occurred with the decrease in L* and increase in b* values. The tarocco is the richest orange variety in vitamin C (RAPISARDA and INTELISANO, 1996; RAPISARDA *et al.*, 2001). Recent studies have shown that the content of this substance in the juice of some clones, varies between 60 and 90 mg/100 mL (RAPISARDA and RUSSO, 2000). In figure 8 it may be seen that the vitamin C level in the just prepared slices, is perfectly in line with the above values. However, during storage there was a clear decrease of this parameter in all examined clones, with the exception of the "Meli" tarocco, in which, even if the trend proved diminishing, there were no statistically significant differences. The clone with the highest vitamin C loss was the "Rosso" with a decrease of roughly 40% in 14 days of storage, whereas in the other clones the variation was between 10% ("Meli") and 30% ("Gallo"). It should be pointed out that the "Rosso" tarocco was the one with the highest vitamin C content (78.9 mg/100 mL) at the beginning of storage, so this decrease, in the course of the storage, did not lead to an excessive reduction in the antioxidant defences of the slices.

Microbiological analyses

In the present study the number of CFU/g, of all microbial groups detected, was lower than established by microbiological criteria for MTV, both at the production day and at the last day of fridge storage. Only mould and yeast counts, at the 15th day of storage were high (1x10^{9} CFU/g) so, in the present work, the microbiological results are shown only for samples until the 12th day of storage. All clones, packaged in the three films, show of CFU/g values lower than 10 until the 6th storage day, except the tarocco Meli, in both HP and MP films (the MVC numbers were 4.3x10^{3} and 3.0x10^{3} CFU/g, respectively) and sanguinello Nucellara in all three different films (PVC numbers, at the 6th day of storage, were 4.0x10^{2} in LP film; 2.8x10^{2} in MP and 3.4x10^{2} CFU/g, in HP film) (data not showed). These results have to be considered very good, since different studies on minimally transformed vegetables,

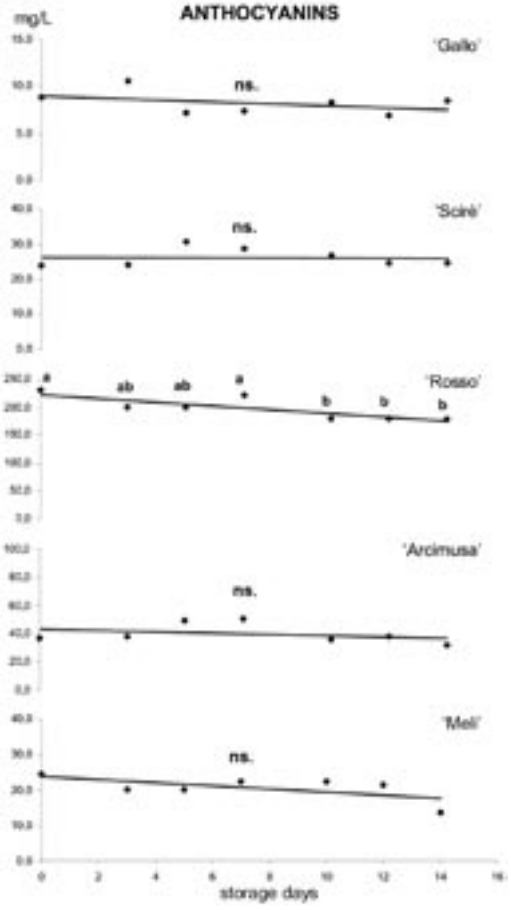


Fig. 7 - Orange slice anthocyanin variation of five clones of cv. tarocco during storage days at 4°C.

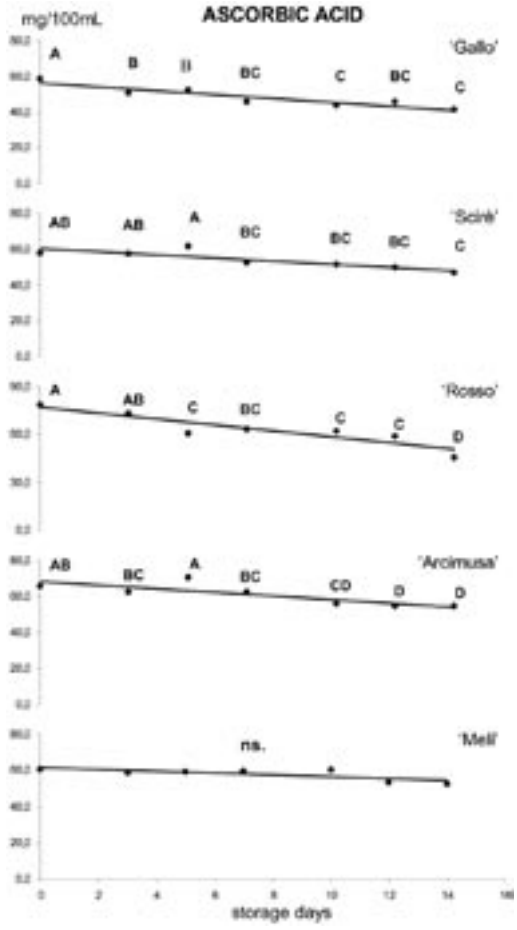


Fig. 8 - Orange slice ascorbic acid variation of five clones of cv. tarocco during storage days at 4°C.

have reported counts of mesophilic bacteria, after production, ranging from 10^3 to 10^9 CFU/g, and despite such high counts, product quality is still considered acceptable (NGUYEN-THE and CARLIN, 1994). The best clones of oranging to be transformed were the tarocco Gallo and Arcimusa. For both clones the lower CFU/g were reached in the LP film, followed by HP and MP film. Figures 9, 10, 11 and 12 show the microbiological evolution during storage time of tarocco Gallo slices packaged with LP, MP and HP films and under vacuum, respectively. As can be seen, the best results were obtained when the tarocco Gallo was packaged under vacuum (Fig. 12). In this case the number of CFU/g was lower than 1, even at the 12th day of storage, for all microbiological groups tested. The positive effect of vacuum condition is the result of a lowered product respiration and reduction of the overall rate of metabolic process, resulting in a better retention of the physiological state of the product and consequent inhibition of the growth of spoilage microrganisms (NGUYEN-THE and CARLIN, 1994). When the slices were packaged in LP film, the CFU/g numbers, for the microbial groups tested, were below 2 until the 12th day of storage. Figures 9 and 10 show the increased values reached when MP and HP films were used. These data are in agreement with other studies where it has been said that microbial growth in the ready to eat fruits is not the most relevant problem even if it can increase the spoilage (Senesi *et al.*, 1996).

Regarding the isolation of *Listeria*, after identification tests, none of the 19 presumptive *Listeria* strains were identified as *Listeria* spp. Eleven isolates, being gram-positive, catalase positive, non spore forming and able to grow at high NaCl concentrations, were identified as *Staphylococcus spp.*; 3 as *Pediococcus pentosaceu*

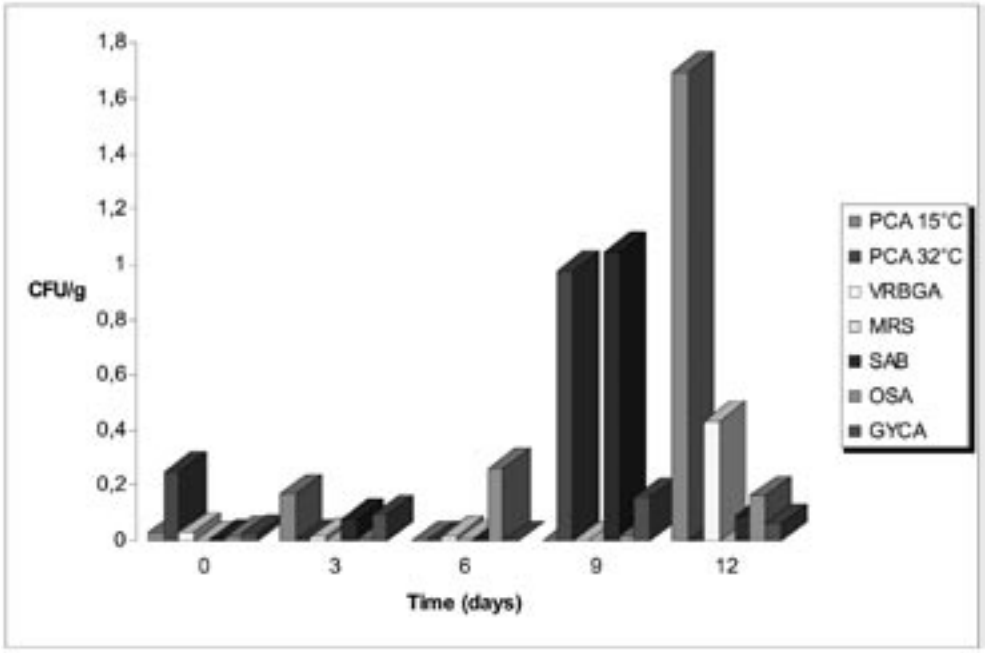


Fig. 9 - Microbiological evolution during storage time at 4°C of tarocco Gallo slices packaged in low permeability film.

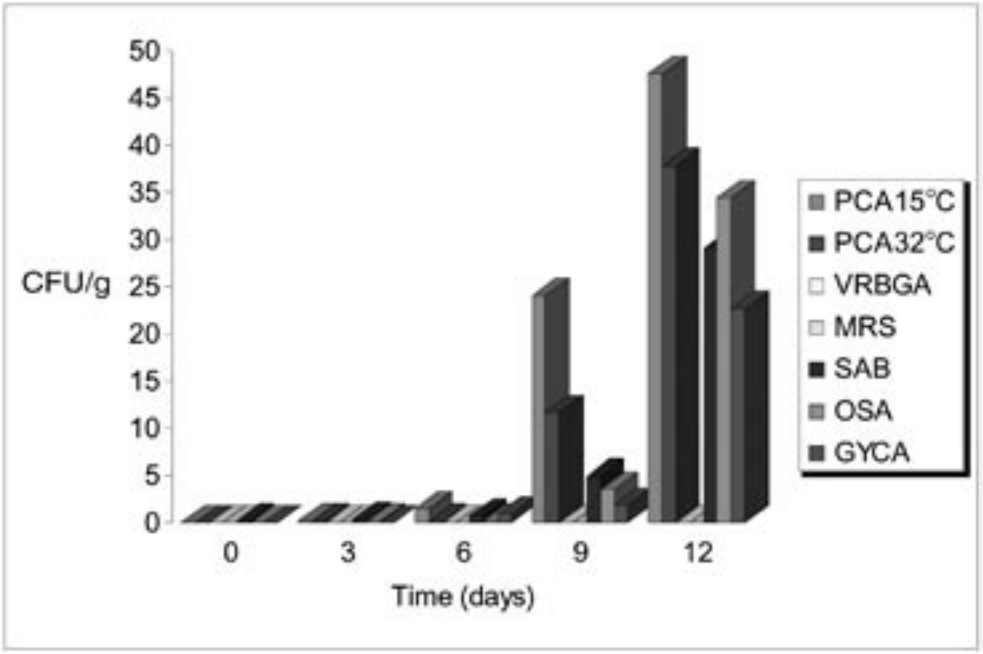


Fig. 10 - Microbiological evolution during storage time at 4°C of tarocco Gallo slices packaged in medium permeability film.

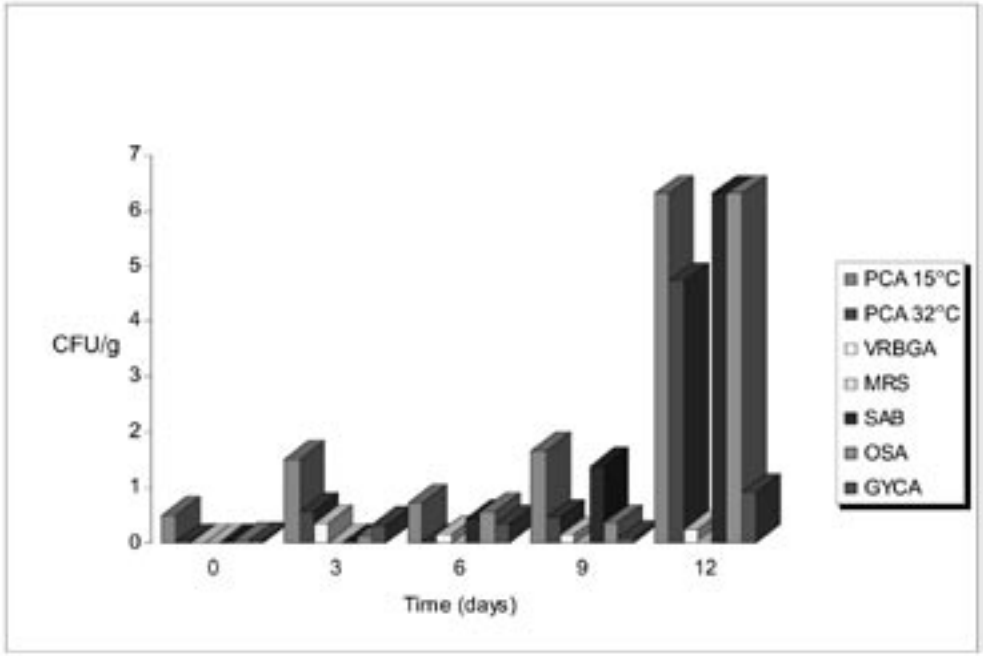


Fig. 11 - Microbiological evolution during storage time at 4°C of tarocco Gallo slices packaged in high permeability film.

and, 1, being strictly aerobic, able to grow in 5% NaCl and producing a yellow pigment as *Micrococcus* spp. The isolation of non-*Listeria* strains is reported in the literature for sea foods (Emberek *et al.*, 1997), where they have been identified as *Corynebacterium*. In the present case, the isolation of gram-positive non-*Listeria* strains could be due to the inhibition of acriflavine by the low pH value of oranges and the presence of carboxylic acids, as previously reported (Beumer *et al.*, 1996). To date no data are published on the natural occurrence of *Listeria* in orange slices. To assure the absence in the product it is necessary to set up a suitable isolation procedure to assess the microbiological risk in minimally transformed orange slices (Szabo *et al.*, 2000).

Sensory analysis

In this study, sensory analysis was used to screen the different cultivars. The Gallo clone, showed the best characteristics, during the shelf-life, so that a longer storage could be hypothesized, while the qualitative evaluation of Meli and Sanguinello decreased quickly. On the basis of these results the influence of packaging films on sample shelf-life was studied. Considering the effect clone x storage on sensory attributes, the HP film, generally, showed the lowest incidence of significant differences between samples at different storage times and fresh slices, followed by the MP film (Figs. 15 and 14). The LP and under vacuum packaging show, statistically significant differences, between fresh slices and a few days (even 2) storage product for many of the attributes considered (Figs. 13 and 16). The graphic results show Gallo clone is

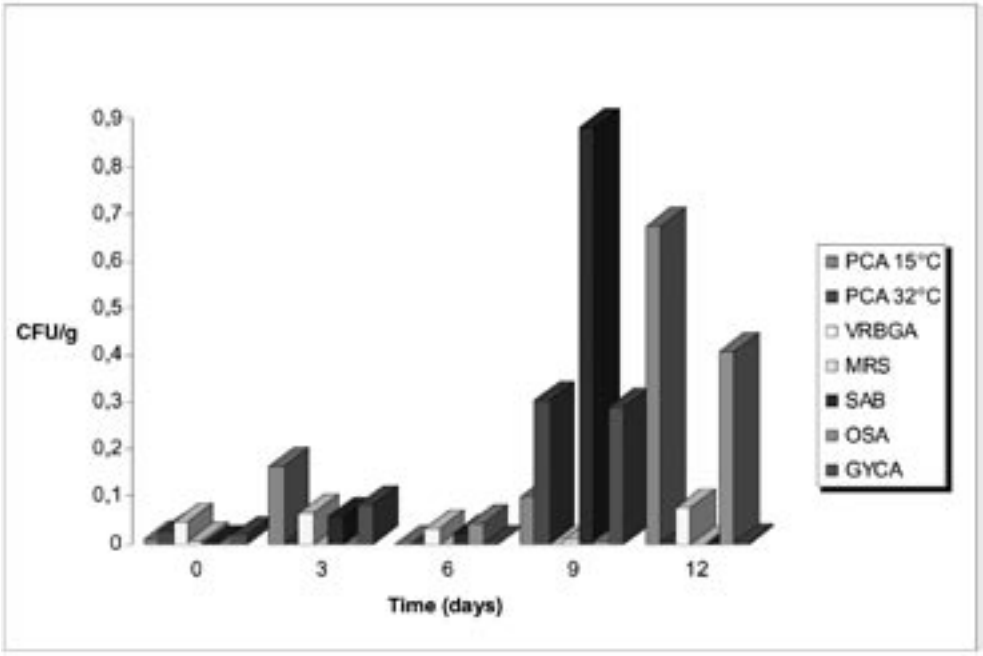


Fig. 12 - Microbiological evolution during storage time at 4°C of tarocco Gallo slices packaged under vacuum conditions.

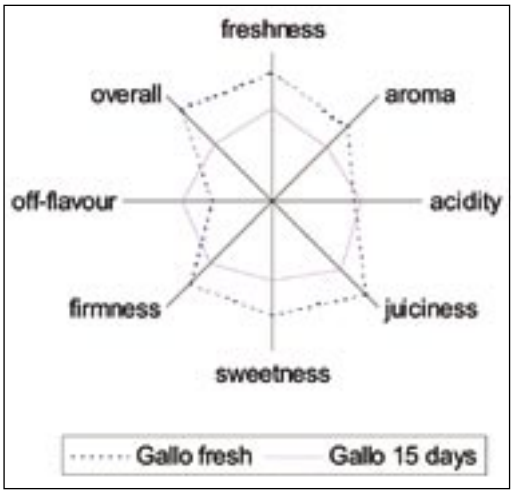


Fig. 13 - Sensory changes of tarocco Gallo slices packaged in low permeability film.

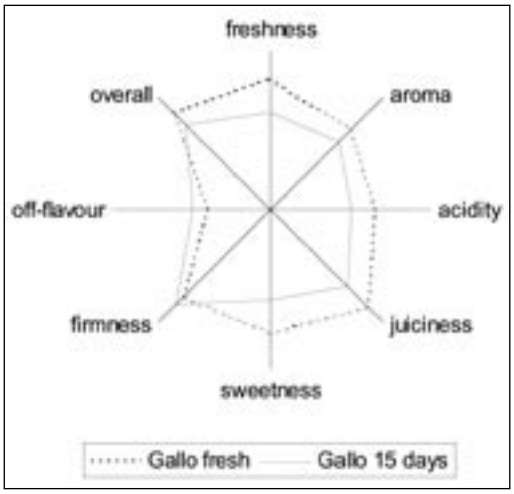


Fig. 15 - Sensory changes of tarocco Gallo slices packaged in high permeability film.

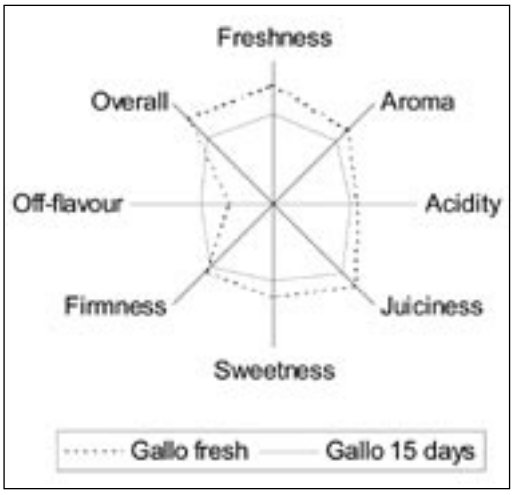


Fig. 14 - Sensory changes of tarocco Gallo slices packaged in medium permeability film.

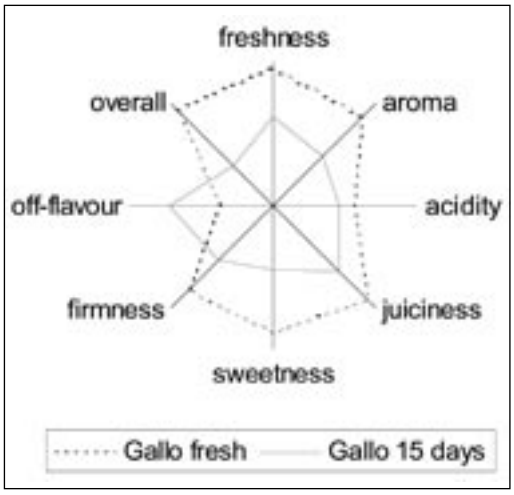


Fig. 16 - Sensory changes of tarocco Gallo slices packaged under vacuum conditions.

the most suitable for processing, while the experimental trial for Meli and Sanguinello had to be stopped before the fixed time (8 and 10 days respectively) due to evident decay of the samples.

CONCLUSIONS

Demand for fruit and vegetable products, due to the rising health consciousness of society, has led to a continual increase in the quantity and diversity of products

available to the consumer. Refrigeration and packaging technology have extended shelf-life and preservation of nutritional characteristics, but at the same time there has been an increased significance of psycrotrophic and anaerobic microrganisms such as *L. monocytogenes*. The results of the present work showed that the highest microbiological and sensory stability was achieved with tarocco Gallo and tarocco Arcimusa, for which the shelf-lives, at fridge temperatures, were longer than 12 days. Regarding the packaging conditions, even though the microbiological results are not in agreement with the sensory ones, this study shows that the MP film can be proposed as a compromise to prolong the shelf-life period over 10 days, at 4±1°C. The microbial safety of MTV should be ensured, in addition to maintaining acceptable sensory and microbial quality. The lack of a suitable isolation procedure for *L. monocytogenes* requires due caution with an extended shelf-life of refrigerated minimally transformed orange slices.

ACKNOWLEDGEMENTS

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LECTURE

COMBINED TECHNOLOGIES TO IMPROVE QUALITY OF RECONSTITUTED APPLE CUBES DURING PROCESSING AND STORAGE

TECNOLOGIE COMBINATE PER MIGLIORARE LA QUALITÀ DI CUBETTI DI MELA RICOSTITUITI DURANTE IL PROCESSO E LO STOCCAGGIO

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ABSTRACT

Freeze-dried apple cubes were rehydrated in water and in 15% high fructose corn syrup (HFCS) solution in order to obtain products with "adjusted" a_w values to be used as ingredients in many food formulations. HFCS-rehydrated products showed more limited changes in colour and texture over process time as well as lower aw values. The combination of a dipping treatment in a 1% AA solution prior to freezing and a 1% AA addition in the HFCS reconstitution solution limited product browning both during drying and rehydration processes. Quality changes of the HFCS rehydrated products were also studied during storage under air and modified atmosphere packaging. The use of an N_2O-containing atmosphere limited colour and texture changes over storage time. This could be related to the inhibition of enzymatic processes determined by this compound.

RIASSUNTO

Cubetti di mela liofilizzati sono stati reidratati in acqua ed in sciroppo zuccherino (HFCS) al 15% al fine di ottenere prodotti con valori di attività dell'acqua aggiustati da utilizzare come ingredienti nella formulazione di prodotti alimentari. I prodotti reidratati in HFCS hanno mostrato durante il tempo di trattamento valori

- Key words: ascorbic acid, dried fruit, modified atmosphere, quality, rehydration -

di a_w più bassi e modificazioni di colore e consistenza più limitate. La combinazione di un trattamento di immersione in soluzione di acido ascorbico (AA) prima della liofilizzazione e di un processo di reidratazione in HFCS addizionato di un 1% di AA ha permesso di limitare l'imbrunimento enzimatico dei prodotti durante l'essiccamento e la ricostituzione. È stato ulteriormente investigato l'effetto del confezionamento in atmosfera modificata sulla qualità dei prodotti reidratati durante lo stoccaggio. L'utilizzo di N_2O nell'atmosfera di confezionamento ha permesso di minimizzare i cambiamenti di colore e consistenza nel tempo di stoccaggio; questo risultato può essere spiegato attraverso l'inibizione dei processi enzimatici determinata da questo composto.

INTRODUCTION

Fruit pieces are often present in the formulation of foods such as baked products, dairy derivatives and ice cream and their qualitative attributes contribute to the global acceptance of the product. In general, fruit ingredients require specific functional properties in order to be compatible with the food system without affecting its shelf-life. In a formulated food the compatibility of the fruit with other components depends, mainly, on the equilibrium of the respective water activity values. Fruit pieces with medium aw values can be obtained by individual or combined, traditional or innovative, dehydration techniques. In the last years, studies have shown an increasing interest in obtaining fruit derivatives by reconstitution of preliminarily dehydrated products characterised by medium-high aw values and peculiar qualitative properties. Mastrocola et al. (1995, 1998a,b) studied the physico-chemical characteristics of dehydrated fruit pieces reconstituted in water solution with different sugar composition and concentration. The reconstitution in sugar solution gave apple and strawberry derivatives with different functional properties depending on the adopted process conditions. Moreover, different process parameters could be easily modulated to accelerate the rehydration process (BARBANTI *et al.*, 1998). These results show an interesting flexibility of this process that provides specific products that can be used in different food formulations.

The rehydration of dried fruit could cause several changes in product quality (MALTINI *et al.*, 1993), thus, besides optimising the process to reach specific physico-chemical properties of the fruit derivatives, it is necessary to evaluate and choose single or combined actions to prevent quality changes due to the development of chemical and enzymatic reactions during reconstitution and storage.

The objective of this work was to investigate the effect of stabilization treatments, such as antioxidant addition and modified atmosphere packaging, on the quality attributes of reconstituted freeze-dried apple cubes during storage time.

In order to inhibit the enzymatic browning reaction, which is one of the most relevant cause of quality decline in apple derivatives, the use of ascorbic acid in different steps of the production process was investigated.

Different packaging techniques were also investigated in order to improve the stability of the reconstituted apple cubes in refrigerated conditions. In particular, reconstituted products were packed in air, under vacuum, in a conventional modified atmosphere containing 5% O_2, 10% CO_2 and 85% N_2 (MA1) and in an innovative modified atmosphere containing 5% O_2, 10% CO_2 and 85% N_2O (MA2).

MATERIALS AND METHODS

Sample preparation

Trials were performed on apples (*Malus domestica*, cv Golden Delicious) of the same batch. After hand-washing, peeling, and coring, apples were sliced and then cut to give uniform cubes of 1x1x1 cm. A batch of these apple cubes were immediately frozen in a forced-air cabinet (Alaska, mod. EF 600, Bologna, Italy) operating at -40°C. A second batch, prior to freezing, were preliminarily dipped in a solution containing 1% ascorbic acid (AA) for 1 min under stirring conditions. After draining for 1 min in a plastic screen, apple cubes were immediately frozen. Both batches of frozen apple cubes were then freeze-dried in a pilot plant drier (Edwards Alto Vuoto, Mini Fast 1700, Milan, Italy), at a shelf temperature of 25°C. The dehydrated products were packed in polyethylene laminate pouches and maintained at room temperature until reconstitution.

Reconstitution procedure

In the first step of the study, freeze-dried apple cubes not dipped in AA solution were reconstituted in distilled water and in a 15% (w/w) high fructose corn syrup (HFCS) aqueous solution (Frudex, Cerestar, Ferrara, Italy).

In a second step of the study, the freeze-dried apple cubes, previously dipped in a 1% AA solution prior to freezing or not, were alternatively rehydrated in a 15% (w/w) HFCS aqueous solution or in a 15% (w/w) HFCS solution added with 1% (w/w) AA.

The samples obtained from the combination of the different treatments were marked as A, B, C, D according to the following scheme:

Sample	Dipping in ascorbic acid (1% w/w)	Reconstitution in 15% HFCS	Reconstitution in 15% HFCS + 1% AA
A	-	+	-
B	+	+	-
C	-	-	+
D	+	-	+

Fixed amounts of freeze-dried apple cubes (40 g) and reconstitution medium (600 mL) (weight ratio 1:15) were placed in 100 mL glass beakers and the product was submerged under a plastic screen. All trials were performed at room temperature using the same stirring rate (100 rpm). Fruit pieces were withdrawn from solutions after 5, 20 and 40 min, drained for 5 min on a plastic screen and blotted. The rehydrated apple cubes were then packed in plastic pouches under normal air for up to 24 h at 4°C.

Packaging

Freeze-dried apple samples obtained from fruit cubes preliminarily dipped in 1% ascorbic acid solution were reconstituted for 20 min in a 15% (w/w) corn syrup with HFCS water solution as above containing 1% ascorbic acid (sample D). After drainage and blotting, aliquots of rehydrated apple cubes (25 g) were

packed in polyamide/polyethylene pouches with gas permeability (at 23°C) as follows: O_2: 30 cm^3/m^2-24h bar; N_2: 7 cm^3/m^2-24h; CO_2: 90 cm^3/m^2-24h, H2Ov: 1.8 g/m^2-24h (85% R.H.). Pouches were packed using a laboratory packing machine (S100-Tecnovac, San Paolo D'Argon, Bergamo, Italy). In order to evaluate the effect of atmosphere composition in the headspace of the plastic pouches, different packaging conditions were chosen: air, under vacuum and two different modified atmospheres: MA1: 5% O_2, 10% CO_2 and 85% N_2; MA2: 5% O_2, 10% CO_2 and 85% N_2O.

Analytical determinations

Total solid determinations were carried out in a vacuum oven for 12 h (AOAC Methods 22.013, 1980).

Water activity was evaluated at 25°C by means of a dew point hygrometer (AquaLab mod.CX2, Decagon Devices, Inc., Pullman, WA).

Colour measurements were carried out with a Tristimulus Colorimeter (Chromameter-2 Reflectance, Minolta, Japan), equipped with a CR 100 measuring head. Standard Commission International d'Eclairage (C.I.E., 1978) conditions, with illuminant C (6774°K) were used. The instrument was standardised against a white calibration tile before each measurement.

Color measurements were taken on the surface of a homogeneous layer of rehydrated apple sample, avoiding free space between the cubes. Data reported are the average of at least 10 replications.

Firmness of fruit pieces after reconstitution was measured with a Universal Testing Machine (Instron International LTD, High Wycombe, UK), mod. 4301, using a standard Kramer Shear press cell (model CS1) with a crosshead speed of 50 mm/min on 50 g of product. From the load-distance graph, maximum shear force (maximum peak load) was considered as an index of firmness.

pH values were determined using a AMEL 334-B pHmeter.

Mass transfer determinations

Water uptake and solid gain due to rehydration were calculated in accordance with Mastrocola *et al.* (1995).

Statistical analysis

One - and two - way ANOVA analysis were conducted using the Statistica '99 software (Statsoft, Tulsa, OK).

RESULTS AND DISCUSSION

Rehydration of dried product in sugar solutions rather than in water could be used to obtain specific aw values and to modulate product functional and textural properties (MASTROCOLA *et al.*, 1995, 1998a, b). Freeze-dried apple cubes were rehydrated using 15% HFCS water solution in order to reach aw values compatible with the formulation of these product in complex food systems with high moisture content. Products rehydrated in water were taken as control. Rehydration was performed for different times in order to evaluate the effect of this parameter on

some quality properties of the final products and in particular, colour, rehydration parameters and texture.

Table 1 reports the data of water uptake, solid gain and moisture of apple cubes reconstituted in water or in 15% HFCS solution for different times. The results indicate that solid and water transfer among apple cubes and the sugar solution take place mainly during the first period of rehydration, due to the highly porous structure of freeze-dried products that allows water to penetrate easily into the food structure (MASTROCOLA *et al.*, 1998a,b).

The comparison of the mass transfer parameters of samples rehydrated in 15% HFCS and those in water showed that the latter had higher values of solid loss, water gain and moisture; these results could explain their lower firmness. Textural data of samples rehydrated in 15% HFCS and water for different times are reported in Table 2.

Reconstitution times above 20 min determined only a limited increase in water gain, while, longer contact between the reconstitution solution and apple cubes led to a solid gain and firmness decrease.

Fig. 1 reports data of the colorimetric value a*/b* which was considered as an index of browning (Senesi and Pastine, 1996). Samples rehydrated in water had rehydration time values of a*/b* colour index significantly higher than samples rehydrated in 15% HFCS. This fact indicates that the reconstitution in sugar solution could limit the development of enzymatic browning (EB) reactions due to the osmotic reduction of a_w at the cut surface. Enzymatic activity can be present during drying and subsequently during rehydration of dried plant tissue. The preservation of enzymatic activity could be improved by using dehydration methods such

Table 1 - Water gain, solid gain, moisture and water activity values of apple cubes rehydrated in water or Frudex for different reconstitution times (mean ± standard deviation).

Rehydration media	Rehydration time (min)	Moisture (% w/w)	Water gain (g/100 g)	Solid gain (g/100 g)	a_w
Water	5	78.29±2,83	376.34±13,76	-11.11± 2,96	0.984±0.002
Water	20	82.31±2,61	404.32±12,11	-26.41± 2,57	0.992±0.003
Water	40	83.33±0,28	425.54±12,31	-34.63± 4,65	0.993±0.003
HFCS	5	69.27±0.49	327.22±13.44	50.25± 7.32	0.973±0.002
HFCS	20	71.97±0.96	352.95±19.28	42.37±10.09	0.977±0.003
HFCS	40	73.82±1.64	360.51±20.64	31.98± 9.44	0.977±0.004

Table 2 - Values of max shear force of apple cubes rehydrated in water or HFCS solution for different times.

Rehydration media	Rehydration time (min)		
	5	20	40
Water	219.6a	119.4c	99.1d
Frudex	239.5a	165.9b	126.9c
Numbers followed by the same letters are not significantly different at a $p<0.05$ level.			

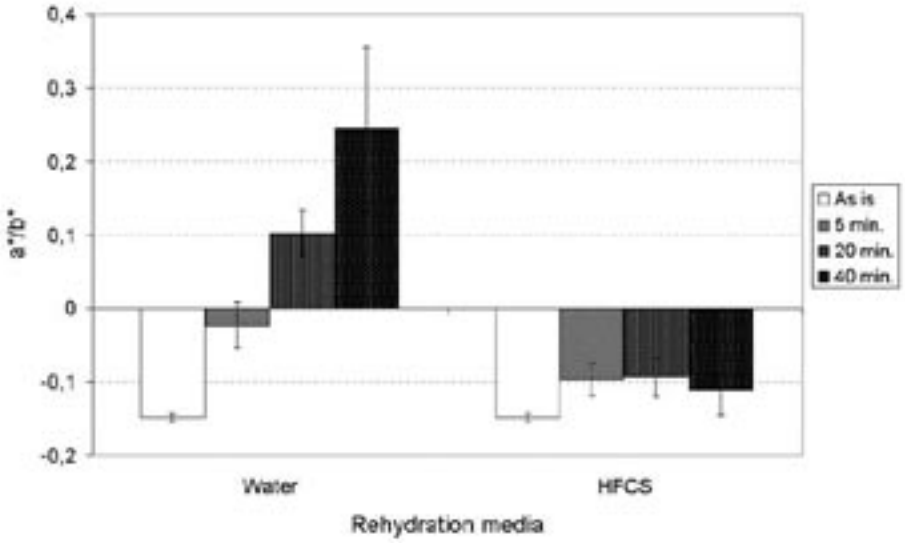


Fig. 1 - a*/b* values of apple cubes rehydrated in different media over rehydration time.

as freeze-drying, that is aimed at obtaining material with uninjured biochemical pathways (LEWICKI *et al.*, 2001).

Effect of combined treatments during processing of reconstituted apple cubes

The effect of using ascorbic acid at different steps of the production of the reconstituted apple cubes was investigated in order to inhibit the EB reactions during processing. To this aim part of the fresh cut products were preliminarily dipped in 1% ascorbic acid solution (pH 2.71) before freeze-drying and eventually freeze dried apple cubes (either dipped or not) were reconstituted in a 15% HFCS solution with or without the addition of AA.

L* values of differently processed apple cubes rehydrated in HFCS solution and of control sample in water are reported in Table 3. Control sample showed L* values significantly lower than HFCS samples while no meaningful differences ($p<0.05$) were evidenced among the latter. Values of a/b* of apple cubes (both

Table 3 - Values of L* of products reconstituted in water or HFCS solution for different rehydration times

Rehydration time (min)	Water	A	B	C	D
5	59.10b	61.58b	65.39a	66.61a	65.08a
20	53.52c	63.83ab	63.10ab	65.64a	65.92a
40	51.19c	63.93ab	66.20a	66.33a	65.79a

Numbers followed by the same letters are not significantly different at a $p<0.05$ level.

dipped or not) reconstituted for different times in Frudex with or without AA addition, are reported in Fig. 2. Pretreatment of fresh apple cubes through dipping in AA solution prior to freeze-drying gave better retain of the initial colorimetric parameters and limited discoloration during the reconstitution step, similar results were obtained by the use of AA in the sugar solution. The use of combined treatments (dipping and reconstitution in AA-enriched HFCS solution) did not further improve the product colour.

Differently pre-treated apple cubes were rehydrated in different solutions for fixed times and stored for up to 24 h in order to better evidence the effect of each process variable on the inhibition of enzymatic browning. The a*/b* colorimetric data of the differently processed apple samples over storage time are reported in Fig. 3.

Reconstitution in 15% HFCS rather than in water reduced the rate of colour browning even if HFCS reconstituted samples showed, regardless of rehydration time, significant colour changes over 24 h of storage (Fig. 3a). Samples dipped in AA solution before freeze-drying presented significantly lower a*/b* values ($p<0,05$) than untreated samples but the protective effect of AA against browning decreases as rehydration time increases (Fig. 3b). Diffusive effects which take place during reconstitution could favour the migration of AA from the surface of the cube to the sugar solution and limit the efficiency of the pre-treatment during the subsequent storage.

The reconstitution in HFCS with AA added inhibited enzymatic browning in both dipped or undipped freeze-dried apple cubes regardless of rehydration time.

The results obtained in this preliminary research suggest that, even in apple

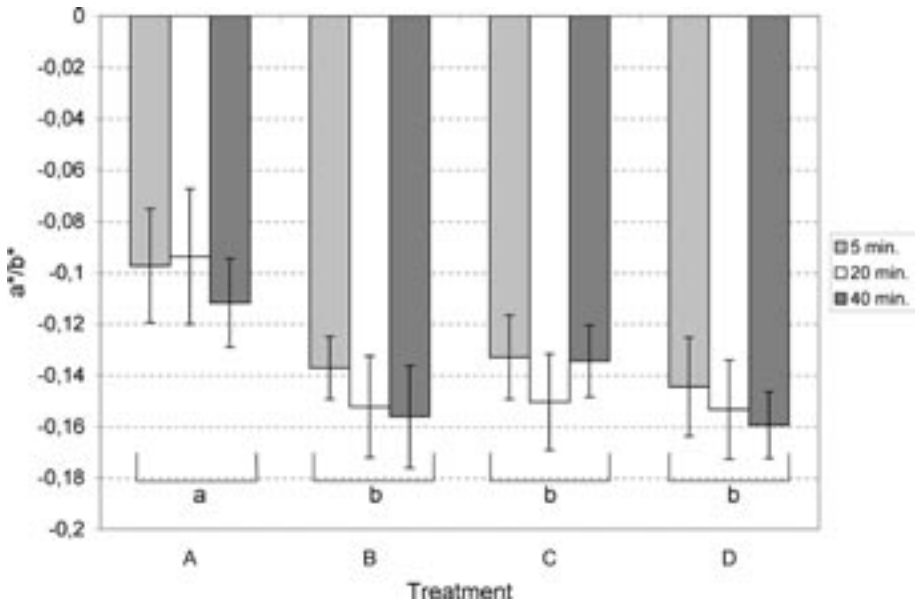


Fig. 2 - Changes of a*/b* values over rehydration time of apple cubes pretreated differently and reconstituted in HFCS solutions.

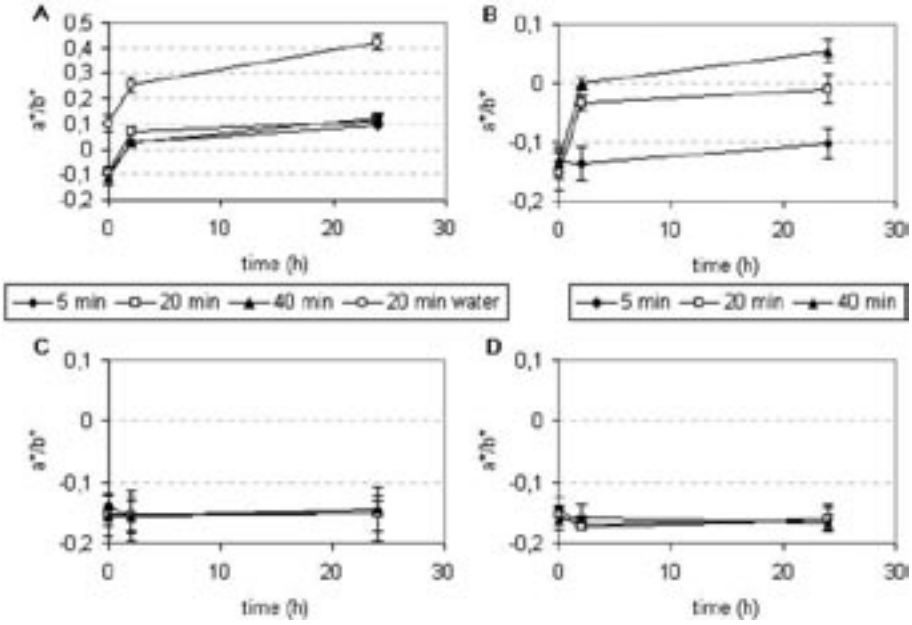


Fig. 3 - Changes of a*/b* values during 24 h storage of apple cubes pretreated differently and reconstituted in HFCS solutions.

cubes not dipped prior to freeze-drying, the reconstitution in HFCS with 1% AA added seems to inhibit enzymatic browning of the product during both processing and subsequent storage under chilling conditions. On the other hand, the preliminary dipping of fresh apple cubes with AA prior to dehydration could limit enzymatic browning reaction during processing of freeze-dried apple (a*/b* values of dipped and untreated products were respectively -2.70 and 0.00 and were significantly different among them) and hence improve the acceptability of the semi-manufactured products.

Effect of packaging conditions

Effectiveness of different packaging conditions in keeping quality and inhibiting enzymatic browning of reconstituted apple products was investigated. Apple cubes obtained from AA-dipped freeze-dried samples and reconstituted in HFCS with 1% AA for 20 min were chosen for this purpose. Besides traditional packaging atmospheres (air and under vacuum), modified atmospheres were also considered: in particular, a ternary gas mixture (N_2-CO_2-O_2) commonly used in minimally processed fruit preservation (MA1) and an innovative gas mixture obtained by substituting nitrogen with nitrous oxide (MA2) were chosen. Both modified atmospheres contained O_2 level of 5% and CO_2 level of 10% in order reduce the activity of the enzymes responsible for the oxidation of phenolic substrates (BURTON, 1982; ROCHA and MORAIS, 2001). Figs. 4 and 5 report L* and a*/b* values respectively as a function of storage time in refrigeration conditions. The different packaging conditions significantly affected colour parameter evolution. After just one day of

storage the samples packed under MA1 and under vacuum showed a significant decrease in lightness (Fig. 4), most evident in the apple cubes packed in the former conditions. These colour changes were also evidenced by visual observation of the samples that had a more saturated yellow colour than that shown by apple cubes packed in air and MA2 conditions. During storage, only limited changes in L* and a*/b* values were observed in the differently packed samples up to 8 days. Above

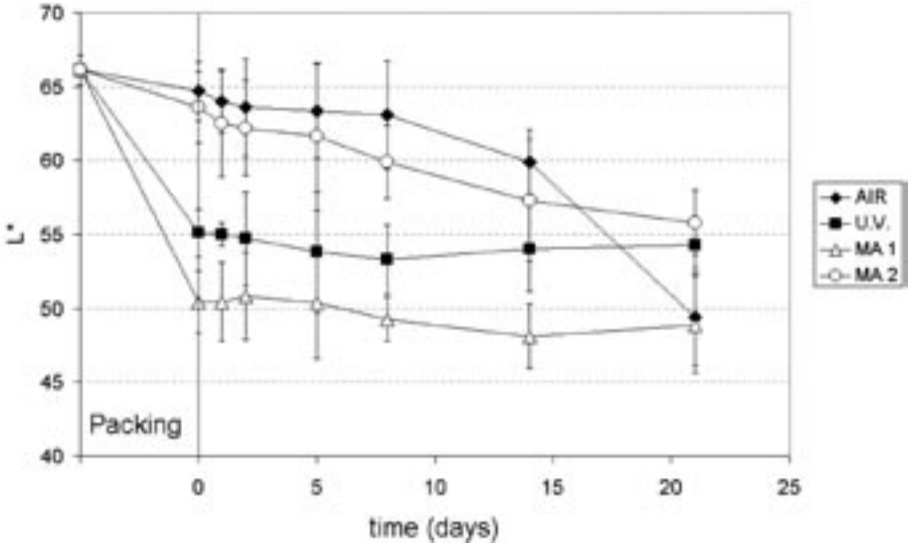


Fig. 4 - Changes of L* as a function of storage time at 4°C of apple cubes reconstituted in HFCS and packed under different conditions.

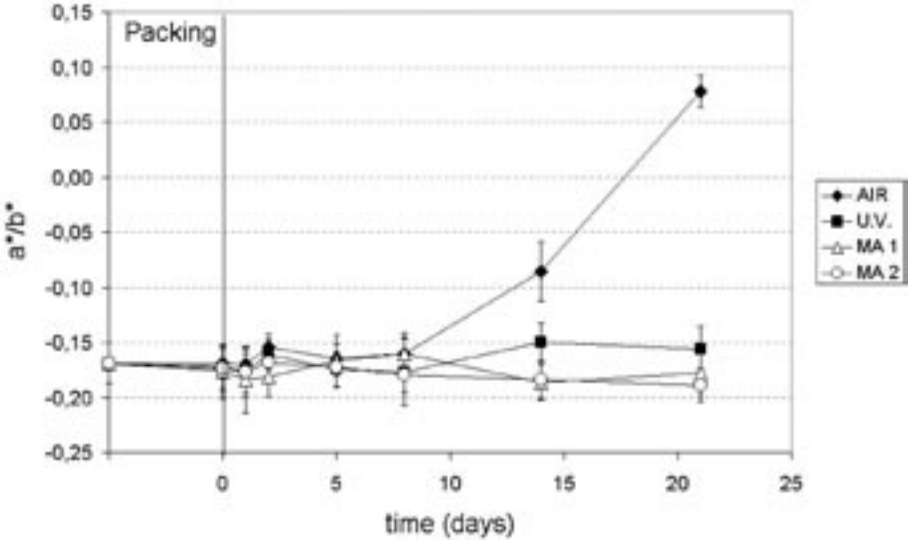


Fig. 5 - Changes of a*/b* values as a function of storage time at 4°C of apple cubes reconstituted in HFCS and packed under different conditions.

this storage time, apple cubes packed in air showed a progressive increase in a*/b* values and decrease in the lightness parameter. After 15 days these colour changes were significant; there was visible decay and the product was unacceptable.

Textural evaluations were also performed to show structural changes during storage; firmness (maximum load) data of the differently packed apple cubes are shown in Fig. 6. Just after vacuum packaging a marked firmness decrease was observed due to air extraction and mechanical compression during packaging. No further changes in firmness of the apple cubes packed under vacuum were observed during storage. All other samples showed a progressive firmness loss during storage time. These textural changes were particularly marked in air and more limited in MA2 packed products. In fresh fruits the softening process is an enzymatic-mediated event that results in the hydrolysis of pectin substance in the middle lamellae and cell wall with a parallel increase in soluble pectin (GLENN and POOVAIAH, 1990). The use of modified atmosphere delays softening by slowing respiratory rates and hence ripening processes (SMITH and RAMASWAMY, 1996; Watada *et al.*, 1996). The preservative effect of nitrous oxide on textural properties could be due to the ability of this compound to inhibit respiration by enzyme binding or to act as an antagonist to ethylene action (GOUBLE *et al.*, 1995). The results of other studies conducted to investigate the effect of nitrous oxide on quality parameters of MA packed minimally processed fruits showed that the ability of this compound to retard product softening was largely dependent on the application of pre-treatments before MAP application (ROCCULI *et al.*, 2002).

In order to test the microbial stability of this type of product, total plate counts and yeast counts were carried out during storage of HFCS rehydrated samples under air atmosphere and chilled condition. After 21 days of storage, HFCS reconstituted sample showed very low total microbial loads (Log CFU/g < 3). Yeast populations were less than 10^5 CFU/g over 21 days of storage. A yeast load of 10^5 CFU/g was

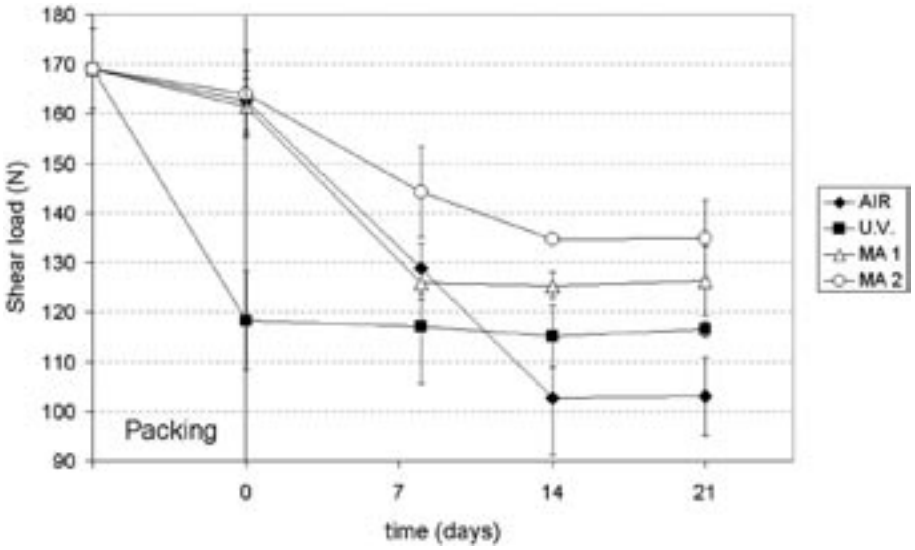


Fig. 6 - Firmness as a function of storage time at 4°C of apple cubes reconstituted in HFCS and packed under different conditions.

taken as a reference considering that the sensory changes of foods are not evident until the spoilage yeast population (except for microorganisms producing undesirable volatile metabolites) reaches 10^5-10^6 CFU/g (FLEET, 1992).

CONCLUSIONS

Freeze-dried apple cubes were reconstituted in water and 15°Bx high fructose corn syrup (Frudex) solution for 5, 20 and 40 min. A 20 min reconstitution time minimized colour and textural changes during processing and maximized water and solid uptake values. The reconstitution in sugar solution rather than water gave a product with improved colour and textural characteristics and with lower water activity values. The addition of 1% ascorbic acid in a dipping solution used prior to freeze-drying and in the reconstitution solution gave dried products with minimum changes in colour and inhibited enzymatic browning for more than 24 h. MAP with low O_2 concentrations played a key role in the inhibition of enzymatic browning during storage in refrigerated condition. After 21 days of storage samples packed under vacuum and in two modified atmospheres (MA1 and MA2) showed more limited changes in colorimetric and textural parameters than air packed sample. Product packed in N_2O-containing atmosphere (MA2) showed minimal changes in colour and loss of texture over storage time; this could be related to the inhibition of enzymatic processes by this compound. The proper combination of acidification with ascorbic acid during processing and of an innovative modified atmosphere packaging could minimize quality changes of reconstituted apple cubes during processing and storage.

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SPECIFIC SPOILAGE ORGANISMS AND SHELF-LIFE OF GREEN OLIVES DIRECTLY FERMENTED IN READY-TO-SELL PACKAGES

MICRORGANISMI ALTERANTI E SHELF-LIFE DI OLIVE DIRETTAMENTE FERMENTATE IN BUSTE PRONTE ALLA VENDITA

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ABSTRACT

The aim of the present study was to evaluate the effect of 4 different packaging films on the fermentation process of green olives, inoculated with *Lactobacillus planturam and Lactobacillus casei* as starter cultures, in ready-to-sell packages. The olives were prepared following the Spanish-style and the fermentation process was monitored until 50 days after packaging. In all batches acid lactic bacteria (LAB) counts (CFU/mL of brine) showed high levels, highlighting the highest values between 30 and 50 days of packaging. The dominant LAB isolates were identified as *Lactobacillus plantarum* species by genotypic methods, confirming the presence of one of the two starters used. Moreover, the dynamics of propionic acid bacteria and yeasts were followed by classical methods showing no differences among all batches.

RIASSUNTO

La presente ricerca ha avuto lo scopo di valutare l'effetto di 4 pellicole, a differente permeabilità, sulla fermentazione, direttamente in contenitori pronti alla vendita, di olive verdi in salamoia inoculate con colture di *Lactobacillus plantarum* e *L. casei*. È stato inoltre valutato l'effetto delle pellicole sulla presenza di micror-

- Key words: green olives, packaging, shelf life, specific spoilage organism -

ganismi alteranti. Le olive sono state preparate seguendo il metodo Sivigliano, ed il processo fermentativo è stato monitorato fino a 50 giorni dopo il confezionamento. Per tutte le confezioni testate la conta su piastra dei batteri lattici (LAB), espressa in UFC/mL, si è mantenuta costantemente elevata raggiungendo i valori più elevati nel periodo compreso tra 30 e 50 giorni dal confezionamento. I microrganismi dominanti, identificati mediante approcci molecolari, sono risultati appartenere alla specie Lactobacillus plantarum, confermando la presenza dell'inoculo. Il dinamismo di microrganismi alteranti quali lieviti e batteri proponici, valutato oltre i 50 giorni, non è stato influenzato dalle diverse pellicole utilizzate.

INTRODUCTION

The world production of table olives is 1,180 tons a year and the fruit is the major fermented vegetable in western countries. Production of naturally fermented green olives is widespread in Sicily and the demand for easily prepared and ready-to-sell green olives, together with globalization of food trade and distribution from centralized processing, pose major challenges for food safety and quality. Traditionally, the preparation of Spanish-style fermented green olives, like other natural vegetable fermentations, is a spontaneous lactic acid fermentation based on an empirical process, which relies upon indigenous microrganisms present in the raw material and environment (FERNÁNDEZ DIEZ, 1983; FERNÁNDEZ *et al.*, 1995). Briefly, the process consists of a treatment with NaOH and fresh water for a few days and subsequently brining process with a 5-10% (w/v) NaCl solution. In this step of spontaneous fermentation the olives are handled in order to favour the growth of *Lactobacillus plantarum* in the brines, which is thought to be essential for preservation as well as for the final product quality (VEGA LEAL-SANCHEZ *et al.*, 2003). Previous studies highlighted that yeast population coexists with *L. plantarum* until the end of the fermentation process and during storage (RUIZ-BARBA and JIMÉNEZ-DÍAZ, 1995), contributing to the sensorial characteristics of the product (FERNÁNDEZ DIEZ *et al.*, 1985). Sometimes, lactic acid amount is not enough to preserve the olives and spoilage occurs through subsequent contamination by other microrganisms. The use of *Lactobacillus plantarum*, as starter culture, has a potential to improve the microbiological control of the process by increasing the lactic acid yield (FERNÁNDEZ DIEZ, 1983; GARRIDO FERNÁNDEZ *et al.*, 1995). In the Spanish-style green olives, as a consequence of NaOH-treatment, the pH of brine increases becoming unsuitable for LAB but optimum for Specific Spoilage Organisms (SSO) (de CASTRO *et al.*, 2002). High pH values, together with low NaCl amounts, allow the growth of microrganisms, such as *Clostridium* spp. and *Enterobacteriaceae*, with consequent depreciatory fermentations, especially during the first fermentation stage. A recent study has outlined that the use of *Enterococcus casseliflavus* together with *Lactobacillus pentosus* species as starters, produces a quicker acidification of brines, consumption of carbohydrates and decrease of pH value (de CASTRO *et al.*, 2002).

The aims of the present study were to evaluate the effect of 4 different packaging films: i) on the fermentation process of green olives directly fermented in packages ready to sale, inoculated with starter cultures and ii) on the growth of spoilage microrganisms.

MATERIAL AND METHODS

Samples

The samples were experimentally prepared in the laboratory following the Spanish-style process using Etna Nocellara olive variety. Briefly, fresh olives were treated with NaOH solution (about 2% w/v) for 16 h and washed twice with tap water and rinsed with water added of lactic acid. Then, the olives were brined in a NaCl solution (8% w/v) and inoculated with *Lactobacillus planturam* type strain (20174) and *Lactobacillus casei* wild strain (T19), previously isolated from Sicilian brine. The strains were added to brine olives to obtain a final concentration of 10^7 CFU /mL. Finally, the treated olives were divided into equal portions, containing 10 olives (corresponding at about 70 g) dipped into 300 mL of brine and packaged using a Delta 30 vacuum machine (Delta System Vacuum, Brindisi, Italy) with 4 different types of film pack, provided by CRYOVAC (Rho, Milano). In detail: film pack with an O_2 permeability of $2.447x10^{-6}$ cm^3 m m^{-2} sec^{-1} atm^{-1} (high permeability, HP); film pack with an O_2 permeability of $1.241x10^{-8}$ cm^3 m m^{-2} sec^{-1} atm^{-1} (low permeability, LP); film pack with an O_2 permeability of $1.242x10^{-7}$ cm^3 m m^{-2} sec^{-1} atm^{-1} (medium permeability, MP1) and film pack with an O_2 permeability of $3.716x10^{-7}$ cm^3 m m^{-2} sec^{-1} atm^{-1} (medium permeability, MP2). For each film pack used, 20 brine batches were obtained and a vessel batch was used as control. The brine batches were stored at room temperature for a period up to 133 days.

Microbiological analyses

Brine samples were diluted in Ringer solution and the number of CFU/mL of different microbial populations was determined by pour plates into the following selective media: MRS (Oxoid) and MSE (Biolife) added of cycloeximide (Sigma) (100 µg/L) incubated at 32°C for 4 days, for lactobacilli and *Leuconostoc* respectively; KAA (Oxoid) was incubated at 32°C for 48 h for *Enterococcus* count. Propionic acid bacteria count was determined using following selective medium: Yeast Extract 1%, Na-lactate 1%, Tripticase 1% KH_2PO_4 0,25%, agar 1,5%, incubated at 32°C for 7 days. These selective media were incubated under anaerobic conditions. Moreover, VRBGA medium (Oxoid) was used for *Enterobacteriaceae*, incubated at 37°C for 48 h. PCA (Oxoid) was incubated at 32°C for 48 h, for aerobic mesophilic count; Sabouraud dextrose agar (Oxoid) with chloranphenycol was incubated at 25°C for 48 h, for yeasts and moulds count; Brilliant Bile Green broth (Oxoid) at 37°C for 48 h and 42°C for 48 h for total and fecal coliform counts respectively, determined by the MPN method for a three tube series. These media were incubated under aerobic conditions. All plates were subjected to microbiological enumeration.

Isolation and preliminary identification of LAB and propionic acid bacteria

A total of 30 colonies of LAB and 13 colonies of propionic acid bacteria, from the highest serial dilutions, were purified on MRS agar medium and on YEL agar, respectively. All strains were subjected to microscopic observation, Gram staining and catalase reaction. The growth of LAB isolates was tested at different NaCl concentrations (2, 8 an 12% w/v). Propionic acid bacteria isolates were also subjected to β-hemolysis and biochemical tests, which were carried out by using purple broth base (Himedia). The latter medium was used according to the manufacturer's in-

structions, and added with mannitol, raffinose, inositol, sorbitol and starch (10 g/L). The tubes were incubated in anaerobic conditions at 30°C for 6 days. The results were considered positive when bromocresol purple changed from purple to yellow.

Extraction of total DNA from LAB

Cell cultures (1.5 mL) in the late exponential growth phase were centrifuged at 8,000 rpm for 10 min and the cell pellets were washed and resuspended in 0,5 mL of TE-buffer (10mM Tris-HCl; 1 mM EDTA, pH 8,0). The suspension was poured in a 2 mL screw-capped tube containing 0.3 g of sterile zirconium beads (diameter, 0.1 mm), homogenized in a bead-beater (Biospec Products, Bartlesville, OK) at 5,000 rpm for 3 min and cooled on ice. The homogenate was centrifuged at 13,000 rpm for 5 min and the supernatant fluid was stored at -20°C until use.

PCR-RFLP analysis of the 16S rDNA

PCR amplification was performed with Taq Dna polymerase Recombinant (Invitrogen-Life Technologies, U.K.) using primers 7f 5'-AGAGTTTGATC/TA/CTGGCTCAG-3' and 1510rev 5'-ACGG(C/T)TACCTTGTTACGACTT-3' (Lane, 1991). Reaction mixtures consisted of 20 mM Tris HCl (pH 8,4), 50 mM KCl, 3 mM MgCl2, 50 mM each dNTP, 1.25 U of Taq polymerase, 5 pmol of each primer and 1 µL of appropriately diluted template DNA in a final volume of 50 µL. DNA from isolates and type strains was amplified in a PCR Express Thermocycler (Hybaid) programmed as follows: 94°C for 3 min; 30 cycles of 94°C for 30 s, 52°C for 30 s, and 68°C for 1.5 min; and finally 68°C for 7 min.

Restriction fragment length polymorphism (RFLP) of 16S rDNA PCR products was performed by restriction enzyme digestion with Hae III, Hin6 I and Alu I (MBI Fermentas), followed by electrophoresis of the products on a 2% (wt/vol) agarose gel in 1X TBE buffer (89 mM Tris–borate, 89 mM boric acid, 2 mM EDTA; pH 8.0) containing ethidium bromide. The ReadyLoad™ 100 bp DNA Ladder (Invitrogen-Life Technologies, U.K.) was used as size standard. Gel was visualized after electrophoresis on a 1.2% agarose gel containing ethidium bromide.

RESULTS AND DISCUSSION

Microbiological profile

Microbial dynamics of LAB during fermentation period of batches, packaged with the 4 different film packs, were evaluated in the selective media and are shown in Figs. 1 and 2. In detail, the lactobacilli counts (CFU/mL), evaluated in MRS medium, showed a decrease until the 28th day of fermentation process and an increase from the 28th to 50th day with the highest value in LP film pack (1x10^{8} CFU/mL) (Fig. 1). The Leuconostoc count (MSE medium) was, for all batches, almost constant during the fermentation period except for the LP film pack batch that showed the highest value (1x10^{8} CFU/mL) from the 35th to 49th days (Fig. 2). The *Enterococcus* count (KAA medium) showed, in all batches tested, the lowest values approximately at the 30th day and a constant increase until the 50th day of fermentation (data not shown).

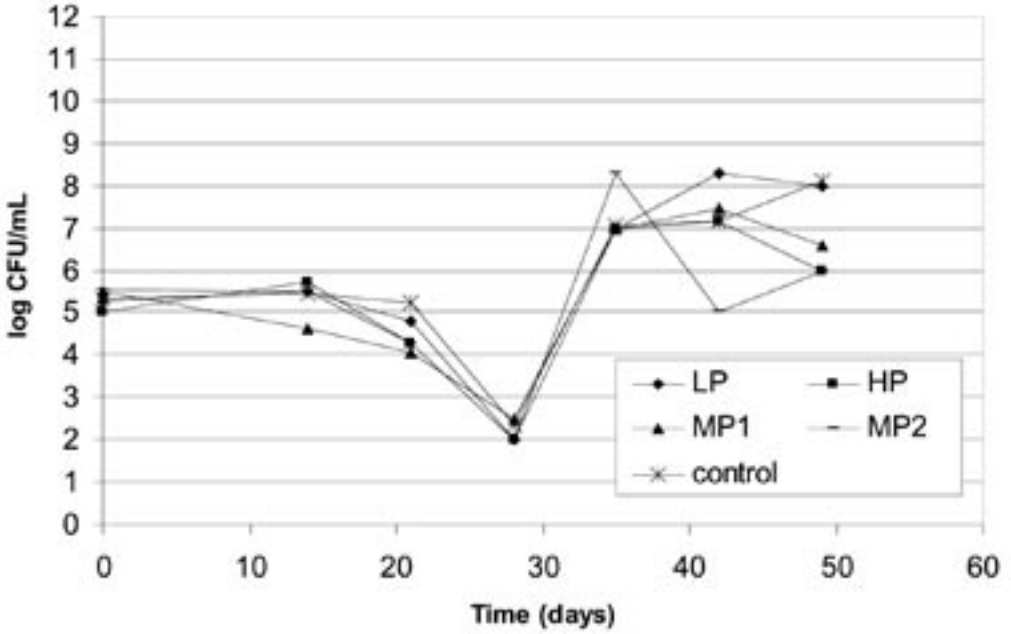


Fig. 1 - Changes of microbial population in MRS agar medium of brine batches packaged with different films (LP – Low Permeability; HP – High Permeability; MP1 and MP2 – Medium Permeability).

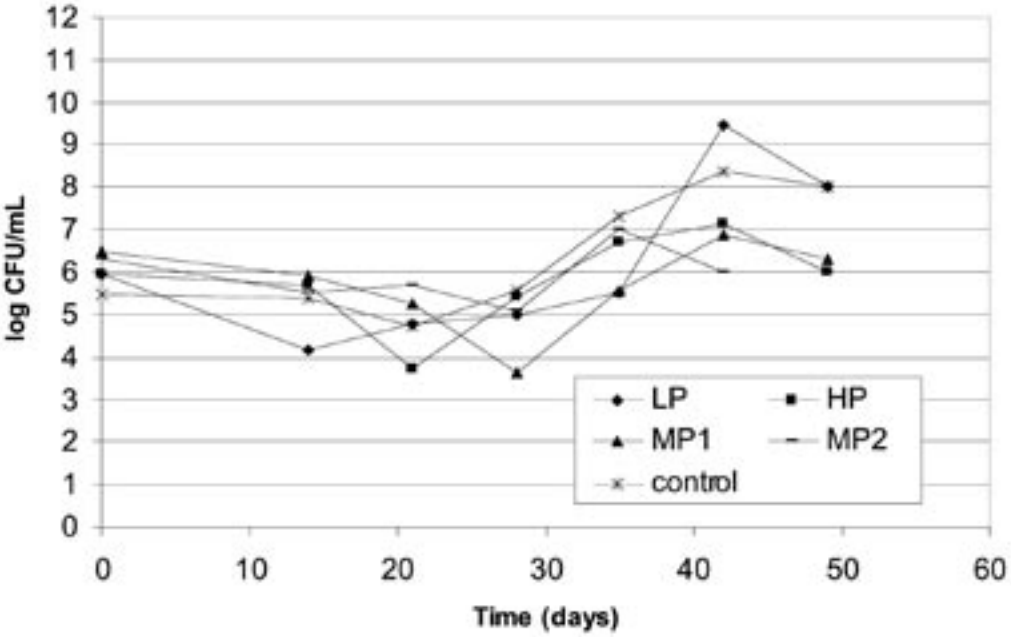


Fig. 2 - Changes of microbial population in MSE agar medium of brine batches packaged with different films (LP – Low Permeability; HP – High Permeability; MP1 and MP2 – Medium Permeability).

As highlighted by microbial enumeration, the highest values of LAB were found in the batches packaged with LP film pack, that having low O_2 permeability, could create microaerophylic conditions that encourage LAB growth.

In Fig. 3 yeast and mould population counts showed an increasing trend, reaching the highest value ($1x10^9$ CFU/mL) after 40 days of fermentation in all batches. The yeast and mould enumeration was comparable among all samples, the batch HP showed a dark brine, probably due to oxidation activity, and a massive presence of moulds.

Regarding the *Enterobacteriaceae* counts, as expected, the number of total and faecal coliform decreased constantly after the fermentation period (data not shown).

As reported in the literature (POM B39, 2001), the propionic acid bacteria are able to grow after the fermentation process. The counts, shown in Fig. 3, were found to reach the highest value approximately at 80 days ($1x10^5$ CFU/mL), decreasing until the 133rd day with the lowest value ($1x10^2$ CFU/mL) in MP2 sample. Propionic acid bacteria counts, in vessel control, showed low values between 56 and 86 days, reaching at the 133rd day, a value similar to film pack.

Genotypic identification of LAB

In order to monitor the fermentation process during the first 50 days, 30 representative lactic acid bacteria (LAB), from selective media with the highest serial dilution, were isolated, purified and identified by PCR-RFLP of the 16S rDNA, using Hae III, Hin6 I and Alu I restriction enzymes (MBI Fermentas). The profiles were compared to profiles obtained from type and wild strains used as starter. The results showed three distinct profiles. Sixteen strains appeared to be identical to *Lactobacillus plantarum* type strain 20174 (Fig. 4 lane 19) and 1 strain to *Lactobacillus casei* wild strain. The remaining 11 strains showed a unique profile (data not shown). The results indicated the dominance of *Lactobacillus plantarum* species during the fermentation process as guarantee that lactic fermentation occurred. The high isolation frequency of strains with different profiles, belonging to other species, will be subject of further investigations.

Preliminary identification of propionic acid bacteria

Propionic acid bacterial isolates from selective medium were subjected to phenotypical and biochemical tests, including the acid production from mannitol, sorbitol, raffinose, inositol and starch and results are reported in Table 1. All 13 strains were positive to the catalase reaction, negative to β-haemolysis test and Gram positive. No strain fermented inositol and starch; only the strains OPR 10, OPR11, OPR 12, fermented mannitol, sorbitol and raffinose, showing the same biochemical profile as *Propionibacterium jensenii* (DE CARVALHO *et al.*, 1995).

CONCLUSIONS

This study assayed the influence of packaging with different oxygen permeability films on Spanish-style green olive fermentation. The results have demonstrated

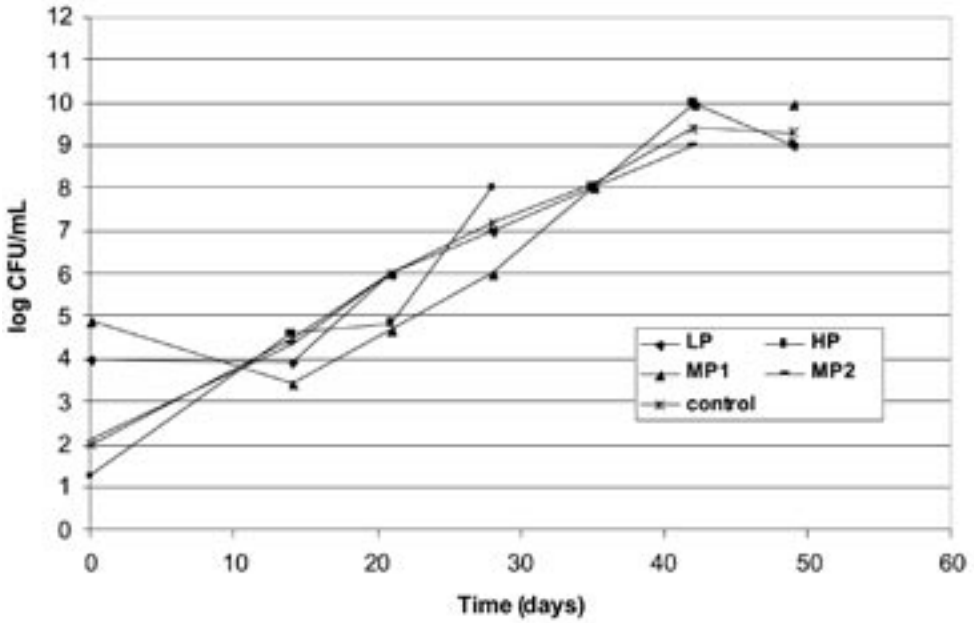


Fig. 3 - Changes of microbial population in Sabouraud Dextrose agar medium of brine batches packaged with different films (LP – Low Permeability; HP – High Permeability; MP1 and MP2 – Medium Permeability).

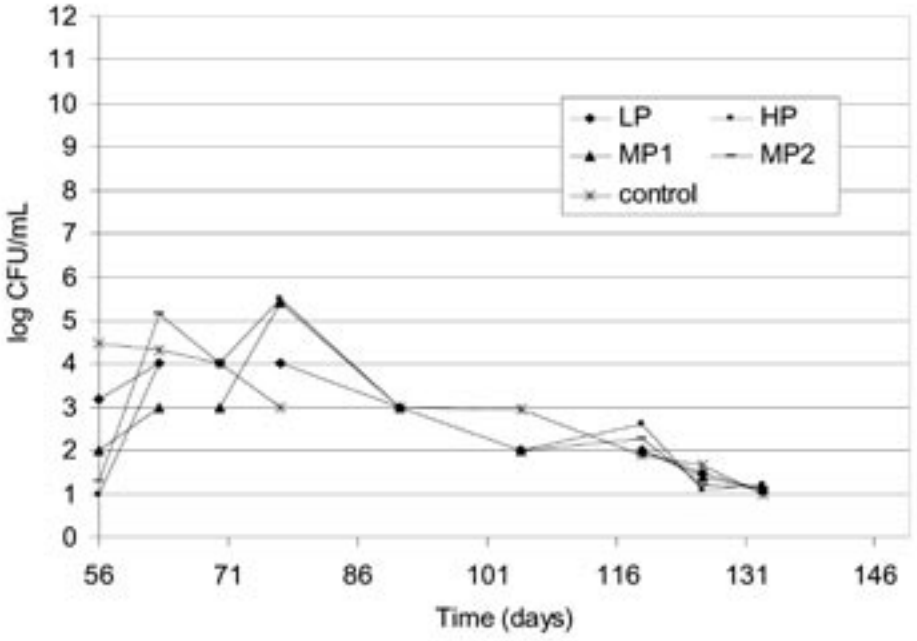


Fig. 4 - Changes of microbial population in YEL agar medium of brine batches packaged with different films (LP – Low Permeability; HP – High Permeability; MP1 and MP2 – Medium Permeability).

Table 1 - Origin, and biochemical characteristics of the presumptive propionic acid bacteria.

Strains	Batches	Gram staining	Catalase reaction	Colour of pigment	β-haemolysis	Acid from:				
						MAN	SOR	RAF	INO	Starch
OPR5	LP	+	+	transparent	-	-	-	-	-	-
OPR6	HP	+	+	transparent	-	-	-	-	-	-
OPR7	HP	+	+	transparent	-	-	-	-	-	-
OPR9	MP2	+	+	transparent	-	w	-	-	-	-
OPR10	CONTROL	+	+	white	-	+	+	+	-	-
OPR11	MP2	+	+	yellow	-	+	+	+	-	-
OPR12	MP1	+	+	orange	-	+	+	+	-	-
OPR13	MP2	+	+	cream	-	w	-	-	-	-
OPR14	LP	+	+	white	-	w	-	-	-	-
OPR15	HP	+	+	light-brown	-	w	-	-	-	-
OPR16	HP	+	+	white	-	w	-	-	-	-
OPR17	HP	+	+	light-brown	-	w	-	-	-	-
OPR18	HP	+	+	white	-	-	-	-	-	-

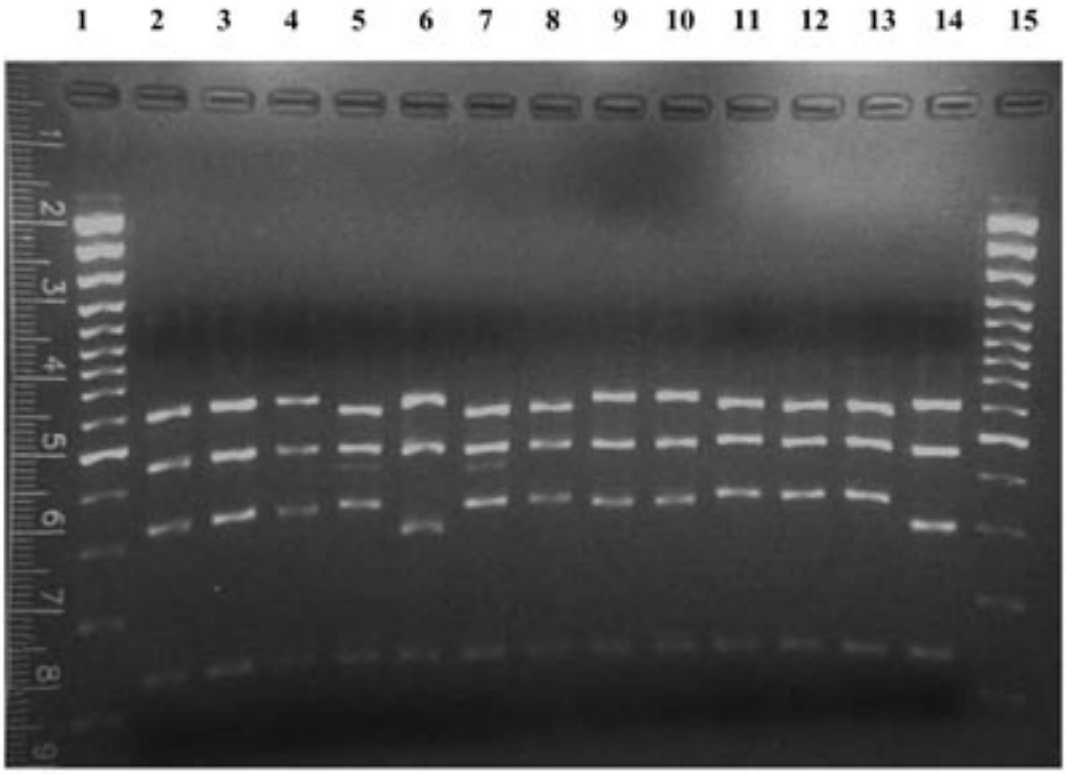


Fig. 5 - Restriction patterns of 16S rDNA with the endonuclease *Hae*III. From left to right: lane 1: 100 bp DNA ladder; lane 2: MDS1; lane 3: MDS2; lane 4: MDS3; lane 5: MDS 4; lane 6: MDS 5; lane 7: MDS 6; lane 8: MDS 7; lane 9: MDS 8; lane 10: MDS 9; lane 11: MDS 10; lane 12: MDS 11; lane 13: *L. plantarum* 20174; lane 14: *L. casei* T19; lane 15: 100 bp DNA ladder.

that fermentation process was carried out successfully in all film pack tested, using *Lactobacillus plantarum* and *L. casei* as starter cultures.

Regarding LAB population, the highest value of CFU/mL was found in the brine packaged with low permeability film pack, suggesting that such film could be suitable for olive brine, fermented directly in film pack ready to sale.

No influence was found on yeasts, *Enterobacteriaceae* and propionic acid bacteria populations in the different packages.

The propionic acid bacteria, found after the fermentation process, reached the highest value approximately at the 80th day, decreasing until the 133rd day. The only difference for propionic acid bacteria was found in vessel control, that showed lower values between 56 and 86 days.

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INFLUENCE OF TEMPERATURE ON THE QUALITY FACTORS OF SHREDDED CARROTS

EFFETTO DELLA TEMPERATURE SULLA QUALITÀ DELLE CAROTE ALLA JULIEN

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ABSTRACT

A mathematical model was developed in order to describe the effect of temperature on the degradation process of shredded carrots. Shredded carrots were stored in an air flux system in the range of temperature 0°-20°C. Storage time varied with temperature to observe the complete decay of pH and colour over time. The experimental data collected at 10°C were used to validate the model. The evolution of the pH over time was reasonably well describes by a Weibull equation, while a first order equation described the colour change. The rate constants of both models varied with temperature according to an Arrhenius - type relationship with activation energy equal to 71±2 KJ/mol for the pH, and 89±5 KJ/mol for the colour.

RIASSUNTO

Un modello matematico è stato sviluppato per descrivere l'effetto della temperatura sui processi di degradazione delle carote tagliate alla julien. Il prodotto è stato condizionato in un sistema a flusso continuo di aria in un range di temperatura

- Key word: kinetics model, shredded carrots, temperature -

0°-20°. Il tempo di conservazione è stato stabilito in funzione della temperatura in modo da osservare il completo decadimento dei parametri pH e colore. Il modello è stato validato a 10°C. Il modello di Waibull descrive bene la variazione del pH nel tempo, mentre la variazione del colore segue una cinetica del primo ordine. Le constanti cinetiche di entrambi i modelli variano con la temperatura secondo una relazione di Arrhenius con una energia di attivazione di 71±2 KJ/mol per il pH e 89±5 KJ/mol per il colore.

INTRODUCTION

Consumer demand for freshness and convenience food has led to the development of many novel products and in particular fresh pre-cut fruits and vegetables. The main concern with these novel products is the detrimental effect on the product quality, exerted by minimally processing operation, i.e. washing, peeling and shredding. Fresh-cut produce in fact is highly perishable with a shelf-life shorter than intact products (BARRY-RYAN & O'BEIRNE, 1999, ZHU *et al.*, 2002, FONSECA *et al.*, 1999). The major objective of scientists and technologist working in this area is to extend the shelf-life of these products while maintaining high quality standards. Refrigeration during storage is essential for preserving the overall quality of minimally-processed food and vegetables since microbiological growth and physical phenomena, which affect the visual appearance of processed vegetables, are very sensitive to storage temperature and temperature fluctuation which may occur along the distribution chain (JACXSENS *et al.*, 2002). Knowledge of the time-temperature history experienced by a ready-to-eat vegetable in the cold chain is of fundamental importance to predict the quality loss and thus the shelf-life of these products. Temperature abuse, such as storage at ambient temperature or improper cooling (T>7°C) has been identified as the major factor in outbreaks of food-borne diseases. In order to predict minimally processed food shelf-life or design the most convenient package for these products, adequate mathematical models are necessary to assess the impact of different parameters and variables, such as temperature and gas composition inside the package, on the quality decay of the product. In fact the decay of the global quality of a commodity is the function of several ambient factors and it is the result of several mechanisms of deterioration. Due to the nature of food systems, describing the evolution of their quality during the storage period is a quite complex task which involves defining one or more appropriate indices directly correlated to the food quality perception. These indices can be derived from sensory evaluation, and chemical or physical measurements (ZOBOLI, 1999, PRZYBYLSKI & ZAMBIAZI, 2000). According to Horta and co-workers the most appropriate characteristics of shredded carrots which can be used to study the effect of the temperature are pH and colour, since other possible characteristics vary significantly during shelf-life or their variability from sample to sample is too high. Moreover there is experimental evidence that both can be correlated to the quality evolution of the carrots during shelf-life (AMANATIDOU *et al.*, 2000; GARCIA-GIMENO, & ZURERA-COSANO, 1997, TALCOTT *et al.*, 2001).

Quality food deterioration is commonly described by a mathematical equation of the type:

$$-\frac{dQ}{dt} = kQ^n \tag{1}$$

where: Q is an appropriate quality index, t is the time, K the rate constant of the quality index decay and n is the pseudo-order of the reaction.

A negative sign is used if the deterioration leads to a decrease of Q and a positive sign for an increase of the index. Usually, quality deterioration processes of foods stored under controlled environmental condition can be described with zero order or first order decay equations. In fact food quality loss at room temperature proceeds quite slowly and moreover many food products become unacceptable as soon as quality diminishes 20-30% with respect to the initial value, thus the quality decay to an unacceptability threshold can be even described by a simple zero order model (LABUZA, 1984). However, there are situations where this approach is too simplistic. Some microbial, enzymatic and chemical degradation kinetics require a more accurate description such as the one provided by the Weibull distribution equation (CARDELLI & LABUZA, 2001; FERNANDEZ *et al.*, 2002; CHUNA *et al.*, 1998). The Weibull distribution probability hypothesized a density function given by (HAHN & SHAPIRO, 1967):

$$f(t) = \frac{\beta}{\tau}^{\beta-1} * \exp\left[-\left(\frac{t}{\tau}\right)^{\beta}\right] \quad t>0 \tag{2}$$

With $\tau>0$ and $\beta>0$, whose cumulative distribution is:

$$F(t) = \int f(t)dt = 1 - \exp\left(-\left(\frac{t}{\tau}\right)^{\beta}\right) \tag{3}$$

τ is a scale parameter and $1/\tau$ has the same meaning as the reaction rate constant in eq. 1 while the shape parameter β acts as a behaviour index.

Equation 1-3 can predict the quality evolution on time of packaged commodities provided an estimate of the parameters and their dependence on temperature. Kinetics constant variation with temperature frequently follows an Arrhenius-type equation:

$$K = Ko \exp\left(-\frac{Eatt}{R}\left(\frac{1}{T} - \frac{1}{Tref}\right)\right) \tag{4}$$

Where Ko is the rate constant at a reference temperature, Eatt, the activation energy in KJ/mol, R, the ideal gas constant in KJ/mol K, and Tref, an absolute reference temperature in K. When the package experiences a given time-temperature history T(t), the value which the quality function, F(Q)t, assumes at the time t, can be found according to the equation 1 or 2 by integrating K(T(t)) dt or $1/\tau$ (T(t)) dt from 0 to time t. If the Tref is taken as storage constant temperature to which correspond after time t the same quality F(Q)t, which the packaged food possess

after the time-temperature history T(t), then the response function will be in both cases given by:

$$F(Q)t = \int K(f(T))dt \quad (5)$$

where K(f(T)) is the time-temperature dependence of quality loss rate constant. The aim of this paper was the validation of the proposed mathematical model in the cases of pH and colour of shredded carrots.

MATERIAL AND METHOD

Carrots were purchased at the local market. Each set of experiments was performed by using a fresh lot. The carrots were peeled, washed with tap water and shredded. The excess of water was removed with a vegetable centrifuge. Samples of shredded carrots were stored in an air flux system at 0°, 4°, 8°, 10°, 12°, 16° and 20°C. A schematic drawing of the experimental equipment is shown in Fig. 1. It consists of a pump (50 L/h) (A) which conveys ambient air into a humidification chamber (B), provided with four outlets each connected to a plastic box (C). Each box is provided with tight inlets and outlets for standard compressed air tubing through which humidification air enters and comes out continuously. 200 g of shredded carrots were packed in each box. Storage time was a function of the temperature and was chosen in order to observe the complete deterioration of the sample based on previous results (HORTA *et al.*, 2002). At each temperature sampling time was selected in order to get a sufficient number of points to represent the deteriorative process (Table 1). Three replicates were performed at each temperature.

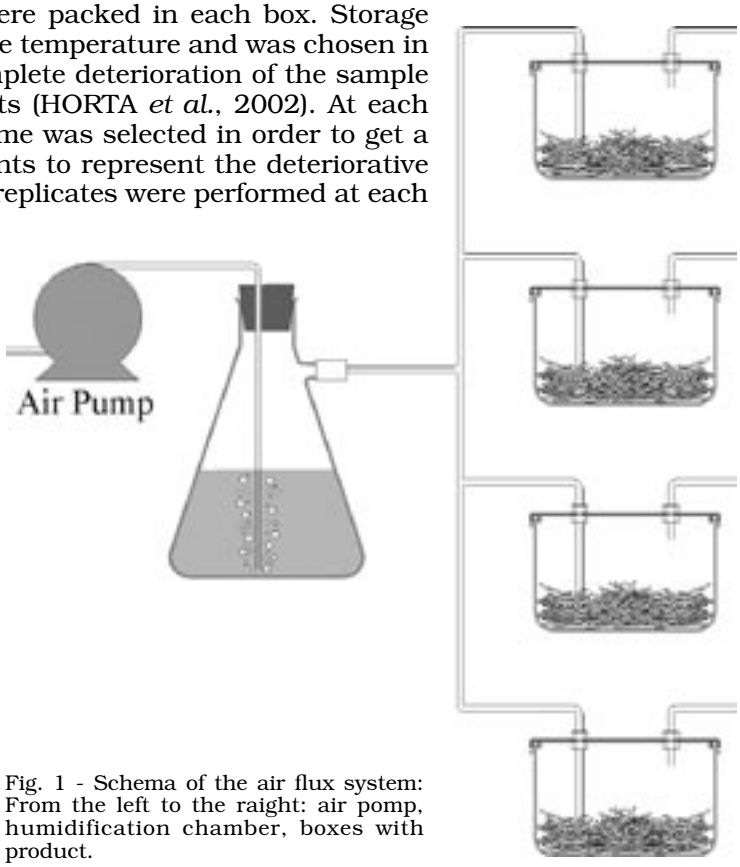


Fig. 1 - Schema of the air flux system: From the left to the raight: air pomp, humidification chamber, boxes with product.

Colour measurement

The colour of the shredded carrots was measured with a tristimulus colorimeter (Minolta Colour Meter CR-300). The diameter of the measurement area was equal to 8 mm. Before performing the colour measurement, the colorimeter was calibrated using a white standard plate (L=100). The colour measurements were performed on the surface of 200 g of shredded carrots

Table 1 - Experimental storage time and sample time to measure the pH and colour of shredded carrots.

Temperature (°C)	Time (days)	Sampling time (days)
0	30	0, 3, 6, 9, 11, 15, 22, 25, 27, 30
4	12	0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14
8	10	0,1, 2, 3, 4, 5, 6, 8, 10
12	6	0, 1, 2, 3, 4, 5, 6
16	6	0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6
20	3	0, 0.5, 1, 1.5, 2, 2.5, 3

placed inside the box. The colour were measured in five different positions: two measurements were performed in each position and the shredded carrots were mixed after the first ten measurements and ten extra measurements were done. Twenty readings were taken for each sample and the figures reported are the arithmetic mean of the various measurements.

pH measurement

Aliquots of 25 g of shredded carrots were homogenized with an equal volume of distilled water. The pH of the homogenate was determined with a standard pH meter (Jenway 3310) equipped with a Schott electrode that was previously calibrated by using buffers solution (pH 4 and pH 7) at 20°C. The pH value was expressed as the average of four independent measurements. Two samples were analysed from each box and two measurements were carried out from each sample.

Data Analysis

Data were submitted to ANOVA analysis ($p<0.05$) (Microsoft Excel) to find out the effect of the storage time and temperature on the main quality indices. Experimental data were fitted to the models by least-squares procedure. As the models were not linear the best fittings were achieved by non linear regression, performed by means of the package STATISTICA (release 5.1, 97 edition, Statsoft, Tulsa, OK, USA). The iterative procedure started from a set of parameters given by the operator. The validity of the solution found by statistical procedure was checked by examining statistical figures.

RESULT AND DISCUSSION

Mathematical modelling of colour variation

During storage shredded carrots lose their typical orange colour and when stored at relatively high temperature, browning of the surface becomes evident. According to the CEI system, several parameters derived from instrumental measurement can be used to represent the colour variation such as the coordinate

a*, b*, and L*. The "a*" coordinate of the CEI system was chosen because it was the one which best described the colour change of the shredded carrots during storage at different temperatures. A first order decay equation of the form:

$$\frac{a^*}{a^*_0} = \frac{a^*_{inf}}{a^*_0} + \left(1 - \frac{a^*_{inf}}{a^*_0}\right)\exp(-kt) \tag{6}$$

was used to describe the variation of a* through time. To account for the natural variability of the raw material, the actual value of the coordinate "a*" was normalized by dividing it by the corresponding value at time zero. In equation 6, K is the rate constant while a^*_{inf}/a^*_0 is a second parameter which in principle may vary with temperature. At first, experimental data were fitted with eq. 6 by considering both K and a^*_{inf}/a^*_0 adjustable parameters. Fig. 2 shows the influence of temperature on these parameters. From (Fig. 2a) it can be noticed that the a^*_{inf}/a^*_0 values at various temperature are randomly scattered around a constant value (~0,6). Therefore a second fitting procedure was performed by keeping the term a^*_{inf}/a^*_0 constant and equal to 0,6 considering only K as an adjustable parameter. Fig. 2b shows the result of this second procedure. The rate constant K was plotted against the reciprocal of the absolute temperature and a reasonably good linear correlation was obtained, thus K(T) can be described by an Arrhenius-type equation. According to the experimental findings, the colour variation of shredded carrots with varying time and temperature is fully described by the set of equations formed by eq. 4, 6 and:

$$\frac{a^*}{a^*_0} = const \tag{7}$$

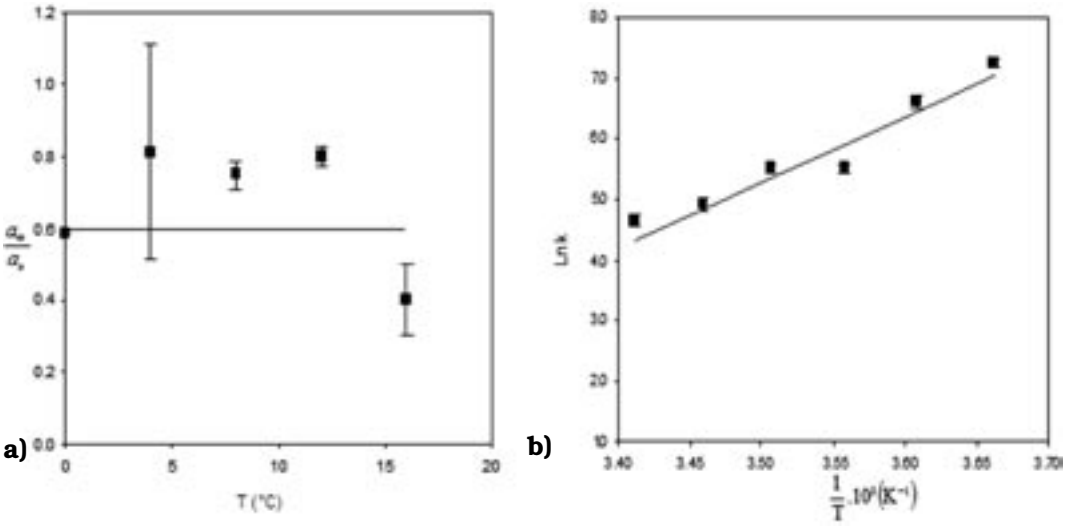


Fig. 2 - Dependence of the first order model parameters from the temperature.

Table 2 - Parameter estimates of the mathematical model in eq. 8 and in eq.11 and relevant statistical data.

Model constant	Estimates±SE	Correlation matrix of parameters		
		$\frac{a^*_{inf}}{a^*_o}$	1/ko	
$\frac{a^*_{inf}}{a^*_o}$ (dimensionless)	0.60±0.06			
ko (1/h)	0.003±0.001	0.96		
Eatt (kJ/mol)	89±5	0.097	-0.089	
R^2_{adj} %	75.5			
		$\frac{pH_{inf}}{pH_o}$	β	1/τ o
$\frac{pH_{inf}}{pH_o}$	0.55±0.01			
β (dimensionless)	2.8±0.3	0.27		
1/τo (1/h)	0.0123±0.0006	0.59	0.22	
Eatt (kJ/mol)	73±4	0.04	0.06	0.18
R^2_{adj} %	88.3			

to get *an estimate* of the parameters which characterise the Arrhenius equation Ko and Eatt, eq. 4 and 7 were incorporated in eq. 6 providing the following relationship:

$$\frac{a^*}{a^*_0} = \frac{a^*\text{inf}}{a^*_0} + \left(1 - \frac{a^*\text{inf}}{a^*_0}\right)\exp\left(-\left(ko\exp\left(-\frac{Eatt}{R}\right)*\left(\frac{1}{T}-\frac{1}{Tref}\right)\right)t\right) \quad (8)$$

which was fitted to the experimental result by using a non-linear regression procedure. This one–step analysis offers the advantage of a great precision because it avoids the statistical error of the estimation of the intermediate parameters K. The parameters evaluated according to this procedure are listed in Table 2. The proposed mathematical model provides a good fit of the experimental data (Fig. 3), a R^2_{adj} is equal to 75.5% and the residuals are randomly distributed around an average value equal to zero (Fig. 4). By considering the high variability of the raw material this result can be considered quite satisfactory.

Mathematical modelling of the pH variation

Fig. 5 shows the variation of pH of shredded carrots stored under air. pH data collected at different times follow a sigmoidal curve, passing from a value equal to 6.4±0.3, corresponding to fresh carrots, to a value which appears to be independent of temperature and is equal to 3.6±0.2.

ANOVA analysis indicated that time has a significant effect on the pH of shredded carrots with a 95% confidence (P0.05<<0.001). By considering the shape of the pH curves vs time at all temperatures one can conclude that a

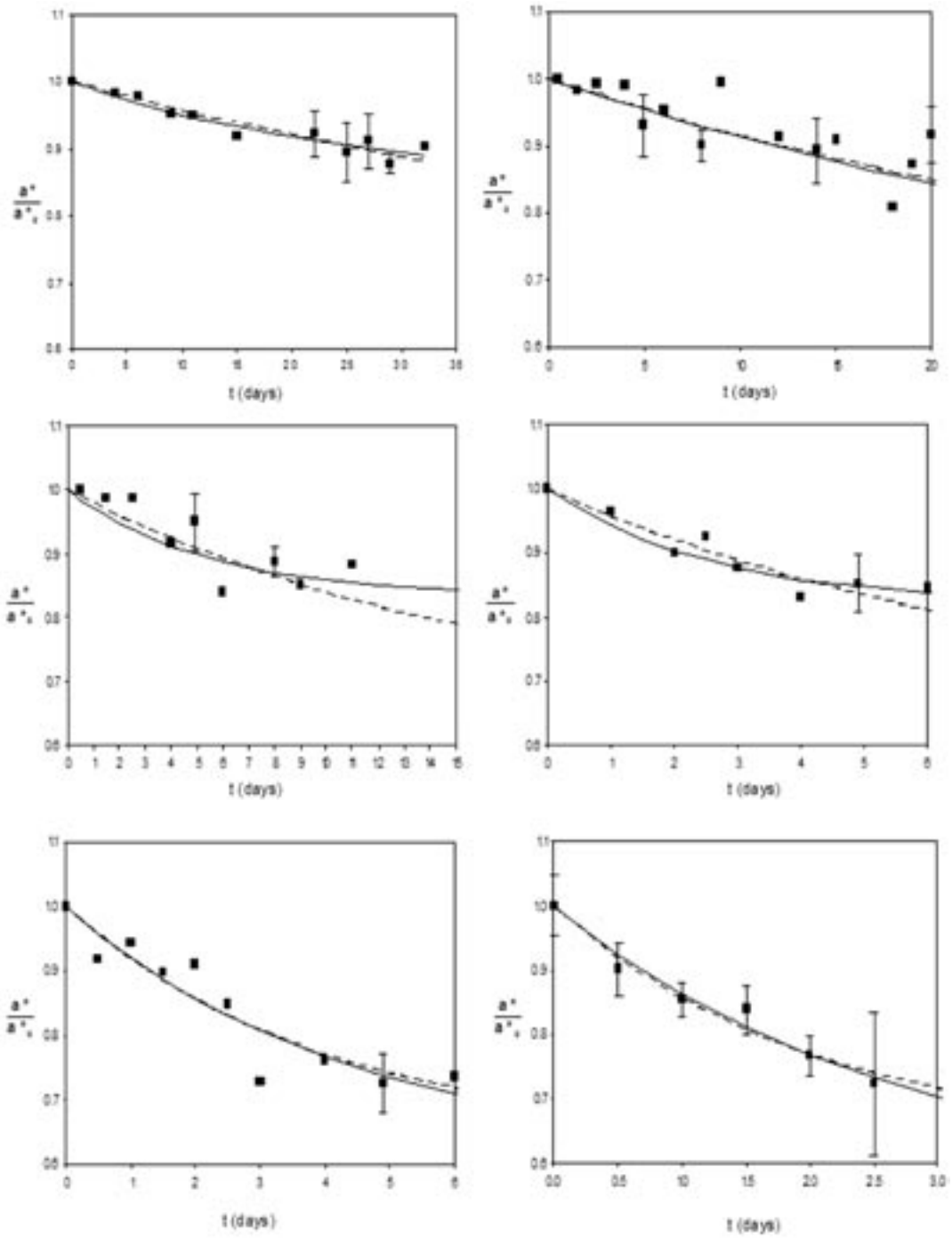


Fig. 3 - Change of the colour (a^*/a^*_o) of the shredded carrots stored in air-flux system over time in a range of temperature 0°-20°C. The dots represent experimental data with the standard deviation, the continous line shows the fit of the first order model to the experimental data at each temperature; the broken line shows the fit of the global model.

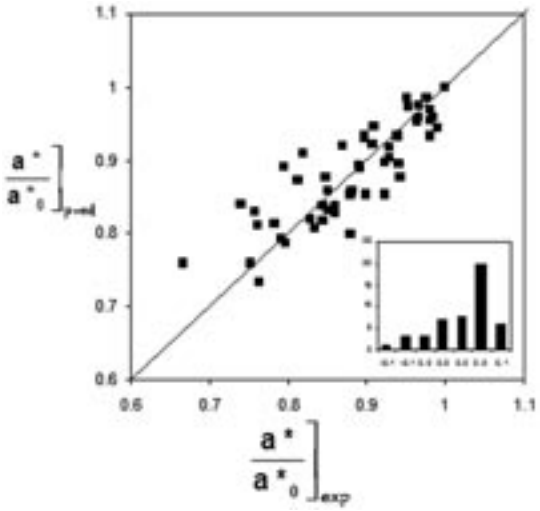


Fig. 4 - Relationship between experimental and predicted values of the colour (a^*/a_o^*) measured during storage of shredded carrots in air flux system in a range of temperature 0°-20°C. Enclosed figure: Frequency distribution of the residual.

first order decay equation is not appropriate to describe the pH evolution on time of shredded carrots. By contrast a Weibull equation of the form:

$$\frac{pH}{pHo} = \frac{pH}{pHo} + \left(1 - \frac{pH}{pHo}\right)\exp\left(-\left(\frac{t}{\tau}\right)^{\beta}\right) \quad (9)$$

where pHo and pHinf are the initial and the steady state values which pH assume respectively, appears to be more adequate. In eq. 9, the term $1/\tau$ has the same meaning as the reaction constant (k) and β is a slope factor (CHUNA *et al.*, 1998). Table 5 summarises the relevant statistical figures which support the hypothesis that Weibull equation is an adequate model to describe the pH variation of shredded carrots with varying time. Again a preliminary investigation was made to assess the influence of temperature on the parameters of eq. 9. Fig. 6 shows the values of pH_{inf}/pH_o, β and $1/\tau$ estimated by non-linear regression of experimental data by using equation 9 at a given temperature. The results plotted in Fig. 6 suggested that pH_{inf}/pH_o as well as β do not vary with temperature, as already reported in the literature (Chuna *et al.*, 1998). Instead $1/\tau$ increases almost linearly with the reciprocal absolute temperature. Hence, an Arrhenius-type equation:

$$\frac{1}{\tau} = \frac{1}{\tau o}\exp\left(-\frac{Eatt}{R}\left(\frac{1}{T} - \frac{1}{Tref}\right)\right) \quad (10)$$

can be used to predict the reaction rate with varying temperature once the parameters Eatt and $1/\tau$ are known. Following the procedure outlined before to define the model relative to the colour of shredded carrots, eq. 10 was incorporated into eq. 9 obtaining:

$$\frac{pH}{pHo} = \frac{pH\,\inf}{pHo} + \left(1 - \frac{pH\,\inf}{pHo}\right)\exp\left(-\left(1/\tau o\exp\left(-\frac{Eatt}{R}\right)*\left(\frac{1}{T} - \frac{1}{Tref}\right)\right)t\right) \quad (11)$$

setting pH_{inf}/pH_o and β equal to a constant and experimental data were fitted to the resulting equation by using a non-linear regression analysis. Tab 6 lists the estimated parameters and the relevant statistical figures. The agreement between the mathematical model and the experimental data is shown in Fig. 5. R^2_{adj} was equal to 89.2% and residual distributed and following a normal distribution with an average equal to zero (Fig. 7 a-b).

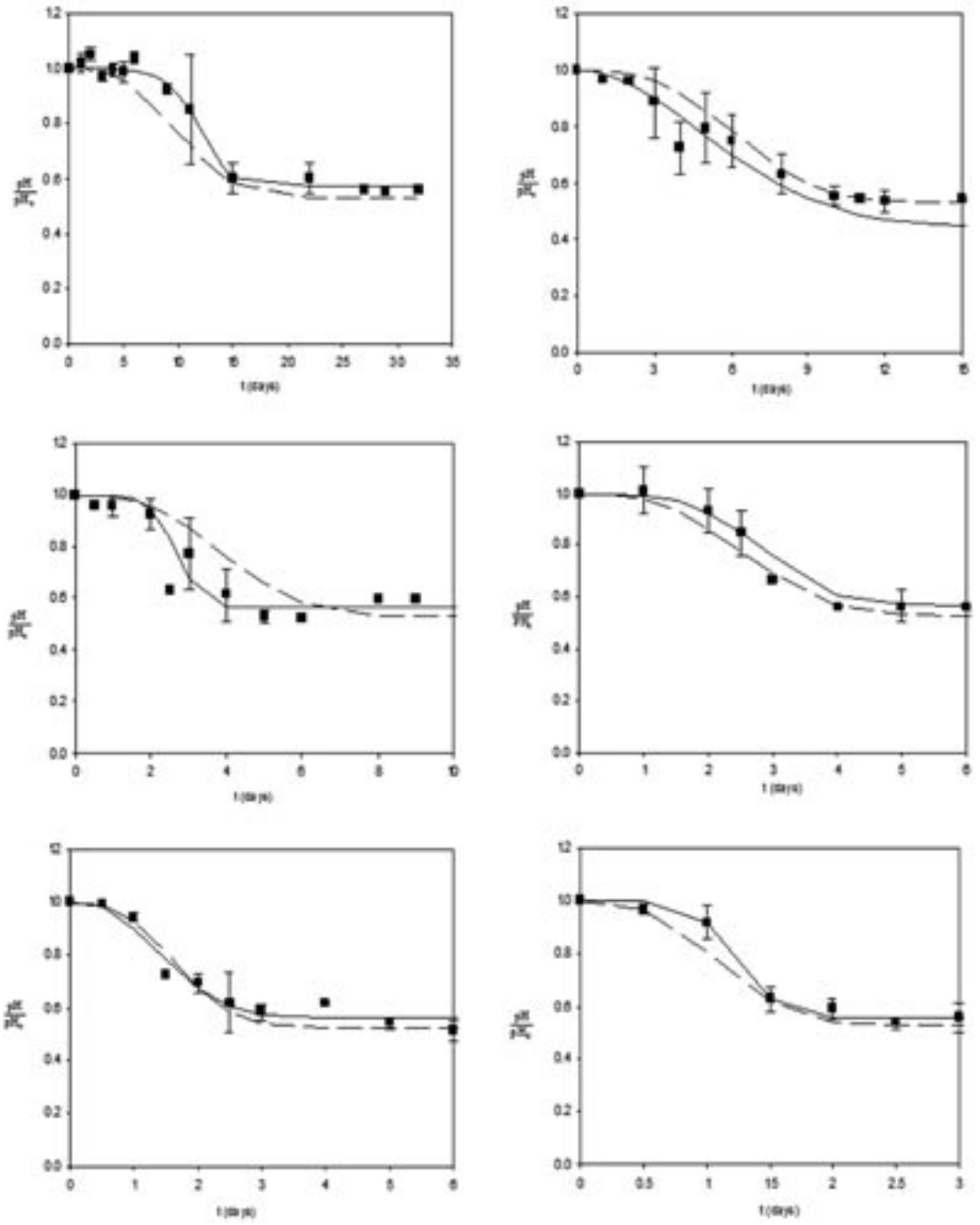


Fig. 5 - Change of the pH/pH_0 of the shredded carrots stored in air-flux system over time in a range of temperature 0°-20°C. The dots represent experimental data with the standard deviation, the continous line shows the fit of the Weibul model to the experimental data at each temperature; the break line shows the fit of the global model.

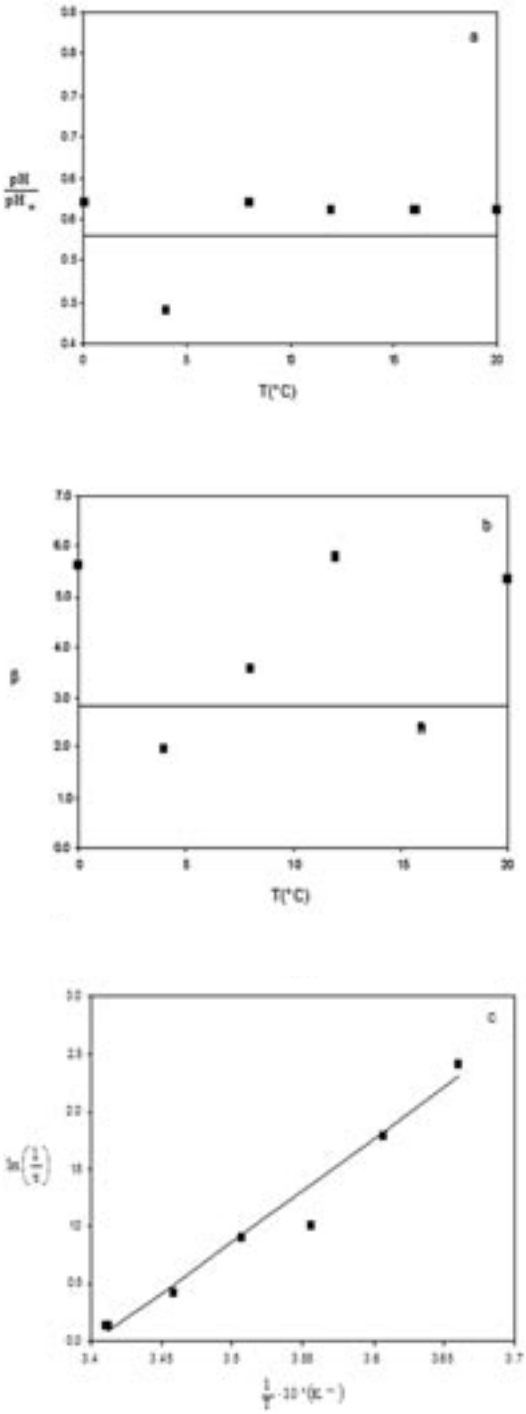


Fig. 6 - Dependence of the Weibull model parameters from the temperature.

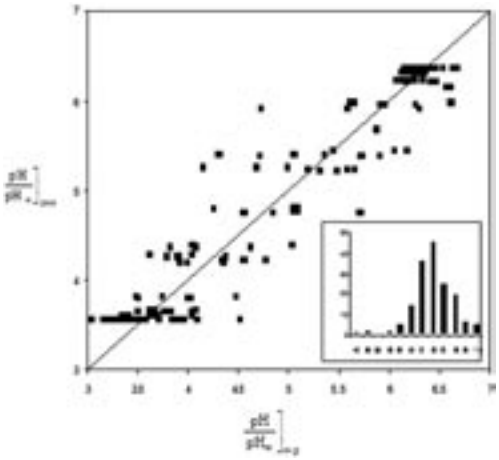


Fig. 7 - Relationship between experimental and predicted values of the pH/pHo measured during storage of shredded carrots in air flux system in a range of temperature 0°-20°C. Enclosed figure: Frequency distribution of the residual.

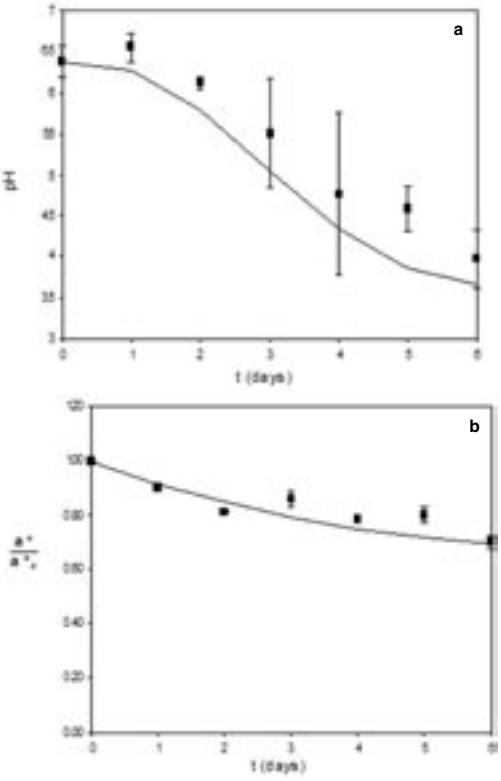


Fig. 8 - Prediction ability of the Weibull model (a) and first order model (b): the dots represent the experimental data at 10°C (a-pH, b-a^*/a^*_o) and the line the values predicted by the models.

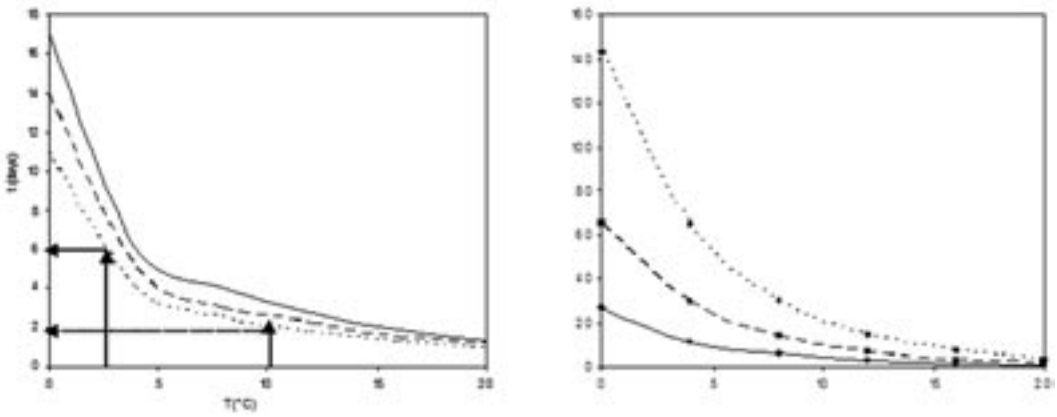


Fig. 9 - Shelf-life prediction - the different lines show different threshold levels (a; pH = ── 5,5 ----- 4.5. b; a* = ── 0.7, ---- 0.9).

CONCLUSION

The results of this investigation showed that a Weibull model, with a rate constant increasing with temperature according to an Arrhenius-type relationship, describe reasonably well the change of pH over time in the range of temperature tested. Among the Lab coordinate system for colour evaluation, a* had the highest sensitivity for the time and temperature changes. A first order model well described the change of colour over time. The reaction kinetics variation on temperature was predicted in a satisfactory manner by an Arrhenius-type equation. To assess the validity of the proposed model, Eq. 8 and Eq. 11 were used to predict colour and pH variation on time of shredded carrots stored in air at 10°C. The agreement between experimental data and model prediction (Fig. 8 a, b) is very good proving the prediction capability of our model. Once defined, the model can be used to predict the shelf-life of shredded carrots at any given temperature. An example is given in Fig. 9 where by way of example the threshold level for pH and colour (a*) values are equal to 4.5 and 0.9, respectively. Accordingly, the predicted shelf-life will be 6 days if the carrots are stored at 4°C and 2 days when stored at 10°C.

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POSTER

EVALUATION OF HMF AS A MARKER OF THE SHELF-LIFE OF HONEY

VALUTAZIONE DELL'HMF: PARAMETRO DI SHELF-LIFE DEL MIELE

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ABSTRACT

Samples of orange and eucalyptus honey were pasteurised at 70°C for different times and then stored for ten months at 25°C. HMF content, diastase activity, pH and total acidity were determined on fresh honeys, on pasteurised honeys and periodically on stored honey samples. Pasteurisation did not induce negative effect on HMF content in orange and eucalyptus honeys. After ten months of storage orange honey shows a HMF content above the fixed EU limit of 15 mg/kg, while eucalyptus honeys, during storage, show a very variable trend of HMF content which prevented the quality of this honey to be evaluated.

RIASSUNTO

Campioni di miele d'Arancio e di Eucalipto sono stati pastorizzati a 70°C per tempi diversi e poi conservati a 25°C per dieci mesi. Il contenuto in HMF, l'attività diastasica, il pH e l'acidità totale sono stati determinati sui mieli non pastorizzati,

- Key words: HMF, honey, storage, thermic treatment -

su quelli pastorizzati e periodicamente sui campioni sottoposti a conservazione. La pastorizzazione non ha indotto alcun incremento del contenuto in HMF in entrambe le varietà di miele. Il miele d'Arancio dopo dieci mesi di conservazione presenta un contenuto in HMF superiore al limite fissato dall'EU di 15 mg/kg, mentre il miele di Eucalipto mostra un andamento del contenuto in HMF durante la conservazione molto variabile e ciò non permette di valutare realmente la qualità di questo prodotto.

INTRODUCTION

The shelf-life of honey is fixed at 24 months and, after this period, it is not considered satisfactory as regards its aroma, biological and nutritional value. Evidently, it is necessary to have parameters that can determine the honey quality and a correct shelf-life. The European Directive n. 110 (2001) utilises HMF (5-Hydroxymethylfurfuraldehyde) as parameter for honey quality: it gives information on the total heat exposure, conditioning and storage of honey.

HMF is formed during acid-catalysed dehydration of hexoses and is connected to the chemical properties of honey, like pH, total acidity, and mineral content (Singh and Bath, 1998). Generally not present in fresh honey, its content increases during heat conditioning and storage (Fallico *et al.*, 2003). Honey processing requires heating to reduce viscosity and to avoid fermentation, usually in air ventilated chambers at 45°-50°C for 4/5 days; moreover a pasteurisation at 70°C for 3-5 min can be carried out to avoid crystallisation for a prolonged period (Gonnet M., 1997).

Codex Alimentarius Commission (2000) established that the HMF content of honey after processing and/or blending must not exceed 40 mg/kg, with the exception of honey coming from countries or regions with tropical temperatures. In this case, HMF content must not exceed 80 mg/kg. The European Union, moreover, established a maximum of 15 mg/kg for honeys which have low enzymatic content (EU Directive 110/2001).

The aim of this research is to verify if the official standards, HMF, diastase activity and total acidity are appropriate for defining shelf-life in fresh and pasteaurised honeys.

MATERIALS AND METHODS

Samples of orange (*Citrus aurantium* L.) and eucalyptus *(Eucalyptus camaldulensis* L.) honey, were picked from stainless steel drums directly provided by local beekeepers [Zafferana Etnea (CT), Sicily]. Honey samples were from the 2002 season and were conditioned in April. The honeys were analysed as fresh honeys (treated at 45°-50°C for 4/5 days), and after a pasteurisation at 70°C for 3, 5 and 7 min. Pasteurised honeys were stored at 25°±2°C from April 2002 to February 2003 and were analysed monthly or every two months. pH, total acidity, and diastase activity were determined on all honey samples according to official methods (AOAC, 1980). HMF levels were determined as follow: 5 g of honey samples were diluted up to 50 mL with distilled water, filtered on 0.45 mm filter and immediately injected in a HPLC (Varian 9012Q) equipped with a Diode Array Detector (Varian, Star 330). The HPLC column was a Merck Lichrospher, RP-18, 5 µm, 125x4 mm, fitted with

a guard cartridge packed with the same stationary phase (Merck, Milan). The HPLC conditions were the following: isocratic mobile phase, 90% water at 1% of acetic acid and 10% methanol; flow rate, 0.7 mL/minute; injection volume, 20 µL. All the solvents were HPLC grade (Merck, Milan). The wavelength range was 220-660 nm and the chromatograms were monitored at 285 nm. HMF was identified by splitting the peak in honey with a standard HMF (Sigma-Aldrich, Milan), and by comparison the spectra of HMF standard with that one of honey samples. The amount of HMF was determined using an external calibration curve, measuring the signal at λ=285 nm.

RESULTS AND DISCUSSION

Tables 1 and 2 report the HMF content, diastase activity, pH and total acidity in fresh and pasteurised honey samples. It is possible to assert that the pasteurisation does not increase the initial content of HMF in orange honey samples (11.0 mg/kg, 10.8 mg/kg after 3 min, 11.7 mg/kg after 5 min, 12.2 mg/kg after 7 min at 70°C, respectively), while a lower level compared to the initial value was observed in eucalyptus honey samples (35.8 mg/kg, 15.9 mg/kg after 3 min, 14.0 mg/kg after 5 min, 15.1 mg/kg after 7 min at 70°C, respectively). Diastase activity does not seem to be influenced by pasteurisation process in orange or eucalyptus honeys. Pasteurised and unpasteurized orange honey samples increased their HMF content during storage, at 25°±2°C, in a similar way (Table 1). Different time of pasteurisation (3, 5 and 7 min) did not influence the HMF formation. After 10 months of storage all honey samples show a HMF concentration above the fixed

Table 1 - Average values for HMF (mg/kg), diastase (nd), pH and total acidity (meq/kg) in fresh and pasteurised Orange honeys.

		Apr.	May	Giu.	Jul.	Sep.	Oct.	Dec.	Feb.
Fresh honey	HMF	11.0	14.6	15.4	19.1	27.1	30.1	32.4	39.8
	Diastase	8.5	8.4	8.2	7.8	7.2	7.2	7.2	7.2
	pH	3.30	3.72	3.72	3.72	3.66	3.60	3.58	3.72
	Total acidity	32.5	33.3	32.7	34.7	29.5	31.5	32.5	27.5
Pasteurised honey 3 min a 70°C	HMF	10.8	14.1	16.1	19.1	26.1	31.0	30.7	41.2
	Diastase	8.4	8.3	8.1	7.9	7.5	7.3	7.3	7.0
	pH	3.43	3.71	3.61	3.70	3.64	3.61	3.55	3.69
	Total acidity	32.0	34.6	32.5	36.2	30.8	32.0	33.5	28.8
Pasteurised honey 5 min a 70°C	HMF	11.7	13.8	14.9	19.1	27.8	33.2	32.5	44.7
	Diastase	8.4	7.8	7.7	7.7	7.6	7.0	6.8	6.8
	pH	3.43	3.73	3.68	3.70	3.63	3.62	3.36	3.59
	Total acidity	33.5	34.4	35.2	35.7	32.0	32.0	35.3	28.5
Pasteurised honey 7 min a 70°C	HMF	12.2	14.2	15.2	19.0	28.0	32.7	34.2	47.6
	Diastase	8.1	8.0	7.6	7.6	7.6	7.6	7.5	6.9
	pH	3.48	3.79	3.75	3.74	3.73	3.71	3.44	3.52
	Total acidity	30.5	32.3	31.0	32.9	29.8	31.0	30.5	27.8

Table 2 - Average values for HMF (mg/kg), diastase (nd), pH and total acidity (meq/kg) in fresh and pasteurised Eucalyptus honeys.

		Apr.	May	June	Jul.	Sep.	Oct.	Dec.	Feb.
Fresh honey	HMF	35.8	40.5	9.7	17.5	25.1	52.2	19.1	23.1
	Diastase	23.5	20.6	19.5	17.2	17.0	16.7	16.4	16.1
	pH	3.73	3.93	3.98	3.72	4.00	4.11	3.79	3.89
	Total acidity	34.5	33.0	34.7	36.5	33.5	34.0	35.3	29.0
Pasteurised honey	HMF	15.9	39.8	0.0	12.9	25.8	55.6	16.1	25.2
3 min a 70°C	Diastase	22.5	20.3	18.5	17.7	17.7	17.0	16.8	16.1
	pH	3.74	3.93	3.98	3.70	3.95	3.98	3.83	3.88
	Total acidity	34.0	34.2	35.7	36.0	34.0	34.8	34.0	30.0
Pasteurised honey	HMF	14.0	16.2	0.0	11.4	23.5	51.2	19.5	23.4
5 min a 70°C	Diastase	19.7	19.0	19.2	18.2	17.4	16.9	16.5	15.8
	pH	3.74	3.94	3.92	3.70	3.96	4.00	3.72	3.90
	Total acidity	34.5	34.7	35.2	36.0	34.3	35.5	35.8	29.3
Pasteurised honey	HMF	15.1	13.5	9.8	18.1	23.5	51.2	21.1	19.1
7 min a 70°C	Diastase	20.0	19.3	19.2	18.3	17.8	16.4	16.3	15.2
	pH	3.74	3.96	3.94	3.74	3.94	3.98	3.76	3.83
	Total acidity	34.0	34.6	35.5	34.5	33.3	34.0	34.5	26.8

limit for this variety (15 mg/kg). Diastase activity slowly decreased with storage time (Table 1).

The trend of HMF concentration in unpasteurised and pasteurised eucalyptus honey samples during storage was variable (Table 2) and it is very difficult to predict the development during storage. Also in this case pasteurisation, did not influence the HMF formation and diastased activity decreased with storage time. Total acidity decreased during storage in both orange and eucalyptus honeys; all values were lower than the legal limits (50 meq/kg) (Table 2).

Twenty four months of shelf-life, does not meet citrus honey the EU HMF legal limit (15 mg/kg). Although the authors confirm their opinion that this limit is too low, they suggest a 12-month shelf-life for this honey. As concerns eucalyptus honey it is important to note the decrease of HMF after sample pasteurization and the unpredictable behaviour of HMF during the 10 months of storage.

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NON-CONVENTIONAL ANALYTICAL INDICES TO EVALUATE THE QUALITY OF THE COVERING OIL DURING THE SHELF-LIFE OF PRESERVED VEGETABLES

INDICI ANALITICI NON CONVENZIONALI PER VALUTARE LA QUALITÀ DELL'OLIO DI COPERTURA DURANTE LA SHELF-LIFE DI CONSERVE VEGETALI

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ABSTRACT

Samples of preserved dried tomatoes were prepared in the laboratory utilising extra virgin olive oil as covering medium. Analytical indices represented by the percentages of the classes of oxidation, polymerisation and hydrolysis of triglycerides, were considered. The results indicated that the overall oxidation, as the sum of oxidized triglycerides plus triglyceride oligopolymers, increased during the first months and then stabilized enabling the product to be consumed at the end of the storage time. The *trans* isomers of unsaturated fatty acids did not change after heat treatment and during the storage period and may be used to ascertain the genuineness of the covering oil.

RIASSUNTO

Conserve di pomodori secchi sott'olio sono state preparate in laboratorio utilizzando olio extra vergine di oliva. Sono stati presi in considerazione indici

- Key words: covering oil; HPSEC; oxidized triglycerides; preserved vegetables; shelf-life; triglyceride oligopolymers -

analitici costituiti dalle percentuali delle classi di sostanze derivanti dalla ossidazione, polimerizzazione ed idrolisi dei trigliceridi. I risultati ottenuti indicano che l'ossidazione totale, data dalla somma delle percentuali di trigliceridi ossidati e oligopolimeri di trigliceridi, aumenta durante i primi mesi di conservazione ma tende poi a stabilizzarsi consentendo così l'impiego alimentare del prodotto al termine del periodo di conservazione. Inoltre gli isomeri *trans* degli acidi grassi insaturi non hanno mostrato variazioni durante la preparazione e il periodo di stoccaggio delle conserve; per cui si può affermare che la loro determinazione può essere utilmente impiegata nell'accertamento della genuinità dell'olio di copertura.

INTRODUCTION

The literature has indicated that, during the shelf-life of preserved foodstuffs, there is a slight increase in the percent of free fatty acids of extra virgin olive oils used as a covering medium as well as a decrease in the peroxide value and an increase in the spectrophotometric indices (Bocca *et al.*, 1990; Paganuzzi *et al.*, 1995). This means that the analyses prescribed by the EC regulation n. 2568/91 and its subsequent amendments and modifications do not permit an evaluation of the oxidative level or of the genuineness of the oil used since some indices exceed the limits set by the law. The need has thus emerged to have suitable analytical parameters to evaluate the quality of an oil in a more realistic fashion. The aim of this investigation was thus to monitor the trend of the levels of oxidation and hydrolysis of the covering oil during the shelf-life of foodstuffs preserved in oil by means of non-conventional analytical indices. The durable life of these products, as suggested by their producers, is longer than 2 years and, to date, no data has appeared in the literature that provides insight into the actual degree of oxidation the covering oil reaches before the best-before date or during the shelf-life of the product. This is an important issue since the intake of rather high levels of oxidation products may also produce adverse effects on human health. The effect of heat processing for microbiological stabilization on the state of oxidation and hydrolysis of the covering oil was also evaluated.

MATERIALS AND METHODS

Preserves of dried tomatoes in oil were prepared in the laboratory in glass jars of 314 ml, each with a metal lid. The dried tomatoes were parboiled in vinegar for 30", dried and arranged in the glass containers; oil was added and the airtight jars closed. The jars were heat-treated in hot water at T = 74°C (core of the product) for 15' and then cooled under cold water. Extra virgin olive oil obtained from drupes of *Coratina* cultivar was used as a covering medium. The jars were then kept at room temperature to simulate the shelf-life conditions of the product in a supermarket. Immediately after preparation and then at 0.5; 1; 3; 6; 12 and 18 months a jar was opened, its oil collected and then analyzed. The analytical techniques used were silica gel column chromatography (CC) to separate polar compounds (PC) from the oil and high performance size-exclusion chromatography (HPSEC) to then separate and quantify the classes of substances contained in the PC. The analytical determinations were performed as described in previous papers (GOMES, 1992; GOMES and CAPONIO, 1999). *Trans* isomers determination of unsaturated fatty acids was carried out as previously indicated (CAPONIO *et al.*, 2003).

RESULTS AND DISCUSSION

It is known that, regarding the vegetables preserved in oil, the lipid contribution of vegetables to covering oil is negligible (GOMES *et al.*, 1998). PC are mainly made up of products of triglyceride oxidation, polymerization and hydrolysis and HPSEC analyses of PC enable triglyceride oligopolymers (TGP), oxidized triglycerides (ox-TG) and diglycerides (DG) to be measured. TGP are mainly found in refined oils (EDER, 1982) although small amounts may also form in virgin olive oils due to oxidation (GOMES, 1995); the amount of TGP present in an oil is considered to be a reliable index of oxidative degradation by many Authors. Oxidized triglycerides comprise all the products of triglyceride oxidation and are thus another important class of substances to determine oxidation of an oil. DG enable a more complete measurement of hydrolytic degradation than do free fatty acids alone. The sum of percent TGP + ox-TG represents the degree of total oxidation of an oil since it is constituted by the classes of substances coming from the oxidation and polymerization of triglycerides. Fig. 1 shows the trend of total oxidation (TGP% + ox-TG%) as a function of the storage time of the products considered. As may be observed, total oxidation increased substantially during the first months of storage and then stabilized after a certain period of time. This trend explains why the commercial product may be consumed according to the durable life information on the label. Heat processing did not cause formation of TGP but an increase in the amount of ox-TG which proved to be 23% greater in the heat-treated oil than in the original oil used as a covering medium. Afterwards, as oxidation progressed, a small amount of TGP appeared. Fig. 2 shows the HPSEC chromatograms of the PC of the extra virgin olive oil used as covering medium before and after the heat treatment and after one year of storage time. The overall increase in ox-TG at the end of the storage period was 62.8% while total oxidation increased by about 97%. Hydrolytic degradation as measured by determining the amount of DG did not increase after heat treatment although it had increased by 28.3% by the end of the storage period.

The *trans* isomers (*trans* oleic, *trans* linoleic + *trans* linolenic) were present at trace levels in extra virgin olive oil used as covering medium and did not change

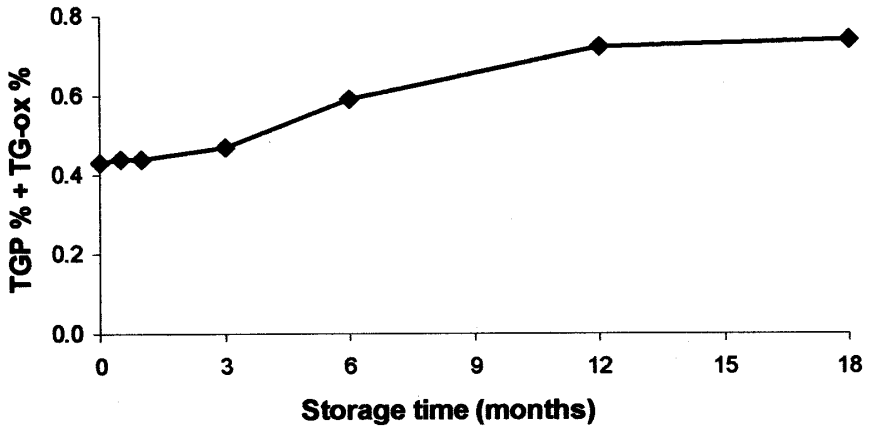


Fig. 1 - Trend of total oxidation of the extra virgin olive oil, used as covering medium of preserved dry tomatoes, during the storage time.

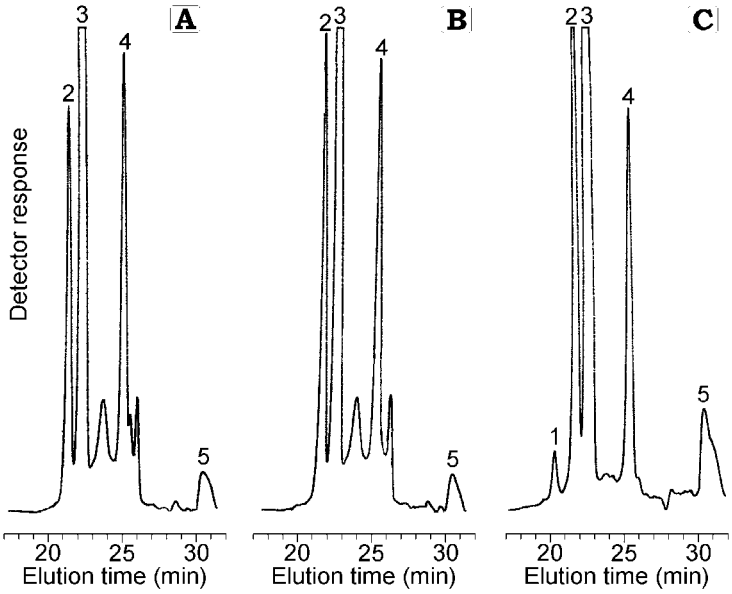


Fig. 2 - HPSEC analysis of polar compounds of the extra virgin olive oil used as covering medium: (A) before and (B) after the heat treatment; (C) after one year of storage time. 1) triglyceride oligopolymers, 2) oxidized triglycerides, 3) diglycerides, 4) free sterols and triterpene diols, 5) free fatty acids.

after heat treatment or during the storage period and may thus be used to ascertain the genuineness of the oil.

In conclusion, the findings of this investigation suggest that the sum of TGP%+ ox-TG% and the percent of amount of DG constitute reliable parameters to evaluate the quality of covering oils by measuring the level of oxidation and hydrolysis, respectively. Genuineness of the same oils may be ascertained by analyzing the *trans* isomers.

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EFFECT OF SUPERHEATED WATER COOKING ON SOME TEXTURAL CHARACTERISTICS OF CUTTLEFISH (*SEPIA OFFICINALIS*)

EFFETTO DELLA COTTURA CON VAPORE SURRISCALDATO SU ALCUNE CARATTERISTICHE DELLA TEXTURE DI SEPPIE (*SEPIA OFFICINALIS*)

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ABSTRACT

In this paper, the results of some technological trials carried out on frozen common cuttlefish (*Sepia officinalis*) are reported; the effects of a superheated water cooking (110°, 120° and 130°C, each temperature for 2, 4 and 6 min) on weight loss percentage, firmness and cohesiveness of cuttlefish samples (mantle and tentacles) were evaluated.

Results have shown that high weight loss is always present, sometimes up to 40-50%. Tentacle firmness showed low values for intermediate treatment times and temperatures, both for just cooked and cooked-frozen-thawed samples; on the other hand, mantle samples did not show relevant firmness changes with temperature and time. Mantle cohesiveness values were always higher then the corresponding tentacle values.

RIASSUNTO

In questo lavoro sono stati riportati i risultati di alcune prove tecnologiche effettuate su campioni di seppia (*Sepia officinalis*); la sperimentazione è consistita sostanzialmente nella valutazione delle variazioni di peso, consistenza e coesività in campioni di seppia (parte anatomica del mantello e tentacoli), trattati con vapore surriscaldato a tre differenti temperature (110°, 120° e 130°C), ognuna delle quali a tre differenti tempi di trattamento (2, 4 e 6 minuti). Inoltre, una parte dei campioni, successivamente alla cottura, è stata congelata, decongelata e quindi analizzata.

I risultati hanno mostrato che il calo peso, sempre crescente all'aumentare

- Key words: cuttle fish; firmness; steam-water cooking; texture analysis -

delle temperature e dei tempi di trattamento, ha raggiunto valori finali di circa il 40-50% sul peso fresco del campione.

La consistenza dei tentacoli è risultata abbastanza bassa per valori intermedi di temperature e tempi di trattamento, sia per i campioni tal-quale e cotti, sia per quelli cotti-congelati-decongelati; al contrario, la consistenza dei campioni anatomici del mantello non è variata al variare delle temperature e dei tempi di trattamento termico. Infine, i valori di coesività del mantello sono sempre risultati maggiori dei corrispondenti valori misurati sui tentacoli.

INTRODUCTION

The market of industrial ready-to-use foods is progressively increasing: frozen or refrigerated pasta-based and rice-based dishes, traditional Italian and foreign meat- and seafood-based recipes are at present available on market shelves. Cephalopods, and in particular the common cuttlefish (*Sepia officinalis*), is often used as ingredient in ready-to-use foods and its textural characteristics are the main quality parameters to be considered (DENIS and ROBIN, 2001; SHUBRING, 2000; COPPES *et al.*, 2002). The production of refrigerated ready-to-use foods may consist of (i) the cooking of the different ingredients (raw materials, as fresh or thawed), (ii) mixing of the ingredients together with salt, sauces and spices and (iii) the packaging in plastic trays (sometimes with modified atmosphere) and final pasteurization; in the case of frozen ready-to-use foods, after cooking, the several ingredients are mixed together and then submitted to an IQF (individual quick freezing) treatment; the packaging in multi-layer pouches and storage at frozen temperature are normally carried out. Hence, both for refrigerated and frozen ready-to-use foods, no further home preparation is required and meals can be made ready-to-eat in a few minutes (from 3 to 7) by reheating them in a pan or in conventional or microwave ovens. The aim of this short paper was to evaluate the effects of a superheated water cooking method on weight loss percentage, firmness and cohesiveness of mantle and tentacles of common cuttlefish samples.

MATERIALS AND METHODS

Frozen cuttlefish (mantle and tentacles), coming from the Atlantic ocean were used for laboratory trials; after thawing at 4°C for 24 hours, mantle and tentacles were separately subdivided in samples of about 100 g each. By using an experimental under-pressure water cooker, samples were submitted to several cooking cycles at three temperatures (110°, 120° and 130°C), each at three different times (2, 4 and 6 min). Each cooking cycle was performed on three repeated samples for average data collection. For one group of samples, the different analyses were performed just after the cooking process; another group, after cooking was sealed in plastic bags and frozen in a static device at -20°C for 45 days; after then, samples were thawed and analysed. On all samples, the weight loss was determined by weight difference before and after cooking,. The quantitative textural assessment on samples was performed according to the literature (BOURNE, 1978, PELEG, 1980, SHERMAN, 1989) by using an automatic TA-XT2i Texture Analyser (Stable Micro System, Godalming, England) and running the texture profile analysis (TPA) test program. Results were expressed by means of response surface methodology, by using a quadratic polynomial model where the response factors (weight loss percentage, firmness, cohesiveness) were

plotted as a function of the heat treatment time (x axis) and temperature (y axis).

RESULTS AND DISCUSSION

Weight loss is a very critical event for this kind of food material and is dramatically influenced by the cooking conditions; in fact, as exemplified in Fig. 1, the weight loss increases with the increasing of the heating time and temperature. The maximum weight loss measured during the cooking trials was about 65% for tentacles (Fig. 1) and about 38% for mantle samples (Fig. 2). The subsequent frozen storage (45 days) and thawing, did not increase the weight loss of samples.

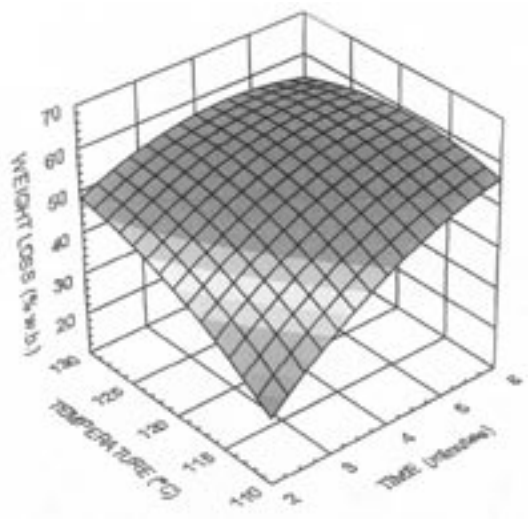


Fig. 1 - Cooked cuttlefish tentacles: weight loss (%).

A low firmness (a popular term describing the hardness) is a key attribute for cooked common cuttlefish. Measures of firmness had a high reproducibility, basicly due to structural homogeneity in both tentacles and mantle samples. In particular, cooked tentacles (Fig. 3) always showed higher firmness values (about 3.000 g) than mantle samples (about 2.100 g, Fig. 4), particularly for short treatment times (2 min) and for the lowest treatment temperature (110°C). For all trials and also for all frozen and thawed tentacle samples, a saddle-shaped surface, Fig. 3, was always obtained; hence firmness decreased until intermediate values of treatment time and temperature, then increased again, probably due to the strong protein contraction during an excess of cooking time. On the contrary, the mantle firmness was always unchanged (Fig. 4), independent of treatment time and temperature; this behaviour was also observed in the case of samples analysed after 45 days of frozen storage.

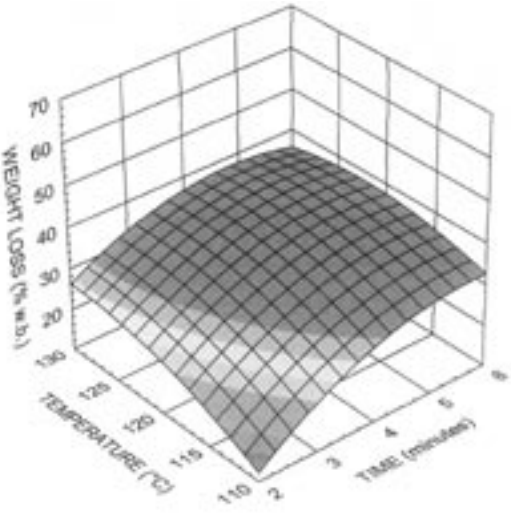


Fig. 2 - Cooked cuttlefish mantle: weight loss (%).

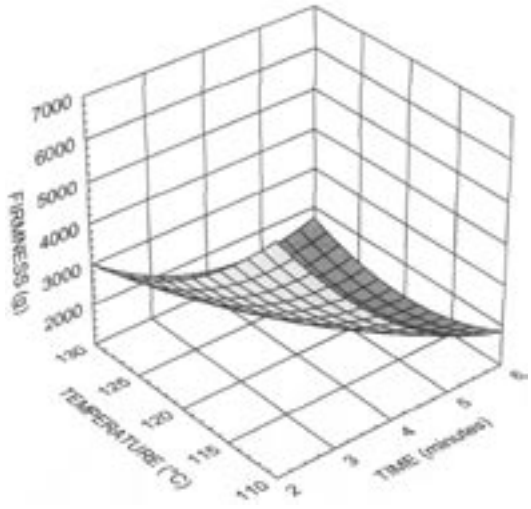


Fig. 3 - Cooked cuttlefish tentacles: firmness (g).

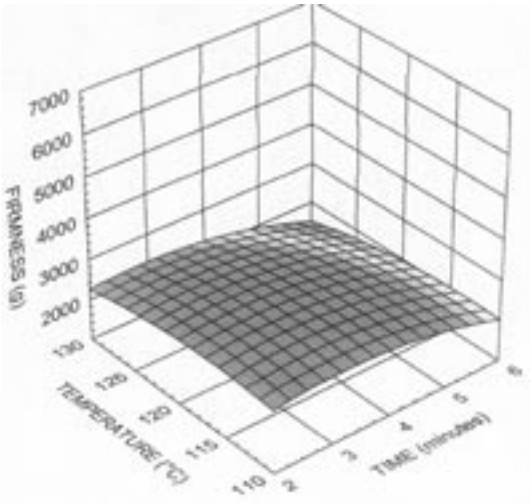


Fig. 4 - Cooked cuttlefish mantle: firmness (g).

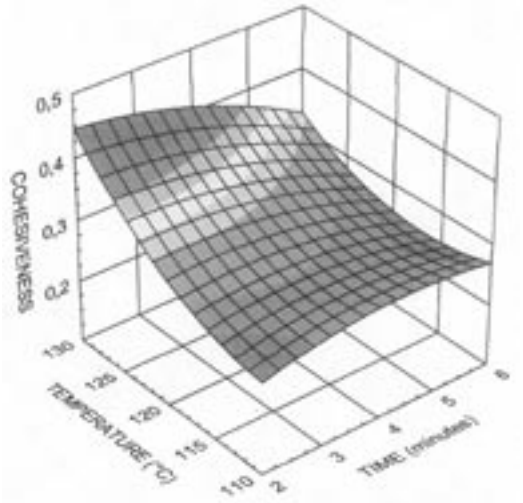


Fig. 5 - Cooked-frozen-thawed cuttlefish mantle: cohesiveness.

Cohesiveness can be defined (i) from a physical standpoint as the extent to which a material can be deformed before it ruptures and (ii) from a sensory point of view as the degree to which a substance is compressed between teeth before it breaks (SZCZESNIAK, 2002). In the case of cuttlefish samples, it was observed that cooked-frozen-thawed samples, both for tentacles and mantle, had higher cohesiveness values than the cooked samples.

For all samples, cohesiveness increased as the heat treatment temperature increased but not treatment time, as the example reported in Fig. 5. Moreover, it was observed that, due to the different anatomic constitution, mantle samples always showed the highest cohesiveness values.

In conclusion, the treatment of common cuttlefish products with superheated water can be carried out in very short times; for intermediate temperatures and times the sample firmness may be reduced without an excessive increase of cohesiveness. The weight loss, always very high in cephalopods, under our experimental conditions, may be reduced to levels of about 30%, if times and temperatures do not exceed respectively 3-4 min and about 120°C.

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THE INFLUENCE OF WATER ACTIVITY ON PHYSICO-CHEMICAL CHARACTERISTICS OF EDIBLE COATINGS

INFLUENZA DELL'ATTIVITÀ DELL'ACQUA
SULLE CARATTERISTICHE FISICO-CHIMICHE DI FILM EDIBILI

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ABSTRACT

The most important factor influencing the barrier properties of polymeric films is the physico-chemical nature of the matrix. When a hydrophylic polymer is equilibrated at a certain relative humidity value, it can change its physico-chemical characteristics, therefore its barrier properties can be modified. The main objective of this paper was to examine the influence of water activity on chemical and physical characteristics of edible chitosan coating.

RIASSUNTO

Il fattore che più fortemente influenza le proprietà barriera dei film polimerici è la natura fisico-chimica della matrice. Quando un polimero idrofilico viene equilibrato a determinati valori di umidità, può modificare le sue caratteristiche fisico-chimiche e quindi, anche le sue proprietà barriera. L'obiettivo di questo lavoro è stato quello di esaminare l'influenza dell'attività dell'acqua sulle caratteristiche fisico-chimiche di film edibili di chitosano.

- Key words: chitosan film; moist treatment; physico-chemical change -

INTRODUCTION

Polymeric materials for food packaging applications can control the exchange of low molecular weight substances, such as gas, flavour and water vapor between the inside and outside of the packaging. The physico-chemical nature of the matrix influences the barrier properties of polymeric films. When an hydrophylic polymer is equilibrated at a certain relative humidity value, it can change its physico-chemical characteristics, therefore its barrier properties can be modified.

The aim of this paper was to examine the influence of water activity on chemical and physical characteristics of edible chitosan coating.

MATERIALS AND METHODS

Chitosan-based films (obtained using chitosan at different molecular weights) were prepared by conventional casting technique from water solutions with the addition of acetic acid.

In particular, the films investigated will be referred to as follows:

- Sample APM: high molecular weight chitosan film;
- Sample BPM: low molecular weight chitosan film;
- Sample 50%: 50% mixture of high molecular weight chitosan and low molecular weight chitosan film.

Water barrier properties, physical properties and thermal behaviour of edible chitosan coatings were investigated. All samples were characterized by means of water vapor permeability measurements (WVP), X-ray diffraction (crystallinity degree), thermal measurements (DSC) and thermogravimetric measurements (TGA).

In order to evaluate the influence of water activity on chemical and physical structure of edible coatings, water barrier properties and thermal behaviour of moist chitosan treated films were investigated.

The same previous samples A, B and C of edible coatings were exposed to different water activity values for several time intervals and subsequent drying as follows:

- Sample 1: Exposed at 75%RH for 5 days + Desiccated for 2 days;
- Sample 2: Exposed at 75%RH for 15 days + Desiccated for 3 days;
- Sample 3: Exposed at 75%RH for 20 days + Desiccated for 3 days;
- Sample 4: Exposed at 33%RH for 5 days + Desiccated for 2 days;
- Sample 5: Exposed at 33%RH for 20 days + Desiccated for 9 days;
- Sample 6: Exposed at 10%RH for 10 days + Desiccated for 10 days.

By means of X-Ray measurements, crystallinity degree was evaluated.

RESULTS AND CONCLUSION

The results of water vapor permeability properties and physical properties of chitosan-based films investigated showed an important correlation between molecular weight, crystallinity degree and barrier properties. In particular, it was observed that as the percentage of low molecular weight chitosan in the film increases, crystallinity level increases and, as a consequence, the permeability coefficient decreases (Fig. 1 and 2).

Results of the 75%RH treated films showed that an increase in exposition time led to a decreased crystallinity level which it probably means that the relative

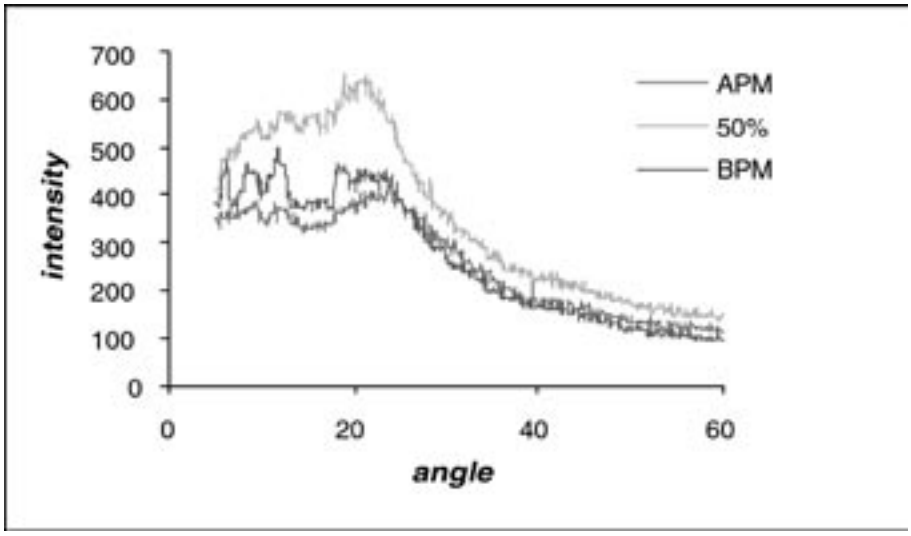


Fig. 1 - X-RAY diffractograms of APM, BPM and 50% chitosan films.

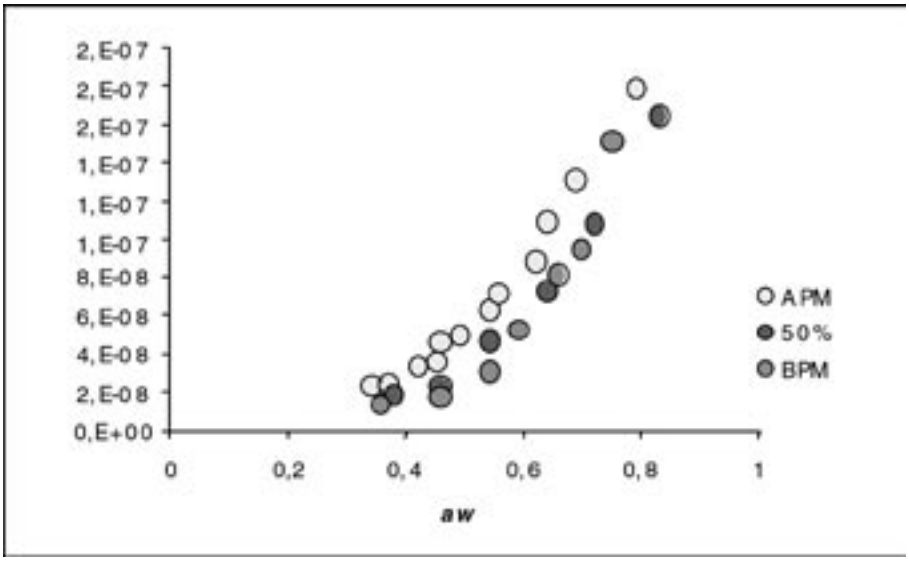


Fig. 2 - WVP of APM, BPM and 50% chitosan films.

humidity chosen was too high. Thus, the films were exposed to lower water activity values for different times.

The results of X-ray measurements on chitosan films exposed to 33% RH showed that, as expected, an increase in exposition time led to an increase of crystallinity level.

Samples exposed to 10%RH value were investigated. Even in this case untreated and moist exposed films showed a marked difference in crystallinity level.

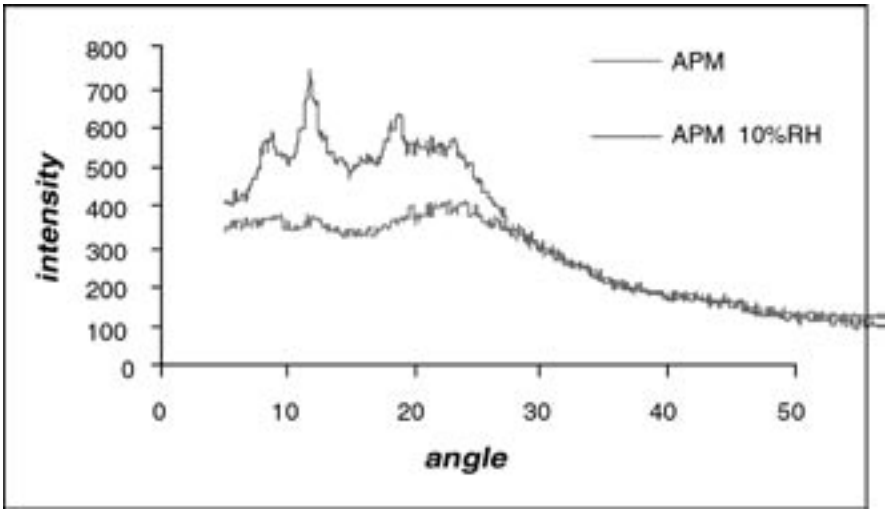


Fig. 3 - X-RAY diffractograms of APM untreated and treated chitosan films.

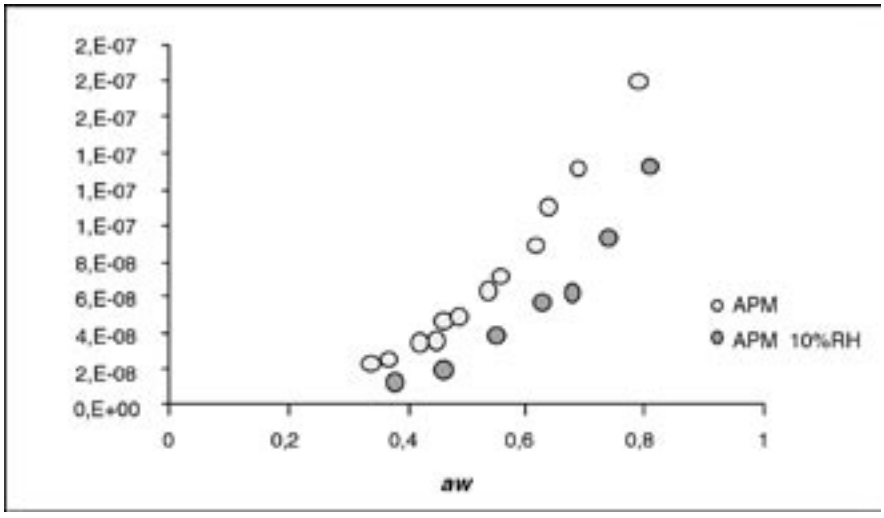


Fig. 4 - WVP of untreated and treated 10%RH APM chitosan films.

In particular, an increase in exposition time led to an increased crystallinity degree and, as a consequence, to an increased permeability coefficient (Fig. 3 and 4).

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INFLUENCE OF PACKAGING MATERIAL ON BREAD CHARACTERISTICS

INFLUENZA DEL MATERIALE DI CONFEZIONAMENTO SULL'INVECCHIAMENTO DEL PANE

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ABSTRACT

The influence of three perforated oriented polypropylene films on the trend of bread ageing was investigated. As bread is packaged hot (80°-90°C), the hole size characteristics are determinant in assuring crust crispness without inducing its wetting. The results emphasize that bread wrapped in one of the films maintained a higher crumb softness during all the storage times.

RIASSUNTO

È stata valutata l'influenza di tre film di polipropilene microforati e del sacco-carta sull'andamento dei fenomeni correlati all'invecchiamento del pane durante la conservazione per 48 ore. Poiché il prodotto viene industrialmente confezionato ancora caldo, è stata sottolineata l'importanza delle caratteristiche geometriche dei fori (dimensioni e densità) nell'assicurare il mantenimento di croccantezza della crosta, evitandone un eccessivo rammollimento. È stato evidenziato che uno dei film considerati ha permesso di mantenere una maggior morbidezza della mollica durante tutto il periodo di conservazione del pane.

- Key words: bread ageing, crumb moisture, oriented perforated polypropylene (OPP) films -

INTRODUCTION

During industrial bread-making processes, loaves are wrapped at a temperature of 80°-90°C. Hence, it is essential that the packaging material allows for both rapid cooling of the product and moisture exchange with the outer environment in order to prevent crust softening, which would make the product unacceptable to consumers and bring about an ideal microclimate for mould growth. At present, perforated oriented polypropylene films best meet the requirements of the industrial bread-making sector.

This work was intended to investigate whether bread packaging by different perforated films may influence the trend of some phenomena connected to bread ageing.

MATERIALS AND METHODS

Bread making trials

A commercial wheat flour (protein: 12.2% db; farinographic stability: 6.5 min) was used for bread-making. Bread-making trials (24 trials over four weeks) were carried out according the straight-dough procedure (AACC 10-10B, 1995). For every trial, two loaves were produced. The weight and the volume of the loaves were determined 15 minutes after baking. After packaging, bread was stored under controlled temperature and moisture conditions (20°C; 60% relative humidity) and analysed after 2, 19, 26 and 48 h (T1, T2, T3 and T4, respectively) from packaging.

Packaging materials

Hot bread samples were wrapped in 20x20 cm bags, which were subsequently sealed. Three perforated oriented polypropylene (OPP) films were used (Table 1). For film PY85 a double-layer film bag (PY85d) was also prepared. The hole size characteristics, previously evaluated by Piergiovanni *et al.* (2003), determined different open surfaces. The water vapour permeability (Water Vapour Transmission Rate – WVTR) of each film was evaluated by the ASTM E-96 (1980) "dish method" at 25°C and 67% relative humidity (Table 1). Another packaging material tested was the so-called paper bag (PB), i.e., a parallel composite structure, whose surface is made of paper (88%) and a cellophane window in the remaining portion.

Sampling and characterisation of bread

Two bread loaves were analysed for each storage time and six 22 mm thick slices were obtained. Moisture evaluation was performed on the bread as a whole

Table 1 - Characteristics of the perforated OPP films used for bread packaging.

Sample	Film Thickness (mm)	Hole Mean Diameter (mm)	Hole Mean Surface (mm^2)	Hole Density (n x cm^{-2})	Open Surface (%)	WVTR ($g\ m^{-2}\ 24h^{-1}$)
PY85	12	1.35	1.43	8.6	12.25	586.9
C250	15	0.54	0.23	21.4	4.96	320.0
C10	15	0.44	0.15	0.9	0.14	76.4

and on different regions of the bread slices: area A, from the core of the slice (27 mm in diameter); area B, intermediate portion of the crumb; area C, bread crust. Crumb softness was determined on specimens A by a compression test using an Instron Universal Testing Machine mod. 4301 dynamometer, at a deformation speed of 20 mm/min. Young module (E) and load at 30% deformation (C30%) were calculated for each curve.

Statistical analyses

Data were subjected to analysis of variance followed by the Fisher test (LSD) to compare means at the 5% significance level by using StatgraphicPlus for Windows 4.0.

RESULTS AND DISCUSSION

Repeatability of bread-making trials

The 48 loaves had an evident crust about 3 mm in thickness, and volume, weight and humidity (corresponding to 730 cm^3, 142 g and 31.4% as means, respectively) characterised by low CV % (<2.5%), indicating high repeatability of the 24 bread-making trials.

Influence of perforated film on bread moisture

A progressive dehydration took place in all samples over 48 h, although its severity differed. Packaging of bread by film PY85 was associated with a considerable moisture loss, as high as 25% of the initial moisture. An opposite behaviour was observed for bread wrapped in film C10, which showed a very low WVTR value and therefore lost only 10% of its initial moisture. Intermediate moisture losses were observed for bread wrapped in film C250, double-layer film PY85 or paper bag.

Evolution of moisture in the three regions of slices during storage is shown in Fig. 1. Important differences among samples can be noted in the crust (area C). Each wrapping material allowed crust to maintain low moisture values assuring crispness characteristics, with the exception of film C10. The bread packaged in this material presented a significantly ($p<0.05$) wetter crust, already at time T2.

Influence of perforated film on bread firmness

The loaf wrapped in a paper bag (PB) was characterised by an E value of 6.65 kPa and a C30% value of 0,58 N at time T1 (Fig. 2). These indices were not significantly different ($p<0.05$) from those found for bread wrapped in film C10 at the same time interval. Conversely, significantly higher softness was observed in samples wrapped in film PY85 (both single- and double-layer) and film C250. For longer storage times a progressive crumb hardening was observed (Fig. 2). The increase in crumb firmness was not shown to be associated with moisture variations of the crumb in the bread core at the time interval considered. Significantly lower E values were only found for bread wrapped in film C250 starting from T2.

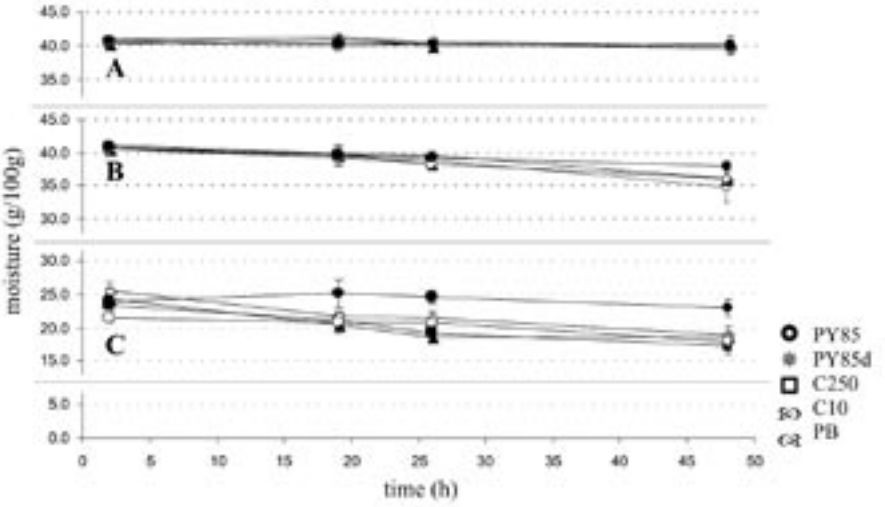


Fig. 1 - Moisture content in the three regions of bread during storage.

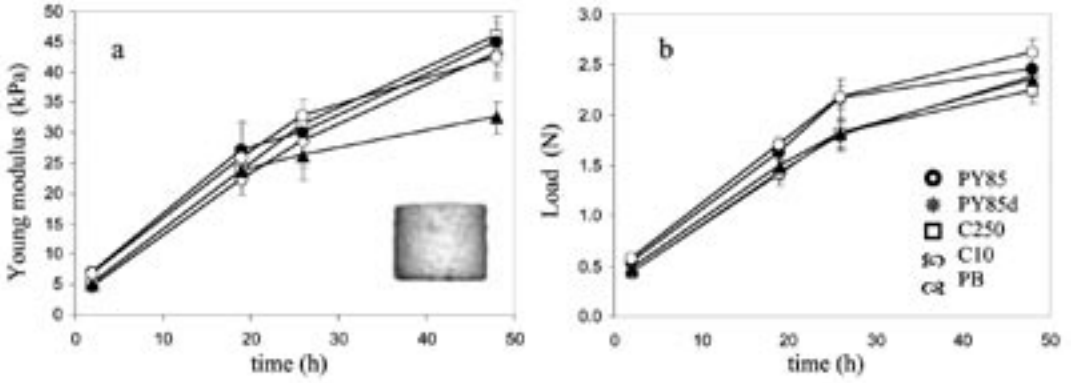


Fig. 2 - Rheological parameters of the central region (area A) of bread during storage: a) Young modulus; b) Load at 30% deformation.

CONCLUSIONS

Some perforated OPP films play an interesting role in modulating moisture variations. Wrapping bread in perforated films may allow both crust crispness and crumb softness to be maintained. Such performances cannot actually be obtained by using alternative industrial bread packaging materials such as the paper bag.

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USE OF RECENT ANALYTICAL PARAMETERS TO EVALUATE THE QUALITY OF REFINED OILS USED AS A COVERING MEDIUM FOR CANNED FISH

USO DI RECENTI PARAMETRI ANALITICI
PER VALUTARE LA QUALITÀ DI OLI RAFFINATI UTILIZZATI
COME LIQUIDO DI COPERTURA DI CONSERVE ITTICHE

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ABSTRACT

An experimental investigation was carried out on several in-oil preserved canned tuna samples to evaluate the degradation level of the refined oil medium by means of recent analytical parameters as well as by the routine analysis prescribed by the EC Regulations. The analytical results show that the HPSEC analysis of polar compounds, separates and quantifies classes of oxidation (triglyceride oligopolymers and oxidized triglycerides) and hydrolysis (diglycerides) substances, giving a much more accurate evaluation of the quality of refined oils used as preserving medium in canned foodstuffs with respect to the routine analyses.

RIASSUNTO

Un'indagine sperimentale è stata condotta allo scopo di valutare il livello di degradazione di oli raffinati utilizzati come liquido di copertura di conserve di tonno utilizzando, oltre alle tradizionali analisi di routine previste dai Regolamenti Comu-

- Key words: canned fish; hydrolytic degradation; HPSEC analysis; oxidative degradation; polar compounds; refined olive oil -

nitari, anche recenti tecniche analitiche quali l'analisi HPSEC (high performance size-exclusion chromatography) dei composti polari dell'olio. I risultati analitici ottenuti hanno evidenziato che per valutare la qualità di oli raffinati usati come copertura di conserve di tonno l'analisi HPSEC dei composti polari, che permette di separare e quantificare le classi di sostanze di ossidazione (oligopolimeri di trigliceridi e trigliceridi ossidati) e di idrolisi (digliceridi) dei trigliceridi, forniva risultati più affidabili rispetto a quelli conseguibili attraverso le analisi di routine.

INTRODUCTION

Amongst their various uses in the food industry, vegetable oils are utilized to cover preserved fish and vegetables and thus protect the food products from contact with air. At times, they may also enhance the organoleptic properties of the preserved food. The types of oils most commonly used to cover preserved tuna are refined olive or seed oils.

Although the sector of foodstuffs preserved in oil is constantly growing, there are still very few contributions in the literature on the analytical assessment of the covering oil and they basically concern procedures for checking the genuineness of the oils used in preserves. This is a major aspect for the inspection of the food preserved in oil, but it is also important to investigate the quality of the oils used as covering media, with a special focus on oxidative degradation. Such investigations would be especially useful considering the harmful effects that high intakes of some intermediate and final products of oxidative degradation may have on consumer health (ALEXANDER 1978; COMBE *et al.*, 2000).

It is known that the quality of refined oils cannot be ascertained by relying only on the traditional routine analyses prescribed by the current legislation (GOMES and CAPONIO, 1997; 1998). Evaluation of the genuineness of refined oils used to cover preserved foodstuffs is further compounded by the fact that the traditional analytical parameters may no longer apply to these oils due to the variations that occur during the technological procedures used to prepare the foodstuffs they cover. Besides, a further cause of variation of these parameters is the release of lipids from the food into the oil during the durable life of the product.

The aim of this investigation was to measure the actual levels of oxidative and hydrolytic degradation of refined oils used to cover preserved tuna by resorting to non-conventional parameters, namely the HPSEC analysis of polar compounds.

MATERIALS AND METHODS

Fifteen commercially available cans of tuna preserved in olive oil were purchased at different retail outlets. Samples of oil were taken from each can, filtered with anhydrous sodium sulphate and kept in a freezer at -18°C until they were analyzed. All the samples were submitted both to the traditional routine analyses and to separation and determination of polar compounds. Fatty acid composition was determined after methylation of the fatty acids as described in the EC Regulation no. 2568/91 following the chromatographic set-up described in a previous paper (CAPONIO and GOMES, 2003). The polar compounds were separated on a silica gel column as described in the IUPAC method (IUPAC, 1987) and analyzed by high performance size-exclusion

chromatography (HPSEC) to determine the main classes of substances of triglyceride oxidation and hydrolysis present. The chromatographic set-up and the procedures for identifying the peaks of interest on the chromatograms were the same as described by the authors in a previous paper (GOMES and CAPONIO, 1999).

RESULTS AND DISCUSSION

Determination of the fatty acid composition of the oils covering the preserved tuna examined revealed that highly unsaturated fatty acids, typical of fish fats, were present in these oils. This means that this measurement cannot be used to effectively evaluate the genuineness of these oils.

Table 1 shows the mean, minimum and maximum values together with the standard deviations obtained in the analyses performed on the oils examined. As may be observed, free fatty acids were always quite low with values ranging from 0.22 to 0.72%. This determination, however, is of very little help in measuring the actual level of hydrolytic degradation of these oils because they have undergone refining. During neutralization of lampante oils, free fatty acids are removed so their determination in this case simply testifies that de-acidification of the oils has occurred.

Determination of the peroxide values yielded a range between zero and 6.0 while measurement of the constants of specific UV absorption at various wave-lengths showed values ranging from 1.478 to 2.787 for K_{232} (conjugated dienes), from 0.240 to 0.983 for K_{270} (conjugated trienes), and from 0.004 to 0.052 for ΔK. Also these analytical parameters constitute an inefficient measurement of the actual oxidative degradation of the refined oils used to cover preserved foodstuffs. In fact, hydroperoxides are degraded and/or modified during bleaching and deodorization so their measurement simply provides indications regarding the resumption of oxidation during the shelf-life of the preserved foodstuff and, as is known, the value of the spectrophotometric constants also depends on the refining conditions used (AMATI *et al.*, 1969; TISCORNIA *et al.*, 1982).

By contrast, HPSEC analysis of polar compounds may provide a reliable measurement of the actual degree of oxidative and hydrolytic degradation of the olive oils used to cover preserved foodstuffs since it has already been used successfully to evaluate the quality of refined olive oils and of oils undergoing heat processing at high tempera-

Table 1 - Analytical characteristics of olive used as a covering medium for preserved tuna.

Determination	Values			Standard deviation
	Minimum	Maximum	Mean	
Free fatty acids (%)	0.22	0.72	0.46	0.16
Peroxide value (meq O_2/kg)	0.0	6.0	2.5	1.8
K_{232}	1.478	2.787	2.077	0.367
K_{270}	0.240	0.983	0.625	0.221
ΔK	0.004	0.052	0.026	0.015
Polar compounds (%)	4.47	6.42	5.53	0.64
Triglyceride oligopolymers (%)	0.24	0.73	0.47	0.13
Oxidized triglycerides (%)	0.43	1.90	0.89	0.43
Diglycerides (%)	2.63	4.45	3.40	0.54

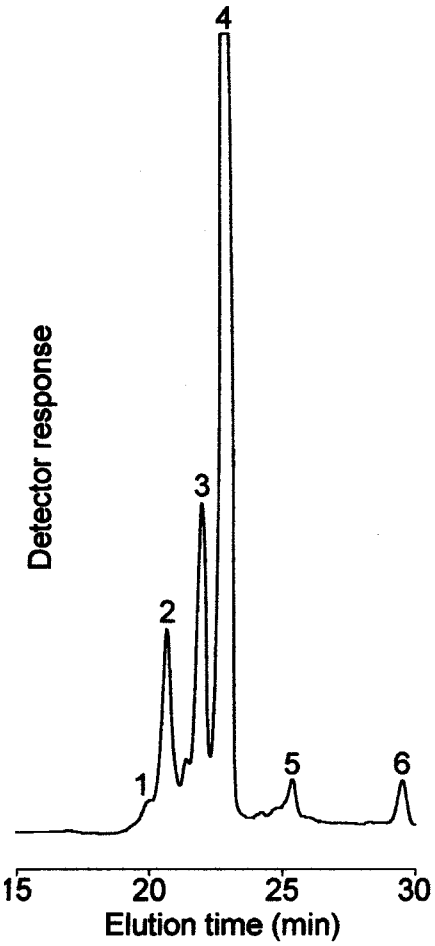


Fig. 1 - HPSEC chromatogram of polar compounds of an olive oil utilized as liquid medium of canned tuna. 1) Triglyceride trimers, 2) triglyceride dimers; 3) oxidized triglycerides; 4) diglycerides; 5) free sterols and triterpene diols; 6) free fatty acids.

tures. HPSEC analyses permit determination of compounds that are not eliminated during the various phases of the refining process (GOMES and CAPONIO, 1997; 1998).

Polar compounds, which comprise substances whose polarity is greater than that of unaltered triglycerides, ranged from 4.47 to 6.42% (mean value: 5.53%, SD = 0.64) and provided an estimation of the overall deterioration of the oils. Their subsequent analysis by HPSEC made it possible to separate and measure the classes of substances of oxidation (triglyceride oligopolymers and oxidized triglycerides) and hydrolysis (diglycerides). Fig. 1 shows a standard chromatogram obtained by HPSEC analysis of polar compounds of one of the samples examined.

The triglyceride oligopolymers ranged from 0.24 to 0.73% (mean value: 0.47%, SD = 0.13), the oxidized triglycerides from 0.43 to 1.90% (mean value: 0.89%, SD = 0.43), and the diglycerides from 2.63 to 4.45 (mean value: 3.40%, SD = 0.54).

All the values obtained for these parameters were similar to the ones reported in the literature for the commercial grades of olive oil and refined olive oil (GOMES and CAPONIO, 1997).

In conclusion, the investigation we carried out confirmed the utility of the HPSEC analysis of polar compounds and suggests that HPSEC should be relied on to determine the quality of refined oils used as a covering medium for preserved tuna.

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POTENTIAL USE OF "PONKAN" AND "PAGE" MANDARINS AS MINIMALLY PROCESSED FRUIT

ATTITUDINE ALLA MINIMA TRASFORMAZIONE DI FRUTTI DI "PONKAN" E "PAGE" (MINNEOLA TANGELO X CLEMENTINE)

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ABSTRACT

The objective of this study was to investigate the potential use of two different cultivars of mandarins, "Ponkan" and "Page" (Minneola tangelo x Clementine), as minimally processed fruits. Fruit was peeled and separated manually in segments, sealed in polystyrene trays using a polyolefinic film and stored at two temperatures 1° or 5°C for 9 days. The following analysis and subjective evaluations were carried out: chemical analysis of the juice, processing characteristics, in-package CO_2, O_2 and C_2H_4 concentration and microbiological assessments. The overall results indicate a better behaviour of both cultivars at 1°C than at 5°C, while Ponkan was more suitable for processing than Page.

RIASSUNTO

L'obiettivo di questo studio è stato quello di valutare l'attitudine alla minima trasformazione di due differenti varietà di mandarini, "Ponkan" e "Page" (Minneola tangelo x Clementine), sbucciati, divisi in spicchi manualmente, confezionati

- Key words: mandarins, minimally processed, modified atmosphere, ready to eat -

utilizzando delle vaschette in polipropilene (100 g di frutti) sigillate con un film poliolefinico. I frutti così preparati sono stati conservati a 1° e 5°C per 9 giorni. Il mandarino "Ponkan", per le sue caratteristiche morfologiche, ha manifestato la migliore attitudine alla minima trasformazione, rispetto al Page che per l'elevata adesione della buccia all'endocarpo e per la compattezza dell'esperidio ha riportato, durante le operazioni di preparazione evidenti danni che hanno comportato un incremento di tutti i processi metabolici e un più marcato decadimento delle caratteristiche nutrizionali e sensoriali. Per gli stessi motivi, la composizione dell'atmosfera interna alle confezioni, ha evidenziato, durante tutto il periodo di conservazione concentrazioni di CO_2 e C_2H_4 più elevate nella varietà Page. I controlli microbiologici hanno indicato esclusivamente la presenza di alcuni microrganismi di tipo ambientale non dannosi per la salute umana.

INTRODUCTION

Minimally processed fruits consist of washed, peeled, sliced or shredded raw vegetables, ready for use and, generally, packaged in plastic bags, stored below 6°C for 8-10 days. They are more perishable than fresh produce as a consequence of tissue damage resulting from processing operations (BRECHT, 1995). Due to specific physiological and morphological characteristics, mandarins are ideally suited for presentation as a ready-to-eat product (PRETEL *et al.*, 1998). Objective of this experiment was to evaluate quality changes during storage of two different cultivars of mandarin manually peeled and separated into segments.

MATERIALS AND METHODS

Fruit was selected for homogeneous size, peeled and divided into segments manually and placed inside polypropylene trays (approximately 100 g of product), and wrapped with a 19 mm thick polyolefinic film denominated MY (Cryovac, Milan, Italy). Film characteristics were: water transmission 109 g/d.m^2 at 38°C and 100% RH; O_2 permeability 3,400 mL/m^2 d bar at 23°C and 0% RH; CO_2 permeability 8,500 mL/m^2 d bar at 23°C and 0% RH). Packages were stored at 1° or 5°C for 9 days. In-package C_2H_4 was assessed by a Varian 3300 GC equipped with a Carbowax 20 M 80/120 mesh Carbograph 1 AW 30 column and a flame ionisation detector. In-package O_2 and CO_2 partial pressure were determined using a gas analyser (Servomex, Crowborough, England). Periodically microbiological assessment was also carried out to verify if spoilage or pathogenic microrganism were present.

The data were subjected to analysis of variance using the "Statgraphics" software (plus 5 version, Manugistics, Rockville, Maryland, USA).

RESULTS AND CONCLUSION

"Ponkan" mandarins were very easy to peel and showed a high aptitude to be separated and segments at the end of the processing operations showed no signs of damage. The peel of "Page" mandarins although soft is strongly adherent

Table 1 - Processing characteristics of the studied cultivars.

Cultivar	Peel thickness (mm)	Peelability (index number)[1]	Separability of segments (index number)[2]	Thickness of membrane (index number)[3]	Segments integrity after separation (index number)[4]
Page	2.2	4	3	1	3
Ponkan	3.7	9	9	2	9

[1] Subjective evaluation on a scale ranging form 1 (least peelability) to 9 (greatest peelability).
[2] Subjective evaluation on a scale ranging form 1 (least separability) to 9 (greatest separability).
[3] Subjective evaluation on a scale ranging from 1 (very thin) to 5 (very tick).
[4] Subjective evaluation on a scale ranging from 1 (very damaged) to 9 (no damage).

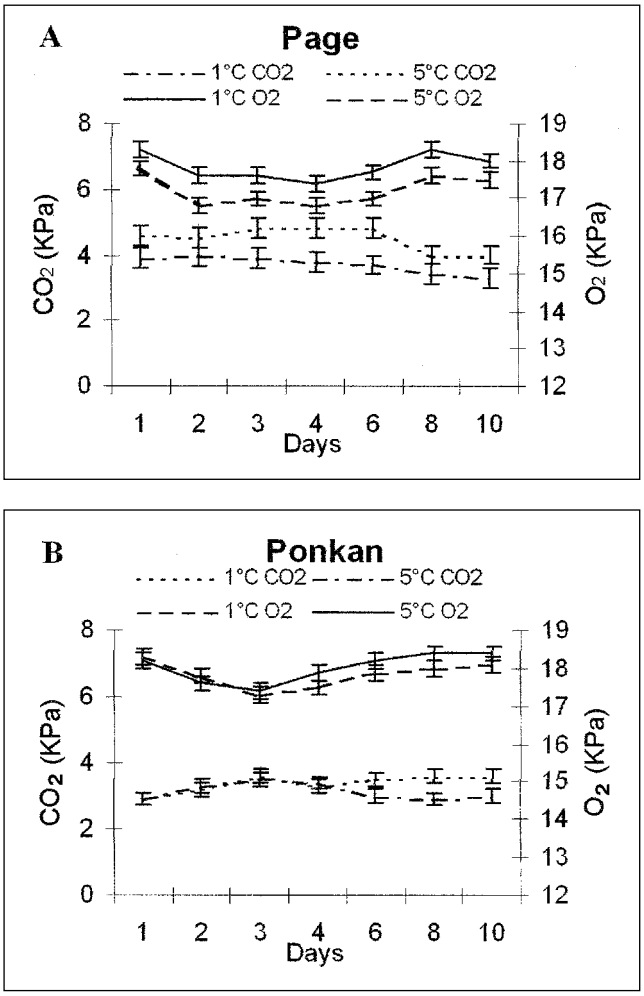


Fig. 1 - In package CO_2 and O_2 evolution during storage at 1° or 5°C of "Page" (A) and "Ponkan" (B). Vertical bars represent LSD values at P≤0.05.

to the endocarp, consequently the operation of peeling and separation of the segments were carried out with difficulty and the final product reported severe wounds and leakage of juice (Table 1). In-package CO_2 and C_2H_4 (data not show) was significantly higher in "Page" than in "Ponkan" mandarins and their levels were positively correlated with physical alteration reported during processing (Fig. 1). Regarding chemical parameters, "Ponkan" mandarins underwent few changes over the storage period and the only significant variation occurred in vitamin C content of the sample stored at 5°C, which declined slightly. In contrast, in "Page" mandarins regardless of the storage temperature, chemical parameters changed markedly: pH values increased progressively whereas both titratable acidity and Vitamin C declined noticeably. In contrast, total soluble solids were fairly stable over the storage period and were not affected by the storage temperatures (Table 2).

The overall results indicate a better behaviour of both cultivars at 1° than at 5°C, while

Table 2 - Effect of different temperatures (1°C and 5°C) on chemical parameters in segments of "Page" and "Ponkan" mandarins manually processed during the storage period.

Storage period	pH		Ascorbic acid (mg/100 mL)		TA (g/100 mL citric acid)		TSS (°Brix)	
	1°C	5°C	1°C	5°C	1°C	5°C	1°C	5°C
Page								
harvest	3.93		38.56		0.91		10.73	
3 days	3.96a	3.94a	36.40a	35.90b	0.79a	0.78a	11.30a	11.00a
6 days	4.00a	3.99a	36.86a	35.46b	0.74a	0.73a	11.16a	10.83 a
9 days	3.99a	4.05a	36.00a	36.33b	0.76a	0.71a	11.30a	11.30 a
Ponkan								
harvest	4.31		51.43		0.90		11.16	
3 days	4.25a	4.27a	49.53a	50.93a	0.89a	0.89b	11.13a	11.00a
6 days	4.43a	4.31a	49.6a	49.03b	0.86a	0.87b	10.73a	10.6a
9 days	4.27a	4.30b	49.43a	47.13c	0.87a	0.87b	11.05a	10.9a

Values followed by the same letters within each storage period do not differ significantly at P≤0.005 by LSD.

"Ponkan" was more suitable for processing than "Page". Microbiological assessments showed only the presence of environmental microorganisms, such as *Bacillus* spp. and *Staphylococcus epidermidis*, whereas no pathogenic germ was detected (data not shown).

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Authors contributed equally to this study.

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METHOD FOR EVALUATING THE BARRIER PROPERTIES OF FOOD PACKAGING VERSUS EXTERNAL POLLUTANTS

METODO PER LA VALUTAZIONE DELLE PROPRIETÀ BARRIERA VERSO INQUINANTI ESTERNI DI IMBALLAGGI PER PRODOTTI ALIMENTARI

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ABSTRACT

The suitableness of materials and articles intended to be used in contact with foodstuffs is regulated by national and European directives. The organoleptic behaviour of the edible parts is also required.

No particular rules are available regarding the package properties versus pollulants.

In terms of problem solving and development of a new packaging solution, it is necessary to understand the barrier versus potential pollution of a package (bottles plus closure).

This study has been carried out in order to define a suitable method to determine the barrier properties of closures, made from different raw materials, for PET water and soft drink containers.

Bottles filled with water and well capped, were put in a contaminated ambient by using an organic compound as "marker". The concentration of the marker in the water was evaluated after several conditioning times. Considering that the only variable was the closure, the different marker concentrations in the water can be related to a different closure barriers.

The 2,4,6 trichloro anisole was used as marker. The test on the water was done by a purge and trap sampling of water followed by a gaschromatography/mass spettrometry techniques (GC/MS) by a single ion monitoring. The sensibility of the method was 0.1 ppb.

The study allowed the barrier properties of the different closures to be verified.

RIASSUNTO

L'idoneità igienico-sanitaria dei materiali destinati al contatto con alimenti è ampiamente regolamentata, anche in dettaglio, da normative nazionali ed europee

- Key words: barrier properties, trichloro anisole -

così come la salvaguardia delle caratteristiche organolettiche della parte edibile è richiesta dalle norme "quadro". Per quanto riguarda invece l'effetto barriera del contenitore verso inquinanti esterni ci si riferisce, a livello normativo, al concetto, importante ma piuttosto generico, della "idoneità tecnologica".

Dal servizio di assistenza, *problem solving* e progettazione di nuove soluzioni di imballaggio che PDC offre agli operatori del packaging, è nata l'esigenza di individuare opportune metodologie di lavoro per "misurare" il potenziale "barriera" di un contenitore o parti di esso nei confronti di possibili inquinanti esterni.

Lo studio presentato riguarda la messa a punto di un metodo di lavoro per poter valutare l'effetto barriera di tappi a vite in materiale plastico di differente composizione, destinati a bottiglie in PET per acqua e soft drinks.

Il metodo si basa sull'esposizione di bottiglie riempite di acqua e chiuse con i campioni di tappo, ad un ambiente contaminato appositamente con un composto organico avente la funzione di "marker". Ad intervalli di tempo prefissati abbiamo analizzato l'acqua delle bottiglie per determinare la concentrazione di "marker" eventualmente migrato. Poichè le bottiglie sono uguali, eventuali differenze di concentrazione del "marker" sono dovute a diverso effetto barriera dei materiali costituenti i tappi.

Come "marker" è stato scelto il composto 2,4,6 tricloro anisolo mentre il metodo di determinazione dello stesso nell'acqua, si basa su di un campionamento "purge & trap" ed analisi in gascromatografia/spettrometria di massa con acquisizione "single ion monitoring". Il metodo raggiunge un limite di determinazione di 0,1 ppb o µ/L.

Lo studio ha permesso di verificare effetti barriera diversi fra i materiali testati.

INTRODUCTION

The compliance of materials and articles which are intended to be used in contact with foodstuffs is regulated by national and European directives. Organoleptic behaviour of the edible parts is also required. No particular rules are available regarding the package properties versus pollulants.

From the PDC experience in terms of problem solving and development of a new packaging solution, the necessity of understanding the barrier versus potential pollution of a package (bottles plus closure), is growing day by day.

This study was carried out in order to define a suitable method to determine the barrier properties of a closures, made from by different raw materials, for PET water and soft drinks containers.

AIM

In this study trichloro anisole was used as a "marker" in order to evaluate the barrier properties of different closures fitted on PET bottles.

The bottles, filled with water, were conditioned at 23°C in sealed bags and polluted internally with anisole.

The water was analysed weekly during the 90-day test period.

MATERIALS AND METHODS

Samples and contamination

Four different closures were used to close the same 500 mL PET bottles.

The samples were put in PE/PET methalised big bags and sealed well.

Table 1 - GC/MS condition.

Column	Capillary column Supelco MND-5S; length 30 m; column ID 0.25 mm; film thickness 0.5 µm
Oven temperature	40°C (2 min) 10°C/min -> 100°C -> 15°C/min -> 250°C
Carrier gas	He 0.8 mL/min constant flow
Injector	Volatile interface 260°C
Detector	Mass selective detector i.e. 70 ev
Acquisition mode	SIM (single ion monitoring) Internal standard (cholrobenzene): 112-114 2,4,6 Trichloro anisole: 195-197-210-167

Inside of each bag, 100 mg of 2,4,6 trichloro anisole were introduced by using a dedicated filter in order to avoid direct contact with the bottles and to allow the internal area of the bag to be contaminated.

One bag was prepared for each data test.

The bags were stored in a thermostat at 23°C for all the test times.

Determination of 2,4,6 trichloro anisole by Purge and Trap and GC/MS analysis

A purge and trap sampler was used. The sample was purged at 70°C for 10 min with nitrogen flow (30 mL/min). The TCA realised was trapped on a tenax/carbon cartridge at 40°C.

The analyses was carried out by thermal desorbption (280°C) of trap followed by GC/MS SIM (single ion monitoring) analysis according to the condition described in Table 1.

Sample preparation

Ten mL of sample (water coming from the bottle) were added with 1 µL of internal standard (chloro benzene solution at 0,11 µg/µL) corresponding to 11 ppb on the sample, and analysed by P&T and GC/MS.

Calibration

A multi-level calibration was carried out by injecting 2,4,6 trichloro anisole standard solutions (from 0.1 up to 10 ppb) in conditions similar to those used for the samples.

The calibration curve is reported in Fig. 1.

A typical chromatogram of a standard solution, is reported in Fig. 2.

Method validation

Validation of the method was obtained by repeatability test, applying the entire procedure to 6 standard solutions at 1 ppb. As shown in Table 2 the value of the RDS % is ±10%.

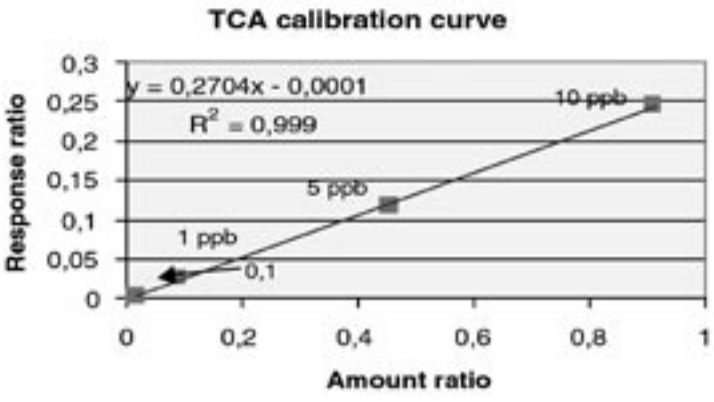


Fig. 1 - Calibration curve of 2,4,6 trichloro anisole.

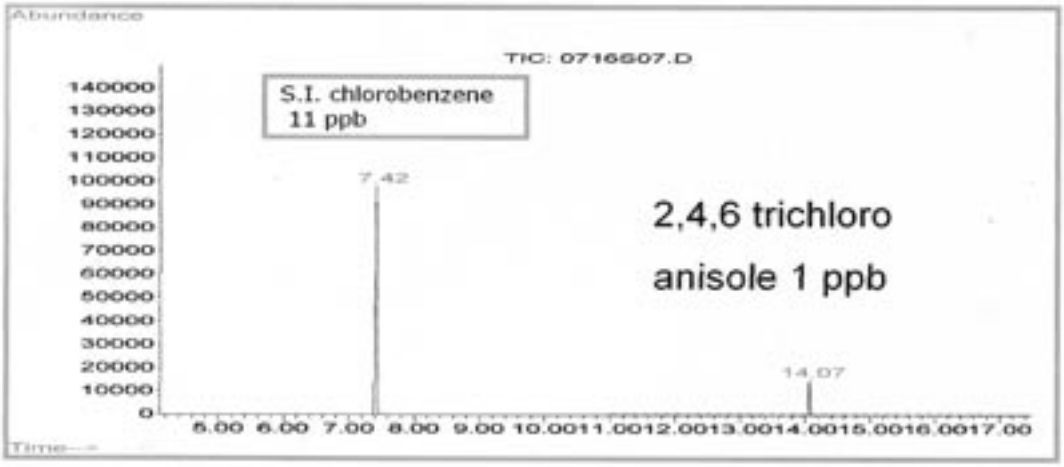


Fig. 2 - Purge and trap and GC/MS analysis of standard solution at 1 ppb.

Table 2 - Repeatability on standard solution of TCA at 1 ppb.

Compounds	P1	P2	P3	P4	P5	P6	Media	DS	RDS%
2,4,6 trichloro anisole	1.11	0.93	0.90	0.87	0.91	1.05	0.96	0.093	9.6
The value of the RDS% is ±10%.									

Table 3 - TCA results.

The results are expressed in ppb of 2,4,6 trichloro anisole					
Sample	T1 Week 1	T2 Week 2	T3 Week 3	T4 Week 4	T5 Week 6
	ppb of 2,4,6 trichloro anisole				
01	<0,1	<0,1	0,4	4,5	14,3
02	<0,1	<0,1	<0,1	<0,1	0,5
03	<0,1	<0,1	<0,1	0,2	1,2
04	<0,1	<0,1	<0,1	0,2	0,9

RESULTS AND CONCLUSION

At every expiry three bottles for each sample were analysed: in Table 3 you can find the results obtained (average of three bottles).

The analytical method is characterised by a detection limit of 0.1 ppb and a RDS % ±10%.

The study allowed the barrier properties versus TCA of the tested closures to be discriminated.

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POSTER

SHELF-LIFE STUDY OF TALEGGIO CHEESE PACKED WITH PAPER OR IN MODIFIED ATMOSPHERE AND COMPARISON OF ANALYTICAL METHODS FOR AMMONIA DETECTION

STUDIO DELLA SHELF-LIFE DI FORMAGGIO TALEGGIO CONFEZIONATO CON CARTA O IN ATMOSFERA MODIFICATA E CONFRONTO TRA METODI ANALITICI PER LA DETERMINAZIONE DI AMMONIACA

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INTRODUCTION

The individuation of appropriate indexes, markers of cheese ripening, is very important in view of increasing the value of fresh cheese quality performances for the consumer.

Taleggio cheese is an Italian PDO (Consorzio) cheese made with whole cow milk, characterised by a soft texture and a short ripening time. The study of shelf-life is very interesting because Taleggio cheese is a "living" product, where the ripening proceeds during marketing, with chemical and sensorial properties changing during storage.

In a previous study (RIVA *et al.*, 2002; BENEDETTI *et al.*, 2002), some parameters related to proteolytic phenomena [NCN/Ntot, NPN/Ntot, NH3/Ntot, soluble peptidic fraction at pH 4.6 (RP-HPLC), caseinic fraction (IEF), aminoacids (Aminoacid Analyzer)], and to aromatic and sensory characteristics measured with image analysis and electronic nose (Electronic nose: Applied sensor's 3200 with an array of 10 MOSFET and 12 MOS sensors), were evaluated. Measures were repeated at

- Key works: ammonia, detection methods, shelf-life, Taleggio cheese -

different times during storage, according to conventional shelf-life studies, in order to define the kinetics of degradative phenomena.

MATERIALS AND METHODS

Tests were performed on 2 sets of Taleggio cheese with normal commercialisation ripening (35 days), stored at 10°C, packed with paper or in modified atmosphere.

Samples packed with paper were analysed every 2 days until 65 days, samples packed in modified atmosphere were analyzed every 3 days until 90 days.

The free-ammonia content was evaluated using the classical titrimetic method [Giuliano and Stein (Giuliano and Stein, 1964) by previus Kjeldahl distillation] or with the potentiometric method [Helaine (HELAINE, 1977; PINELLI, 1991)] in comparison with a new colorimetric method [CDR (Manuale CDR)].

The new CDR method is based on a spectrophotometric determination at 700 nm of a blue-green compound obtained from a reaction between ammonia and a specific phenolic reagent.

RESULTS AND DISCUSSION

The free-ammonia content is able to discriminate the different kinetics of proteolysis in Taleggio cheese packed with paper (Fig. 1) or in modified atmosphere (Fig. 2). At storage temperature of 10°C, the trend of these parameters was in sigmoid transition kinetics. This trend suggests two criteria in order to identify shelf-life limits: maximum tolerance (maximum rate) and maximum prudence (maximum acceleration).

The modified atmosphere packaging determines a sensible slowing of the proteolytic phenomena and a considerable increase of shelf-life. Storage time of Taleggio cheese increased from 20 to 53 days according to the prudential criterion, corresponding to the maximum acceleration of the proteolytic process.

Storage time of the same Taleggio cheese (Table 1) increased from 33 to 63 days according to the tolerant criterion, corresponding to the maximum rate of the proteolytic process.

A good correlation between the data from the new CDR colorimetric method and titrimetric method (but not so good between CDR colorimetric and potenziometric method) has been achieved (Fig. 3), but the CDR method was faster and more reliable in determining the ammonia content in an attempt to monitor the proteolytic process.

CONCLUSIONS

A simple colorimetric method was used for the shelf-life study of Taleggio cheese packed with paper or in modified atmosphere.

It has been demonstrated that the modified atmosphere packaging is a good choice for increasing shelf-life of Taleggio cheese, both considering a stability time of the proteolytic process and the shelf-life based on the prudential criterion and the tolerant criterion.

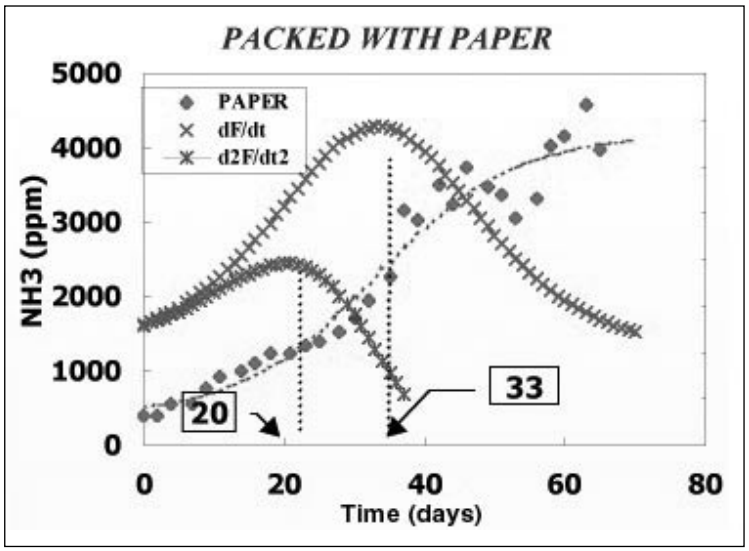


Fig. 1 - Definition of shelf-life limit. A sigmoidal transition curve was used to interpolate the evolution of the ammonia content in Taleggio cheese packed with paper. The first derivative maximum (max speed of decay process) and the second derivative maximum (max acceleration decay process) were evaluated as shelf-life time limit.

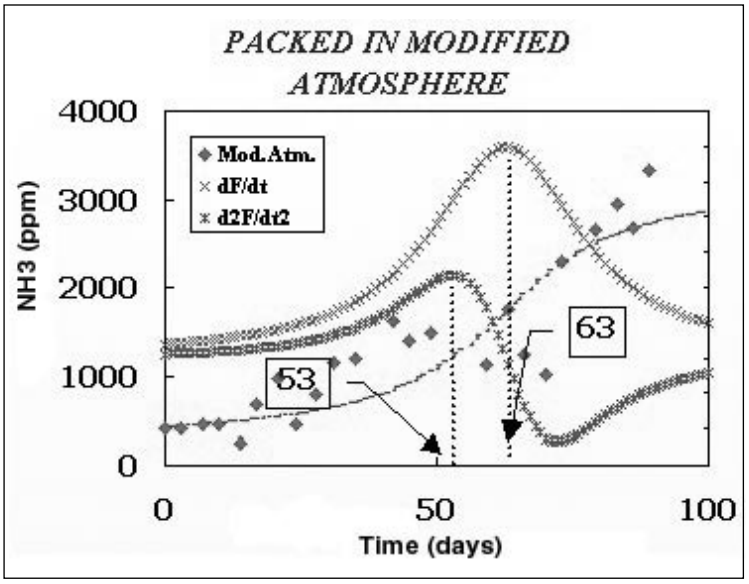


Fig. 2 - Definition of shelf-life limit. A sigmoidal transition curve was used to interpolate the evolution of the ammonia content in Taleggio cheese packed in modified atmosphere. The first derivative maximum (max speed of decay process) and the second derivative maximum (max acceleration decay process) were evaluated as shelf-life time limit.

Table 1.

Shelf-life of packed Taleggio cheese	Tolerant criterion First derivative maximum (days)	Prudential criterion Second derivative maximum (days)
Paper	33	20
Modified atmosphere	63	53

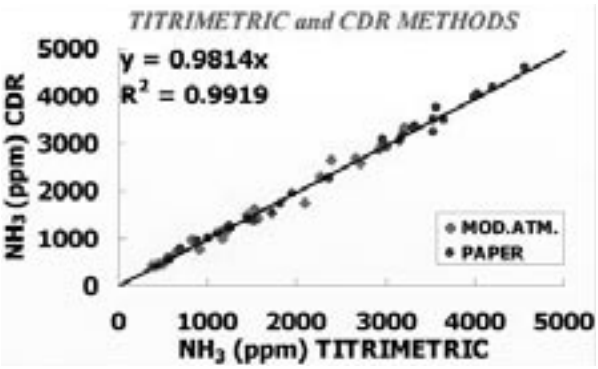




Fig. 3 - Comparison of the titrimetric values and those obtained by the CDR method.

The new CDR colorimetric method has been demonstrated to be able to measure the free-ammonia content of Taleggio cheese, and was faster and more reliable in determining the proteolytic process.

Compared with the titrimetric method, the CDR method has several advantages that make it attractive for both reference and routine purposes.

Firstly, the CDR method produces ammonia results equivalent to the current titrimetric method within a time frame of 8-10 min.

Secondly, no big capital investment is required for laboratories already involved in the analysis of dairy products.

Thirdly, the CDR method is simple and applicable to a very wide range of milk products.

The research confirmed that, also in the case of Taleggio cheese, it is possible to notify the customer abaut the real storage time limits of the product.

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SHELF-LIFE EXTENSION OF MINIMALLY PROCESSED ARTICHOKES

PROLUNGAMENTO DELLA SHELF-LIFE
DI CARCIOFI MINIMAMENTE TRATTATI

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ABSTRACT

In the present work the extension of the shelf-life of minimally processed artichokes by different means was studied. Artichokes were blanched in an aqueous solution of citric, lactic and acetic acids and packed in low permeability pouches in the presence of a mixture of carbon dioxide and nitrogen. Samples were then stored at 4° and 10°C. Colour changes and microbial growth were evaluated during storage time. The combination of heat treatment, acidification, modified atmosphere, packaging and refrigeration extended the shelf-life of minimally processed artichokes for a time sufficiently long for marketing.

RIASSUNTO

Lo scopo di questo lavoro è stato quello di studiare l'effetto combinato di alcune operazioni tecnologiche sulla shelf-life di carciofi minimamente trattati. I carciofi sono stati sottoposti a blanching in soluzione acquosa acidificata con acido citrico, lattico e acetico, confezionati in atmosfera modificata costituita da una miscela

- Key words: artichokes, combined treatments, minimal-processing, shelf-life -

di azoto e diossido di carbonio, e successivamente conservati a 4° e 10°C per 26 giorni. Nel corso della conservazione sono stati analizzati i cambiamenti di colore e lo sviluppo microbico. I risultati hanno evidenziato che l'impiego di soluzioni acidificate per il trattamento di blanching unitamente al confezionamento in atmosfera modificata sono efficaci nel prolungare la shelf-life dei cuori di carciofo per almeno 26 giorni di conservazione a temperature inferiori o uguali a 10°C.

INTRODUCTION

In the last two decades great interest has been shown in the production of minimally processed fruit and vegetables, mainly for their commodity and freshness attributes (GORRIS L., 1999). The products in question are prepared by using appropriate unit operations such as peeling, slicing, shredding, etc. and are presented for sale in packages (MARCHETTI R. *et al.*, 1992) (KING A.D. and BOLIN H.R., 1989). As known, handling and preparation may be responsible for accelerated microbial and enzymatic spoilage, which can lead to colour changes, texture decay, modification of flavour, and loss of nutritional value (SVENSSON S., 1997) (VEROQUAUX P. and WILEY R.C., 1994). The extension of the shelf-life of minimally processed vegetables can be achieved by applying preservation treatments, such as blanching, modified atmosphere, packaging, refrigeration, reduction of pH and water activity, etc. or a combination of these (AGUILERA J.M., PARADA E., 1991). In the present work a combination of technological processes was studied in order to obtain minimally processed artichokes. In particular, blanching was carried out in acid solutions followed by modified atmosphere packaging and refrigerated storage. In fact, although the globe artichoke is one of the most important horticultural crops of Italian agriculture, no minimally-processed artichokes are available on the Italian market. In Italy this product is usually marketed with leaves wrapping the buds. In fact, removal of leaves and shortening of flower stems reduce the storage life because of the activation of enzymatic systems, which can lead to tissue darkening and textural decay (BOLIN H.R. and HUXOLL C.C. 1989). Processed artichokes are usually sold as frozen products or canned in oil or brine. However, the canned artichokes are completely different in taste from the original product, while the frozen ones are, in most cases, strongly acidified and need to be washed after thawing and before cooking.

MATERIALS AND METHODS

Sample preparation

Raw artichokes from a local foodstore were trimmed, cut in four and blanched at 100°C for 5 min in acid aqueous solutions, which were obtained by mixing different percentages (on weight basis) of citric, lactic and acetic acids (Carlo Erba, Italy). The organic acid concentrations used were determined according to a central composite design and their combinations chosen for the blanching procedure were: acetic acid, 0.15%, lactic acid, 0.15%, citric acid, 0.15%. A sample blanched in boiling water was taken as a control. Artichokes were then packed in low permeability pouches filled with a nitrogen/carbon dioxide (50:50 v/v) mixture. The low

permeability pouches consisted of oriented polyammide-polyethylene film supplied by AMB srl (Udine, Italy). The film permeabilities to oxygen, carbon dioxide and vapour were 40 mL m^{-2}/day^{-1}, 120 mL m^{-2}/day^{-1} and 12 g m^2/day^{-1}, respectively. The packed artichokes were stored at 4°, 10° and 25°C for up to 26 days.

Colour measurements

The colour analysis was carried out on artichokes which were previously homogenised with deionised water (the sample: water ratio was 1:1), by using a tristimulus colorimeter (Chromameter-2 Reflectance Minolta, Japan) equipped with a CR-300 measuring head. Colour was expressed in L*, a*, b* Hunter scale parameter, and a* and b* were used to calculate the hue angle (tan-1 b*/a*) (Little, 1975). Data are the average of at least five repetitons.

Microbiological analysis

Aliquots of 10 g of each sample were diluited with peptone water (9 mL), homogenised with Stomacher Lab-blender (mod. 80, Pool Bio-Analysis International, Italy) and dilutions were plated in duplicate into appropriate media. The media and the analysis conditions used were as follows: Plate Count agar (Oxoid, Unipath) incubated at 37°C for 48 h for aerobic mesophilic bacteria (AMB), and at 5°C for one week for psychrotrophic bacteria (PB); Sabouraud agar (Oxoid, Unipath) incubated at 25°C for 4 days for yeasts and moulds.

RESULTS AND DISCUSSION

Table 1 shows the changes of the counts of psychrotrophic and mesophilic bacteria in artichokes, previously blanched in acid solution and packed under N_2/CO_2 atmosphere, during storage at 4° and 10°C. It can be observed that microbial growth was effectively inhibited by combining acidification and modified atmosphere packaging. In fact, neither yeast and moulds nor spores were found in the modified atmosphere packed samples, although they were present in the control

Table 1 - Mesophilic and psycrotrophic bacteria in and N_2/CO_2 atmosphere-packed artichokes, previously blanched in acid solution, stored at 4° and 10°C. Data referred to a sample blanched in water, packed under AO and N_2/CO_2 stored at 4° and 10°C (control) are also reported.

Storage time	Aerobic mesophilic bacteria (CFU/g)			Psychrotrophic bacteria (CFU/g)		
(days)	a 4°C	a 10°C	Control	a 4°C	a 10°C	Control
0	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$> 10^2$
7	$< 10^2$	$< 10^2$	$> 10^6$	$< 10^2$	$< 10^2$	$< 10^6$
14	$< 10^2$	$< 10^2$	$> 10^6$	$< 10^2$	$< 10^2$	$< 10^6$
21	$< 10^2$	$< 10^2$	n.d.	$< 10^2$	$< 10^2$	n.d.
26	$< 10^2$	$< 10^2$	n.d.	$< 10^2$	$9x10^2$	n.d.

a: artichokes; n.d.: not determined.

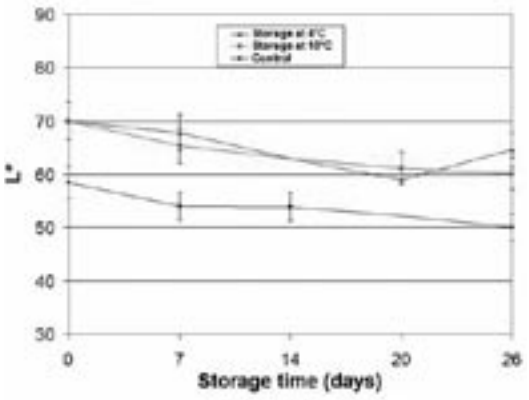


Fig. 1 - Changes in L* values of artichokes subjected to blanching treatment in an aqueous solution of 0.15% citric acid, 0. 15% acetic and 0.15% lactic acid, packed under N_2/CO_2 atmosphere and stored at 4° and 10°C for up to 26 days Data are compared with those referred to artichokes blanched in water and stored at 10°C under modified atmosphere.

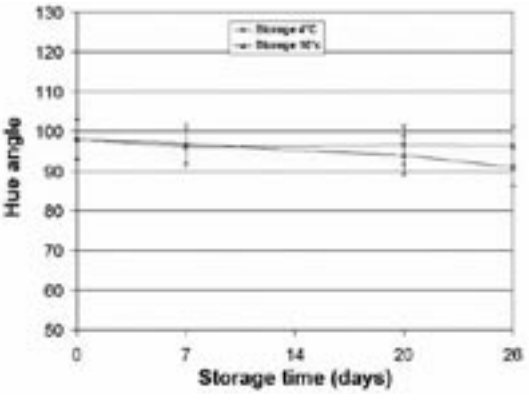


Fig. 2 - Changes in Hue angle values (tan^{-1} b*/a*) of artichokes subjected to blanching treatment in an aqueous solution of 0.15% citric acid, 0.15% acetic and 0.15% lactic acid, packed under N_2/CO_2 atmosphere and stored at 4° and 10°C for up to 26 days.

(data not shown). As regards the temperature effect, storage at both 4° and 10°C was effective in retarding microbial growth, although the mesophilic bacteria in artichokes previously blanched in the acid solution and stored at 25°C reached 10^7 CFU/g after 15 days of storage (data not shown). It is likely that the low pH values of the samples had a predominant role in the microbial stability of artichokes. Fig. 1 shows the changes in L* values of the minimally-processed artichokes studied (data are compared with those referred to artichokes blanched in water and stored at 10°C under modified atmosphere). Blanching carried out in acid solution was effective in mantaining the L* value close to 70 (acceptable value), while a great drop in lightness was observed for the control just after the water-blanching process. A decrease in L* value for the acidified samples was observed within the first seven days of storage, while only a slight further reduction was measured up to 26 days. Fig. 2 shows the changes in hue angle of the considered minimally-processed artichokes during storage at 4° and 10°C. No significant changes of this parameter were observed at both storage temperatures. Since no polyphenoloxidase activity was detected (the methodology described by V. Khan, 1985 was used), since the heating was sufficient to inactivate the enzyme, darkening of blanched artichokes can be attributed to chemical reactions. In particular, interactions between phenolics and iron can take place in the processed products (LATTANZIO *et al.*, 1994). It has been suggested that the phenolic compounds chelate the reduced form of iron to form a colourless complex, which upon exposure to oxygen are oxidised to yield a coloured compound. The addition of citric acid, which is an iron sequestering agent, can explain the higher colour retention for the artichokes blanched in the acid solution compared to the control.

CONCLUSIONS

The use of acid blanching solutions in combination with immersion in acid

mixture, modified atmosphere packaging and storage at low temperature was effective in extending the shelf-life of minimally processed artichokes for up 26 days.

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STUDY ON SORPTION OF FLAVOR COMPOUNDS FROM WINE BY POLYETHYLENE FILM

INDAGINE SULLA MIGRAZIONE DI COMPONENTI DELL'AROMA DEL VINO IN FILM DI POLIETILENE

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ABSTRACT

The sorption of flavour compounds from a red Sicilian wine by a commercial polyethylene film (PE) was investigated. The film samples were stored at ambient temperature (20°C±2) in the dark, and analyzed by means of SPME technique after 15, 30 and 45 days of contact.

Results showed that the investigated PE film absorbs the most volatile compounds, such as n-amyl acetate, 3 methyl 1 butanol, ethyl hexanoate, ethyl octanoate, diethyl succinate, 9 ethyl decenoate, decanol, methyl ethyl decanoate, ethyl dodecanoate and phenyl ethanol, and consequently most of the olfactory portion of the product. Moreover, it was pointed out that the sorption process takes place in the first 30 days.

RIASSUNTO

Lo scopo del presente lavoro è stato quello di valutare l'assorbimento dei componenti volatili (scalping) di un vino rosso siciliano mantenuto a contatto con un film di polietilene (PE) per uso commerciale. La componente volatile trattenuta

- Key words: film, flavor compounds, polyethylene, SPME, wine -

dal film dopo 15, 30 e 45 giorni di contatto con il vino, è stata estratta mediante adsorbimento su resine (SPME) ed analizzata al gas-cromatografo.

I risultati ottenuti hanno mostrato una tendenza del film a trattenere i componenti più volatili del prodotto (n-amil acetato, 3 metil 1 butanolo, etil esanoato, etil ottanoato, dietil succinato, 9 etil decenoato, decanolo, metil etil decanoato, etil dodecanoato e fenil etanolo) e pertanto maggiormente responsabili della sua nota odorosa. Dai dati ricavati si può dedurre che tale processo di scalping avvenga in maniera significativa nei primi 30 giorni di contatto con il vino.

INTRODUCTION

The interaction between food and packaging generally leads to a loss of food quality. In fact, either the diffusion of contaminants from the packaging into the food (migration) or the diffusion of food constituents into the packaging (scalping) brings about the unacceptability of the contained food. Generally, both migration and scalping take place at the same time. The former influences the safety of the packed food, whereas the latter affects its sensorial properties. Polyolefins (such as polyethylene and polypropylene) are generally used as model polymer in migration or scalping studies, because this class of polymers is generally used in contact with food, and also because of its high affinity with lipids (ARORA *et al.*, 1981; IMAI *et al.*, 1990).

In the last years, white and red wine has been commercialized in paper board multilayer structures. As the appreciation of such products is strictly connected with the preservation of the whole flavor compounds, the aim of this work was to identify the volatile flavor compounds of a red wine that migrated inside a polyethylene film (PE).

MATERIALS AND METHODS

The investigated wine was a red mono variety wine (Nerello Mascalese, grape-harvest 2001) which is characterized by a limited phenol content and a lightly marked aromatic profiles.

The tests were performed using a commercial polyethylene film, which was cut in 10 cm^2 pieces, and placed in contact with 250 mL of wine. The samples were stored at ambient temperature (20°C±2) in the dark, and analyzed by means of SPME technique after 15, 30 and 45 days of contact. After contact, the film was rinsed with distillated water and then cut into 1 cm^2 pieces. The film sheets were placed into a glass screw-capped vial and conditioned in thermostatic-bath at 60°C for 15 min. A polydimethylsiloxane (PDMS – 100 µm) fiber, suitable for analyzing aldehydes, alcohols, esters, ketones and hydrocarbon terpenes, was then introduced into the vial for 15 min at the same temperature (JORDAN *et al.*, 2001). The volatile fraction was then desorbed from fiber directly into the GC injector for 3 min at 220°C. Component identification was performed by an GC-MS Shimadzu QP5050A model at the following analysis conditions: Injector 220°C; Transferline 180°C; Oven 50°C x 3', rate 2°C/min → 70°C, rate 5°C/min → 200°C x 150'; Carrier He 1.0 mL/min.; Splitless; CP-WAX 57 CB column (50 m, i.d. 0.25 mm, df 0.2).

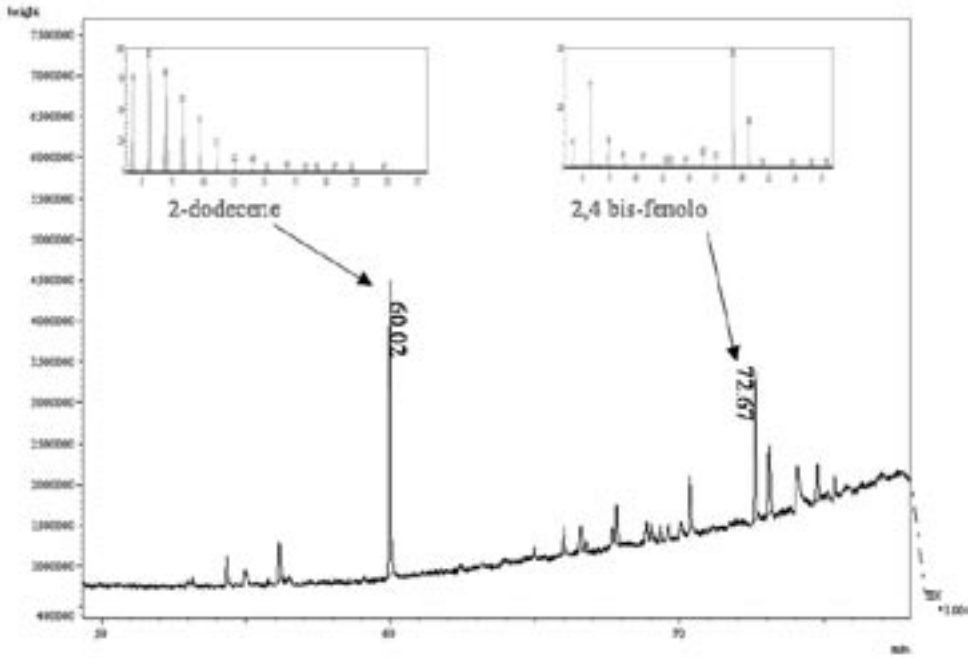


Fig. 1 - SPME of PE film.

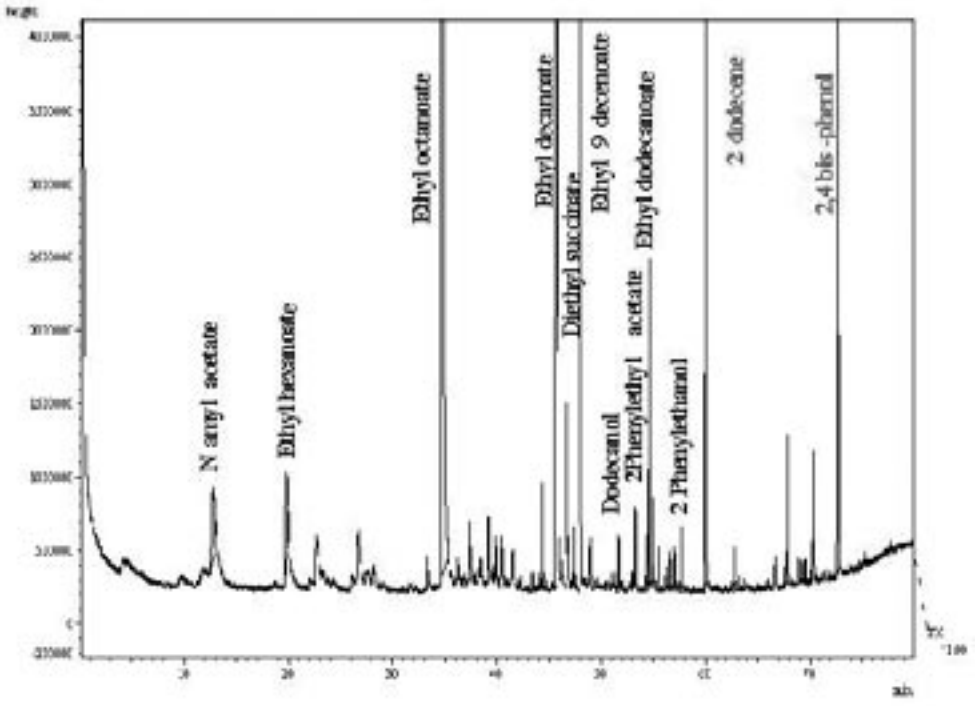


Fig. 2 - SPME of PE film after 30 days of contact with wine.

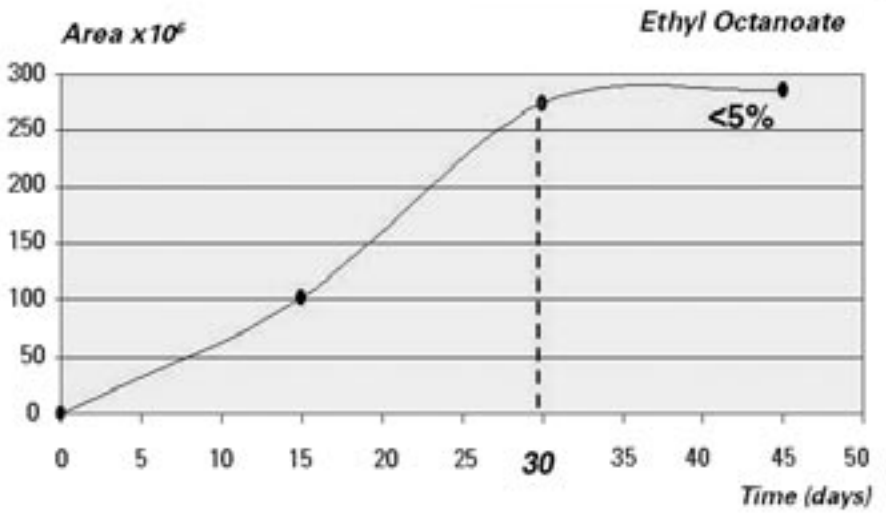


Fig. 3 - The evolution of the peak intensity of ethyl octanoate sorption during the entire period of contact.

RESULTS AND DISCUSSION

Fig. 1 shows the gaschromatographic profiles of the fiber and that of investigated PE film. As shown in the above figure, the fiber is characterized by a flat chromatographic profile, whereas the "as received" PE film is characterized by a chromatogram with two main peaks, identified by fragmentation as 2 dodecene and 2,4 bis-phenol.

After 15 days contact with wine, the analysis of PE film allowed the following food compounds to be identified: n-amyl acetate, 3 methyl 1 butanol, ethyl hexanoate, ethyl octanoate, diethyl succinate, 9 ethyl decenoate, decanol, methyl ethyl decanoate, ethyl dodecanoate and phenyl ethanol. The above mentioned compounds belong mainly to esters and alcohol groups because of the fiber's greater affinity towards these molecules. The analysis of PE film in contact for 30 days with wine revealed an increase of about 100% in all the peaks already mentioned (Fig. 2). However, after 45 days of contact with wine, the peak intensity of the above-mentioned compounds were not significantly different with respect to those detected in the PE film after 30 days of contact. Fig. 3 shows the evolution of the peak intensity of ethyl octanoate sorption during the entire period of contact. Results showed that the investigated PE film does absorbs the most volatile, and consequently most olfactory, portion of the product, and that the sorption process take place in the first 30 days.

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PRELIMINARY STUDY FOR THE EXTENSION OF THE SHELF-LIFE OF A TYPICAL SARDINIAN PRODUCT, "PARDULAS", BY MEANS OF ACTIVE PACKAGING

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ABSTRACT

Some packaging solutions for typical Sardinian sweets (G.T.S.= Guaranteed Traditional Specialty), named "Pardulas" or "Formagelle" have been studied, in order to extend their commercial life to 90 days which at the present time, because of the raw materials used, lasts a few days; such shelf-life does not permit their marketing in other European or extra-European countries.

At the beginning, the finished fresh product was characterized by a global examination, highlighting the primary steps of degradation.

For this reason, the samples, about 24 hours after their production, were checked: microbiologically to evaluate their hygienic-sanitary state, sensorially to evaluate their visual, gustative and olfactory qualities and physico-chemically. The hygienic-safety of the production plant (air, operative procedures and machinery) were checked. A microbiological check on raw materials used was also carried out. The principal steps of degradation were identified:

- High microbial charge because of the high relative moisture of the finished product;
- Loss of moisture with consequent modification of the structural characteristics of the product itself;
- Oxidation of the component fats with the finished product consequently going rancid.

Because of the impossibility of changing the raw materials (G.T.S. mark), the

possibility of using protective atmospheres and innovative solutions such as the active packaging, were examined. Four different types of active packaging were studied:

50% CO_2 + 50% N_2 (MIX);
MIX + oxygen absorbers;
100% CO_2;
100% CO_2 + oxygen absorbers.

The results show that the packaging in MIX without absorbers is more critical than with the other packaging because of a high charge of yeasts and moulds both in the external zone (puff pastry) and in the internal one (stuffing). The sensorial analysis, carried out comparing the fresh samples (24 hours from the production) with the packaged ones, showed differences more from the structural than gustative point of view which was acceptable until about 15 days. Such differences agree with the relative and absolute moisture variations as measured on the products at the different pull dates.

MICROBIOLOGICAL ASPECTS OF HORSEMEAT PACKED IN MODIFIED ATMOSPHERE

ASPETTI MICROBIOLOGICI DELLA CARNE DI CAVALLO CONFEZIONATA IN ATMOSFERA PROTETTIVA

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ABSTRACT

Modified atmosphere is, at the moment, the most widely used technique to preserve fresh foods from spoilage processes. This packaging system, associated with low temperature, considerably prolongs the shelf-life of the meat, by means of a selective control of the microbial development and the maintenance of the bright red colour.

In this work the microbial population growth on slices of horsemeat packaged under three different gas mixtures: 100% CO_2, (MAP A), 65%CO_2-35%N_2 (MAP B), 2%O_2-98%CO_2 (MAP C) stored at 4°C for 20 days was investigated. The meat used was of good quality; indices of safety (*Salmonella*, *Listeria monocytogenes*, sporeforming H2S productors) and faecal coliforms were not found in any of the packaging conditions.

Lactic acid heterofermentative bacteria were the predominant population and their number, already high at the beginning, (10^5 cfu/g) increased during the storage in all conditions tested. MAPA and MAPB, both with high concentration of CO_2, delay the growth of Gram negative bacteria, the principal responsible of the spoilage processes, while in MAP C (2% O_2), an abundant growth of psycrotrophic gram negative bacteria was observed.

Among the three gas mixture studied, MAPA gave the best condition: the meat preserved its red colour and the characteristics of freshness for 9 days. In contrast, under MAPB and MAPC horsemeat rapidly developed off-flavours and brown color and after 6 and 2 days, respectively, was not suitable for retail.

RIASSUNTO

Il confezionamento in atmosfera protettiva attualmente costituisce la tecnica di conservazione più utilizzata al fine di prevenire i processi alterativi degli alimenti freschi.

Questa tecnica associata alla bassa temperatura consente di prolungare la shelf-life della carne attraverso una azione selettiva su microflora presente ed il mantenimento del brillante colore rosso.

In questo lavoro è stata seguita la crescita della popolazione microbica presente

- Key words: horsemeat, lactic acid bacteria, modified atmosphere -

su fettine di carne di cavallo confezionate in atmosfera protettiva e conservate a 4°C per 20 giorni; le miscele adottate sono state: 100% CO_2 (MAPA), 65% CO_2 - 35% N_2 (MAPB), 2% O_2 - 98% CO_2 (MAPC).

I controlli microbiologici effettuati hanno evidenziato una carne di qualità, infatti tutti gli indici di salubrità (*Salmonella, Listeria monocytogenes* e Clostridi solfito riduttori) e di contaminazione fecale cercati sono sempre risultati assenti.

La popolazione microbica dominante è apparsa costituita da batteri lattici il cui numero, già consistente al tempo 0, tendeva ad aumentare durante la conservazione in tutte le condizioni adottate. I confezionamenti corrispondenti alle miscele MAPA e MAPB, entrambi privi di anidride carbonica, hanno consentito un signficativo rallentamento dello sviluppo dei Gram negativi, ritenuti i principali responsabili dei fenomeni alterativi. Sono stati sufficienti invece minime concentrazioni di ossigeno perché la loro crescita procedesse rapidamente, soprattutto quella delle forme psicrotrofe.

Dai risultati è emerso anche che in assenza di ossigeno la carne manteneva più a lungo il caratteristico coloro rosso, abitualmente associato dal consumatore alla freschezza. In particolare nella condizione MAPA che prevedeva il 100% di CO_2 la shelf-life si è protratta per 9 giorni. La riduzione del contenuto di CO_2 ha provocato invece, una accelerazione dei processi degradativi: in MAPB e C, è stato infatti rilevato un rapido sviluppo di sostanze maleodoranti e la comparsa di fenomeni di imbrunimento già dopo 6 e 2 giorni di conservazione rispettivamente, che rendevano la carne non più idonea al consumo.

INTRODUCTION

Consumption of horsemeat has increased recently and is being introduced more often into the diet. The principal reasons can be found in its intrinsic properties: horsemeat in fact, has a high nutritional content, and is characterized by absence of cholesterol and by lower percentage of fat than beef. Another reason is the liberalization of its retail which now is no longer reserved to specialized shops.

The principal problems associated with storage of this product, are determined: by its high glycogen content compared to other meats (2% vs 1%) that favours microbial growth and by the difficulties to preserve the characteristic bright red colour.

Modified atmosphere associated with low temperature is now the best packaging technique, because increases shelf-life through a selective control of

Table 1 - Gas mixture used.

	% gas		
	O_2	CO_2	N_2
MAP A *	-	100	-
MAP B*	-	65	35
MAP C	2	98	-

*: oxygen adsorbitor.

microbial population and preserves the red colour traditionally associated with the fresh product.

In this work the evolution of micro-organisms as an indicator of quality during the storage of horse meat packed in different MAP, was studied with the aim of determining the most suitable one.

MATERIALS AND METHODS

Samples

Slices of horse meat placed on rigid trays and packaged under modified atmospheres were used for the experiments. Three different gas mixture were used (Table 1):

The meat was analysed as soon as arrived to laboratory (t=0) and at established times during the storage at 4°C for 20 days. The following microbial analyses were performed:

- Mesophilic aerobic bacterial count or Total Bacterial Count (TBC) by pour plates in Agar Plate Count incubation at 30°C for 48 h;
- Gram negative bacteria by spread plates in Plate Count Monesin KCl Agar (PMK) aerobic incubation at 30°C for 48 h for mesophilic forms and at 10°C for 10 days for psychrotrophic forms;
- Total and faecal coliforms in Violet Red Bile Agar on twin-layer plates (VRB) incubation at 37°C and 44°C, respectively, for 24 h;

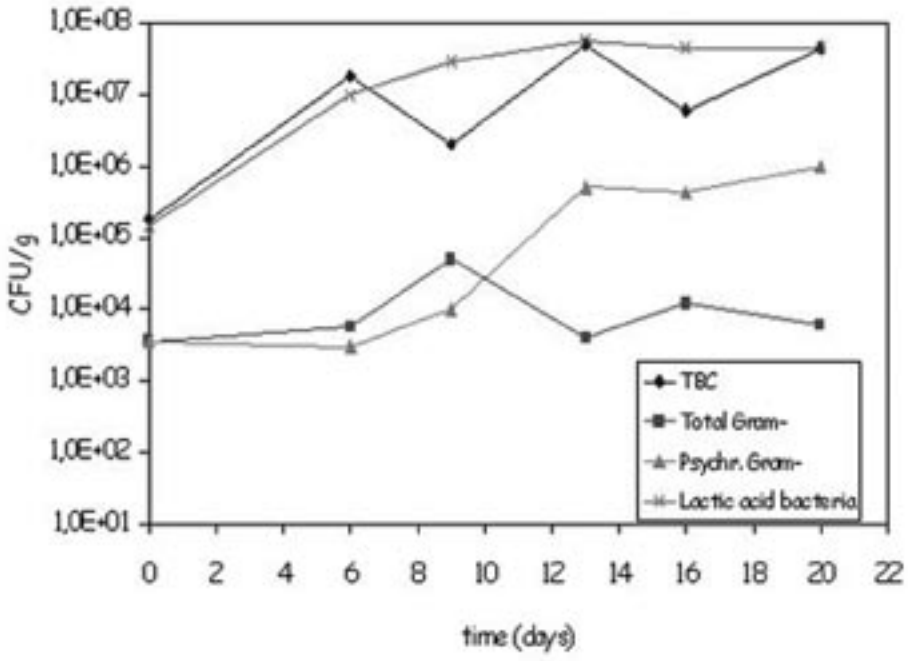


Fig. 1 - Microbial evaluation observed in horsemeat in MAP A and stored at 4°C.

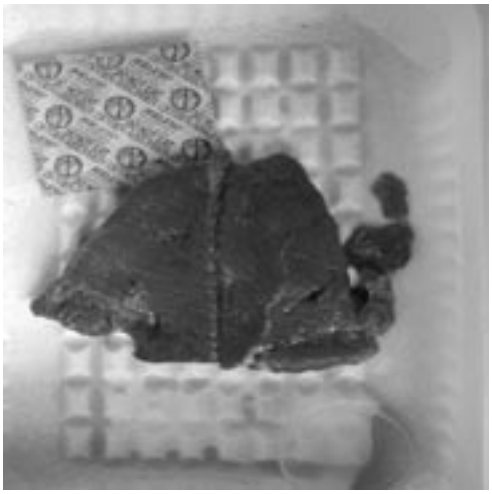


Fig. 2 - Horsemeat stored at 4°C for 9 days.

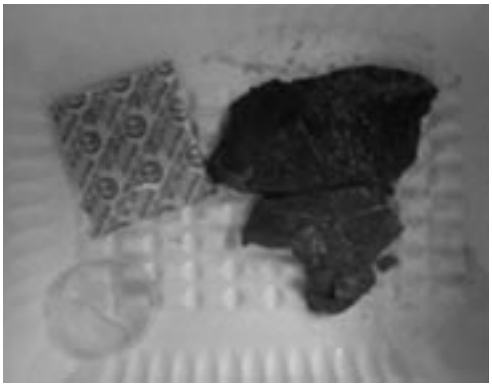


Fig. 3 - Horsemeat stored at 4°C for 13 days.



Fig. 4 - Horsemeat stored at 4°C for 20 days.

- Lactic acid bacteria by pour plates in Man Rogosa Sharpe (MRS) incubation under anaerobic conditions in gas pack jar at 30°C for 48-72 h;

- Spores of *Bacillaceae*, after pasteurisation at 80°C for 10 min, by pour plates in Tryptic Soy Agar, incubation at 37°C for 48–72 h for aerobic and anaerobic facultative species and in Renforced Clostridial Medium (RCM) incubation at 37°C for 48-72 h in anaerobic conditions for strictly anaerobic ones.

- The safety indices: *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes* and spores of *Clostridium perfringens.*

RESULTS AND DISCUSSION

Microrganisms indices of safety and faecal coliforms were always absent.

MAP A

Lactic acid bacteria are the dominant microbial population because their values are often coincident with TBC. A significatively increase the during first 9 days of storage, was observed, after they stabilize on 10^7-10^8 cfu/g of product.

Psychrotrophic Gram negative, the principal responsible for spoilage processes in meat products were much lower; their number at the end of storage was inferior of 10^6 cfu/g of product, considered the limit value for a meat of good quality (Fig. 1).

Under these conditions of packaging and of storage (4°C) the meat preserved its characteristic red colour and texture until 9 days (Fig. 2). Then phenomena of browning along the border that extended during storage, were observed and at 20 days, the meat was already dark, dry and released serum (Fig. 3 and 4).

MAP B

The microbial development in MAP B (65% CO_2 and 35% N_2) is not as different as MAPA: a rapid growth of lactic acid

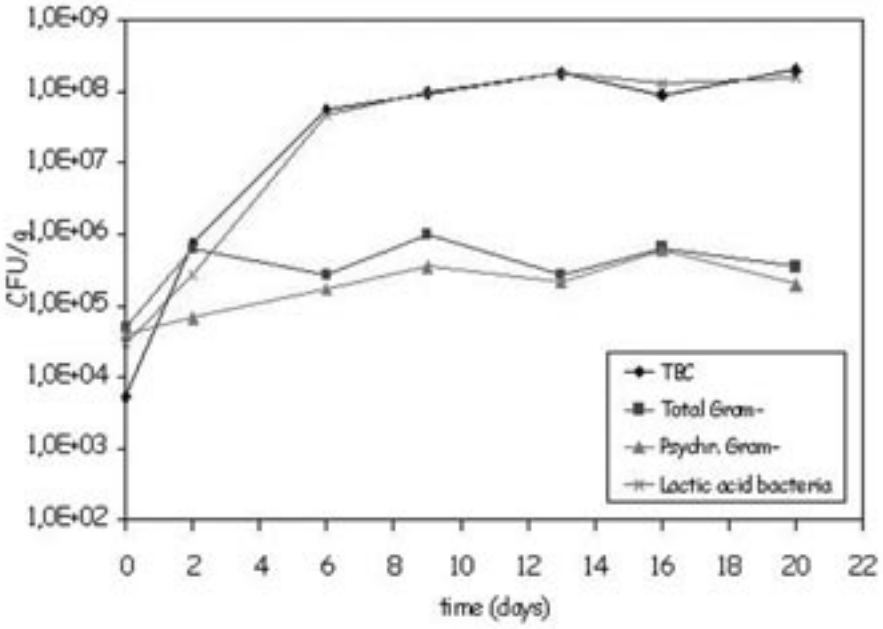


Fig. 5 - Microbial evaluation observed in horsemeat in MAP B and stored at 4°C.

bacteria was observed, while Gram negative values were comparable with those at t=0 (Fig. 5).

However the spoilage processes affecting colour and texture accelerated: after 6 days at 4°C, the meat still had an acceptable colour (Fig. 6), but an abundant release of serum was evident. At 9 days the slices showed wide dark zones and unpleasant smell (Fig. 7).

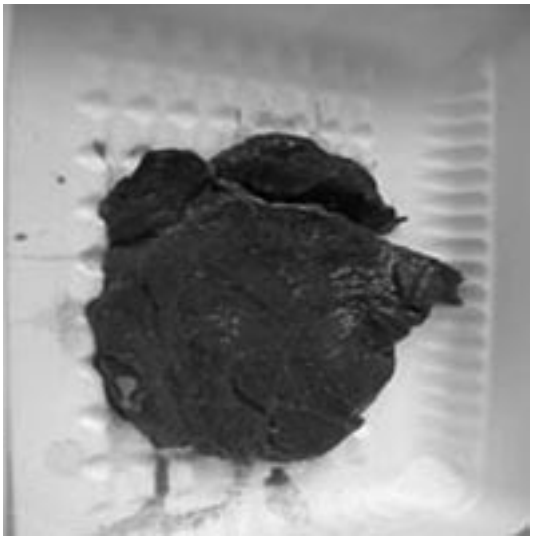


Fig. 6 - Horsemeat stored at 4°C for 6 days.

MAP C

The different gas mixture did not seem to influence the growth of lactic acid bacteria that showed an evolution similar to MAP A and MAP B.

Although the low percentage of O_2, in this condition favours Gram negative bacteria especially psychrotrophic, that at the end of storage the same values of lactic acid bacteria were reached (Fig. 8).

In the presence of oxygen an acceleration of the oxidation processes was also observed: after 6 days the meat was not suitable to use, it was completely black, without texture and had a bad smell similar to acid and fructate (Fig. 9).

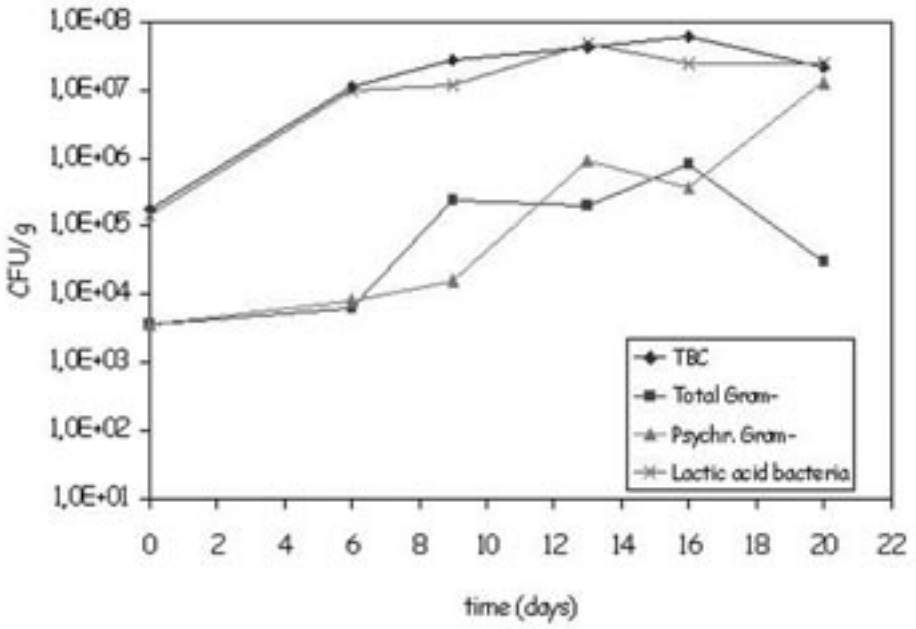


Fig. 8 - Microbial evaluation observed in horsemeat in MAP C and stored at 4°C.



Fig. 7 - Horsemeat stored at 4°C for 9 days

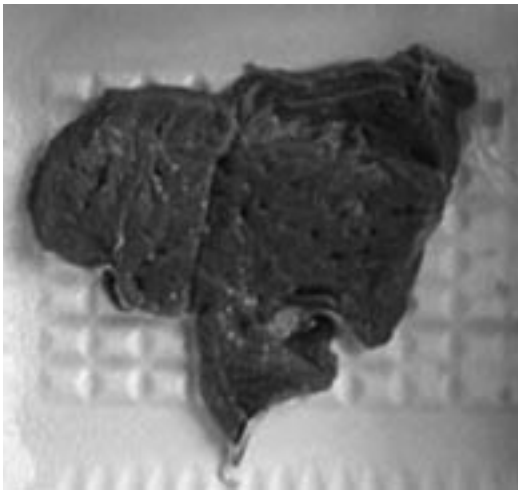


Fig. 9 - Horsemeat stored at 4°C for 6 days.

CONCLUSIONS

The results of the analysis on horse meat, packed in modified atmosphere and maintained at 4°C, show that only in presence of 100% CO_2 it is possible to prolong the shelf-life until 9 days.

This condition encourages the development of acidifying bacteria that through their metabolic activity create an unfavourable environment for the growth of gram negative, less acid-resistant bacteria, responsible for spoilage. The presence of low percent of oxygen is not suitable for the preservation of red colour because it favours a rapid decolouration, the formation of metamioglobin, and the development of aerobic bacteria that accelerates spoilage processes and reduces shelf-life. The packaging in modified atmosphere cannot prescind from microbial quality of meat and the maintenance of cold chain during the retail, that for a product so difficult to store are essential elements to assure the quality.

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