ISSN 1120-1770

ITALIAN JOURNAL OF FOOD SCIENCE

Rivista italiana di scienza degli alimenti

IJFS

Volume XXIII
Number 3
2011

CHIRIOTTI EDITORI

ITALIAN JOURNAL OF FOOD SCIENCE
(RIVISTA ITALIANA DI SCIENZA DEGLI ALIMENTI) 2nd series

Founded By Paolo Fantozzi under the aeges of the University of Perugia
Official Journal of the Italian Society of Food Science and Technology
Società Italiana di Scienze e Tecnologie Alimentari (S.I.S.T.Al)
Initially supported in part by the Italian Research Council (CNR) - Rome - Italy
Recognised as a "Journal of High Cultural Level"
by the Ministry of Cultural Heritage - Rome - Italy

Editor-in-Chief:
Paolo Fantozzi
Dipartimento di Scienze Economico-Estimative e degli Alimenti, Università di Perugia, S. Costanzo, I-06126 Perugia, Italy - Tel. +39 075 5857910 - Telefax +39 075 5857939-5857943 - e-mail: paolofan@unipg.it

Co-Editors:
Bruno Zanoni - Università degli Studi di Firenze, e-mail: bruno.zanoni@unifi.it
Carlo Pompei - Università degli Studi di Milano, e-mail: carlo.pompei@unimi.it
Lanfranco Conte - Università degli Studi di Udine, e-mail: lanfranco.conte@uniud.it
Lina Chianese - Università degli Studi di Napoli Federico II, e-mail: chianese@unina.it
Marco Gobbetti - SIMTREA - Università degli Studi di Bari, e-mail: gobbetti@agr.uniba.it

Publisher:
Alberto Chiriotti
Chiriotti Editori srl, Viale Rimembranza 60, I-10064 Pinerolo, Italy - Tel. +39 0121 393127 - Fax +39 0121 794480
e-mail: info@chiriottieditori.it - URL: www.chiriottieditori.it

Aim: The Italian Journal of Food Science is an international journal publishing original, basic and applied papers, reviews, short communications, surveys and opinions on food science and technology with specific reference to the Mediterranean Region. Its expanded scope includes food production, food engineering, food management, food quality, shelf-life, consumer acceptance of foodstuffs. food safety and nutrition, and environmental aspects of food processing.
Reviews and surveys on specific topics relevant to the advance of the Mediterranean food industry are particularly welcome.
Upon request and free of charge, announcements of congresses, presentations of research institutes, books and proceedings may also be published in a special "News" section.

Review Policy:
The Co-Editors with the Editor-in-Chief will select submitted manuscripts in relationship to their innovative and original content. Referees will be selected from the Advisory Board and/or qualified Italian or foreign scientists. Acceptance of a paper rests with the referees.

Frequency: Quarterly - One volume in four issues. Guide for Authors is published in each number and annual indices are published in number 4 of each volume.

Impact Factor: 5-Year Impact Factor: 0.596 published in 2009 Journal of Citation Reports, Institute for Scientific Information; Index Copernicus Journal Master List 2009 (ICV): 13.19
IJFS is abstracted/indexed in: Chemical Abstracts Service (USA); Foods Adlibra Publ. (USA); Gialine - Ensia (F); Institut Information Sci. Acad. Sciences (Russia); Institute for Scientific Information; CurrentContents®/AB&ES; SciSearch® (USA-GB); Int. Food Information Service - IFIS (D); Int. Food Information Service - IFIS (UK); EBSCO Publishing; Index Copernicus Journal Master List (PL).
IJFS has a page charge of € 25.00 each page.

Subscription Rate: IJFS is available on-line in PDF format only.
2011: Volume XXIII: Ordinary € 50.00 - Supporting € 1,000.00

ITALIAN JOURNAL OF FOOD SCIENCE

ADVISORY BOARD

SCIENTISTS

R. Amarowicz
Editor-in-Chief
Polish J. Food and Nutrition Sci.
Olsztyn, Poland

A. Bertrand
Institut d'Oenologie
Université de Bordeaux
Talence Cedex, France

L.B. Bullerman
Dept. of Food Science and Technology
University of Nebraska-Lincoln
Lincoln, NE, USA

F. Devlieghere
Dept. Food Technology
and Nutrition Faculty of Agricultural
and Applied Biological Sciences Gent University
Gent, Belgium

S. Garattini
Ist. di Ricerche
Farmacologiche "Mario Negri"
Milano, Italy

J.W. King
Dept. Chemical Engineering University
of Arkansas Fayetteville,
AR, USA

T.P. Labuza
Dept. of Food and Nutritional Sciences
University of Minnesota
St. Paul, MN, USA

A. Leclerc
Institut Pasteur
Paris, France

C. Lee
Dept. of Food Science
and Technology Cornell University,
Geneva, NY, USA

G. Mazza
Agriculture and Agri-Food Canada
Pacific Agri-Food Research Centre
Summerland, BC, Canada

J. O'Brien
School of Biological Sciences University
of Surrey Guildford,
Surrey, UK

J. Piggott
Dept. of Bioscience and Biotechnology
University of Strathclyde
Glasgow, Scotland

J. Samelis
Dairy Research Institute
National Agricultural Research Foundation
Ioannina, Greece

M. Suman
Food Research
Lab Barilla C.R. F.lli spa
Parma, Italy

M. Tsimidou
School of Chemistry,
Artisotle University
Thessaloniki, Greece

Prof. Emeritus J.R. Whitaker
Dept. of Food Science and Technology
University of California
Davis, CA, USA

REPRESENTATIVES of CONTRIBUTORS

R. Coppola
Dipartimento di Scienze
e Tecnologie Agroalimentari e Microbiologiche
(DI.S.T.A.A.M.), Università del Molise,
Campobasso, Italy

M. Fontana
Soremartec Italia, Ferrero Group
Alba, Italy

V. Gerbi
Dipartimento di Valorizzazione
e Protezione delle Risorse Agroforestali (DI.VA.P.R.A.)
Sezione Microbiologia ed Industrie Agrarie,
Università di Torino, Torino, Italy

M. Moresi
Dipartimento di Scienze
e Tecnologie Agroalimentari (D.I.S.T..A.)
Facoltà di Agraria, Università
degli Studi della Tuscia, Viterbo, Italy

S. Porretta
Associazione Italiana
di Tecnologie Alimentari (AITA)
Milano, Italy

M. Rossi
Dipartimento di Scienze e Tecnologie Alimentari e
Microbiologiche (DI.S.T.A.M.)
Università di Milano, Milano, Italy

A. Sensidoni
Dipartimento di Scienze degli Alimenti
Università di Udine, Udine, Italy

OPINION PAPER

TECHNOLOGICAL PARAMETERS AFFECTING AGING AND REFINING OF BALSAMIC VINEGAR FROM MODENA IN WOOD RECEPTACLES RELATED TO THE NEW EC REGULATION N. 583/2009

A. DEL SIGNORE
Dipartimento di Scienze, Università "G. d'Annunzio", Viale Pindaro 42, 65127 Pescara, Italy
Corresponding author: Tel. +39 085 4537505, Fax +39 085 4549755
email: signore@sci.unich.it

ABSTRACT

The characteristics of vinegar refined in wood receptacles are due to: the period of refining, the volume of the receptacles, the type of wood, the geographic origin of the wood used, the natural or thermic treatments carried out on the wood and the number of cycles for which the receptacle has been used.

The EC Regulation regarding the Protected Geographic Indication (PGI) for Balsamic Vinegar from Modena requires an aging period of not less than 60 days in wood barrels.

The time of refining, taken as a parameter in isolation from the others, has no relevance whatsoever since all parameters are so closely linked together.

- Key words: aging, characteristics of wood receptacles, Balsamic Vinegar from Modena, refining -

INTRODUCTION

European Commission Regulation N. 583 of July 3, which was published in the Official Journal of the European Union on July 4, 2009, provides for the registration of both Protected Designation of Origin (PDI) and the Protected Geographic Indication (PGI) for Balsamic Vinegar from Modena. In an annex to the regulation there is a summary of the Method of Production (Annex II).

This regulation restates the Italian Ministerial Decree of December 3, 1965 regarding the contents and the method of preparation of Balsamic Vinegar from Modena. In fact, the older Decree can be compared with the more recent European Commission Regulation as follows:

Obviously, since the Regulation recognizes a "new" product in terms of Protected Geographic Indication (PGI), unlike the 1965 Decree, it contains all of the indications needed to characterize the product geographically:

√ indication of the area of production from which the product originated (territory of the provinces of Modena and Reggio Emilia);

√ indication on the land registry map of the parcel on which the grape cultivation takes place;

√ list of producers of must;

√ indication of the types of grapes (Lambrusco, Sangiovese, Trebbiano, Albana, Ancellotta, Fortuna, and Montuni)[1];

√ the assembly of the raw materials, their processing, refining and/or aging in wooden barrels, must, by law, take place in the geographic area of origin, with the exception of the conditioning, which may take place outside of the provincial territories of Modena and Reggio Emilia[2].

Ministerial Decree 3/12/1965	**European Commission regulation N. 583/2009**
- the product is obtained by means of traditional technology, through the alcoholic and acetic fermentation of grape must, or if necessary, partial fermentation or concentration in direct contact with heat.	- the product is obtained from grape must which has been partially fermented and/or cooked and/or concentrated by not less than 20%. The concentration is taken to a density not inferior to 1.240 at 20°C.
- a percentage of vinegar aged not less than ten years is added.	- a percentage of vinegar aged not less than ten years is added.
- with or without the addition of vinegar obtained from the acetification of wine alone.	- with the addition of vinegar obtained from acetification of wine only for a minimum of 10%.
- the acetification, deacidification, aging and refinement of the product take place in barrels made of high-quality woods: sessile oak, chestnut, oak, mulberry or juniper.	- the acetification and refining of the product take place in barrels made of high-quality woods: oak, sessile oak, chestnut, mulberry or juniper, for a period of not less than 60 days.
- the addition of caramel is allowed.	- the addition of caramel is allowed up to a maximum of 2%.
ANALYTICAL AND ORGANOLEPTIC PROPERITIES:	**ANALYTICAL AND ORGANOLEPTIC PROPERITIES:**
Minimum total acidity 6%;	Minimum total acidity 6%;
Alcohol by volume not superior to 1.5%;	Alcohol by volume not superior to 1.5%;
Dry extract, less sugars, a minimum of 30 g/L;	Minimum dry extract 30 g/L;
	Density at 20° C not inferior to 1.06 in the refined product;
	Total sulphur dioxide: maximum 100 mg/L;
	Ashes: not less than 2.5 for a thousand;
	Reducing sugars: minimum 110 g/L;
Colour: dark brown;	Colour: deep brown;
	Transparency: clear and bright;
Taste: sweet and acidic;	Taste: sweet and sour;
Aroma: aromatic, pleasing and distinctive.	Aroma: persistent, delicate, slightly acidic, with woody overtones.

As can be noted, the difference between the Decree and the Regulation consists essentially in the fact that, in the case of the latter, some of the characteristics are specified in quantities. For example: the amount of pure wine vinegar added must be at least 10%; the density must be at least 1.240 at 20°C; the caramel added can be no more than 2%; the refining process must last for at least 60 days.

In the Regulation under discussion, one distinctive point is that aging must take place in special wood barrels for a period of not less than 60 days, beginning from the day on which the raw materials are assembled.

Interestingly, the Regulation is silent regarding the characteristics of the wooden barrels.

Most of the scientific studies published on the aging of vinegar refer either to an aging period of 6 months or to an aging period that is extended to 2 or more years. As regards the barrels (or wooden casks) considered in these studies, research was conducted on barrels that were not very large; often reference is made to the typical size of the *barrique bordolese* (225 L, little more than 2 hL) (GARCÍA PARRILLA *et al.*, 1999). This is because aging periods of less than 6 months and the use of bigger barrels do not allow for an appreciable transformation of the product nor do they allow for the complete effect of the barrel on the contents to come about. Therefore, vinegars aged for less than 6 months, except in particular cases, are generally not the object of study since such an aging period does not produce noteworthy effects even when the barrels are very small (GIACCIO and DEL SIGNORE, 1997; DEL SIGNORE *et al.*, 2000; DEL SIGNORE, 2001a; DEL SIGNORE, 2001b).

It is difficult to distinguish with precision between ripening (short period) and aging (long period). Ripening time is not defined in a technical way; it is referred to as "the time required for the product to develop its organoleptic qualities while it is in the barrel" (GAROGLIO, 1981). Aging instead implies the progressive depletion of some of the ingredients and the substantial transformation of others and aging time is generally considered to start after two years. The process of ripening is characterized by the oxidation reaction of some of the component elements of the product and it is followed by aging which is characterized by reduction reaction.

The evolution, then, depends on the time and even more so on the capacity of the barrels, or in other words, on the relation between the internal surface of the barrels and the volume of vinegar that comes in contact with that surface. The importance of this relation has been widely demonstrated in all of the experiments carried out by the numerous researchers who have dealt with this topic. Moreover, this evolution also depends on the type of wood of which the barrel (or other containers) is made, and on the geographical area from which that wood comes since the essence and the porosity of woods vary from area to area. The treatment that the wood has received (i.e. toasting, boiling, etc.) is also a parameter that effects its properties. In order to better verify all the above mentioned effects, the barrels must be "new".

The Summary regarding "Balsamic Vinegar from Modena" (Protected Geographic indication-PGI) contained in Annex II of the European Commission Regulation n. 583/2009, published in the Official Journal of the European Union on 04.07.2009 L 175/7-11, at paragraph 4.5, deals with "refining". This term comes from the wine sector and refers to the various physical, chemical and biological phenomena that occur in wine during its period of aging, all of which contribute to improving and perfecting the wine's organoleptic properties[3]. Such extensive changes, involving physical, chemical and biological characteristics, cannot take place in a period as brief as 60 days.

In fact, the regulations prescribe 60 days of refining in wood receptacles, but no mention whatsoever is made of the size of the receptacles, which is an essential factor. They do, on the other hand, cite examples of "high-quality wood": "oak, in particular sessile oak, chestnut, mulberry and juniper" but neither cherry (used in the aging of Traditional Balsamic Vinegar from Modena) nor acacia (which is a high quality wood) are mentioned. No consideration is given to the fact that the chemical composition of these woods, even when they belong to the same species (for example, American oak or French oak) can vary greatly. The chemical composition of wood undergoes extensive changes as a result of the heat treatments to which the wood is subjected. Another essential criterion, which is not considered in the Regulation, is the number of times the receptacle has been used, in other words, whether it is old or new. After the second, or at the most third reuse, the wood receptacle is greatly impoverished and no longer has an active effect.

The same paragraph of Annex II refers inappropriately to "aging" in wooden receptacles when, in fact, the process in question is "refining". Moreover, the Inspection Procedure provided for by the Ministry of Agriculture, Food Products and Forests gives a definition for aged "Balsamic Vinegar from Modena PGI", which stipulates that "the finished product is aged for a period of no less than three years in casks, barrels or other wooden receptacles [...]".

In the present work, all of the elements which may exert an influence on the properties of vinegar as a result of time and type of receptacle will be considered. Reference will also be made to products that are stored in glass receptacles.

An analogy with wine, if we may be permitted, would be useful here. Wine stored in metal receptacles has a brighter colour because it does not come into contact with the air, making oxidation very slow (the oxidized product is a bit more "opaque"). Moreover, it has less aroma but a "softer" taste than wine stored in wood. Some experts like RIBÈREAU-GAYON (2007), argue that, for brief periods of storage, it is better to store wine in receptacles made of inert materials like metal and glass, because these receptacles prevent the evaporation and oxidation of some components since there is no contact with the air, and avoid the development of the woody aroma and taste that comes from the disintegration of wood that occurs during even brief periods of storage in new wooden barrels.

PARAMETERS CONSIDERED

In order to bring about the chemical reaction of oxidation (initial phase of ripening) and then that of reduction (which characterizes the aging phase) periods of time of at least 6 months are necessary. When the residence time of vinegar in the receptacles is as brief as 2 months the results are limited to a simple extraction of substances from the wood as a consequence of the contact of the product with the inside of the barrel. Only the substances extracted from the wood have an influence on the properties of the vinegar in this case. It is also essential here to specify that the aromatic substances from the wood will only be transferred to the vinegar if the wood is new: when the wooden receptacles have been used a number of times this parameter of influence is no longer applicable. Interestingly, the Regulation is silent in this regard.

One of the most evident phenomena that occurs when there is contact with wood is the extraction of phenolic substances; this is an indicator which has universally been considered by all of the Authors who have conducted research on the aging of vinegar in general, and on the aging of Balsamic Vinegar from Modena in particular (GARCÍA PARRILA, 1999; GARCÍA PARRILA *et al.*, 1997; KADIM and MANNHAIM, 1999; TESFAYE, 2002; CEREZO *et al.*, 2008; CEREZO *et al.*, 2010). A fraction of the phenols present in vinegar comes from grapes and wine, but those which come from contact with wood are easily distinguishable (GARCÍA PARRILA *et al.*, 1997).

In addition to the complex phenols that come from lignin (polyphenols) there are many other simple phenols that can be extracted from wood, for example, gallic acid, chlorogenic acid and vanillin, syringic acid (and the corresponding aldehyde) and ferulic acid. These simple phenols have been used with success in demonstrating the variations of the product during storage in wood receptacles (GÁLVEZ *et al.*, 1995).

Another indicator which was taken into consideration is the group of furanic substances, in particular two aldehydes: 5-hydroxymethylfurfural (5-HMF) and furanic aldehyde (furfural).

The presence of furanic aldehyde in products that have been aged (wine and vinegar) is in function of the thermolysis of the polysaccharides which are formed in the wood during the toasting of the staves. This treatment brings about a partial reduction of the cellulose which leads to the formation of 5-HMF. The furanic aldehydes (or furfural) originate instead from the pentose of the hemicellulose of wood. Another source of furanic aldehyde is the addition of caramel.

Therefore, in balsamic vinegar the presence of 5-HMF and of furfural, together with consistent quantities of other furanic compounds, is high from the early stages because they are formed during the cooking of the must (as a consequence of the disintegration of the sugars) or after the cooking when the caramel is added (ANTONELLI *et al.*, 2004). It has been observed that if the ratio furfural/5-HMF is higher than one, caramel is not added. This ratio also indicates the origin of the 5-HMF: if the ratio is more than one, the presence of the 5-HMF derives from the thermolysis of hemicellulose, while, if it is less than one, it is due to the addition of the caramel (QUESADA GRANADOS *et al.*, 1996).

Moreover, some of these substances continue to accumulate even during the residence in the wood receptacles as a consequence of the termination of the heat treatment (120°-160°C) of the staves (GARROTTE *et al.*, 2007). It is also to be noted that MASINO *et al.* (2005) have observed that during the aging, a part of the sugars continue to disintegrate and the formation of HMF continues, to the detriment of the sugars present in the balsamic vinegar.

THE PERIOD OF REFINING AND THE RECEPTACLES

The period of refining, the volume of the receptacles, the type and origin of the wood used to make them, the treatment undergone by the wood and the cycles of use of the receptacles cannot be treated as if they were separate and distinct since these six parameters are very closely dependent on each other.

The effect of the dimension of the barrel on the period of refining

It was Singleton (SINGLETON, 1974) who first reported the importance of the ratio surface/volume.

A high level of concentration of phenolic substances can be noted after the first days; from the second week the level diminishes rapidly (KADIM and MANNHAIM, 1999): the concentration of phenols was determined by collecting the product daily at different distances from the barrel wall: at 0.5, 10 and 15 cm, in order to observe the diffusion. After one or two weeks the concentration of the phenols near the barrel wall begins to decrease (this is because an equilibrium has been reached). If the ratio of surface to volume is increased by adding additional wooden surfaces (in the form of wood chips) to the liquid, the effect is as if the barrel had become smaller and the phenols are completely extracted in 4 to 7 days.

Analogously, even the concentration of phenols in the area furthest away from the barrel walls begins to grow, but at a slower speed. In any case, after two or three weeks (depending, obviously, on the size of the receptacle) the differences have disappeared and the concentration becomes uniform everywhere in the barrel. This process can be observed in the figure published in the work of KADIM and MANNHAIM

(1999). Under the experimental conditions reported by the Authors, the surface of the wood was already depleted after a few days; the curve of the component extraction from wood that depends on distance from barrel wall has a high rate of extraction at the outset, but that rate decreases as the distance from the wall increases; depending on the distance from the wall, the liquid and the wood establish an equilibrium with the passage of time. The concentration depends, then, on the area of the barrel from which the sample of vinegar is collected. This can be demonstrated in experiments conducted with new receptacles; if the effects due to the newness of the wood have already been depleted after only a few days, then it follows that with wood that has already been used many times, as happens in big production industries, those effects cannot be found at all.

The experiment cited above involved white wine; our laboratory data (Fig. 1) which refers to balsamic vinegar, are more marked, since the elevated content of acetic acid in vinegar (together with the low content of ethyl alcohol) in contrast with wine, makes it a more aggressive agent on wood, leading to a greater extraction of its components. The concentration of phenolic compounds was determined, in duplicates, by the Folin-Ciocalteau procedure described by OUGH and AMERINE (1988). The experiments regarding balsamic vinegar were carried out using a new 225 L barrique. It must be pointed out here that on an industrial scale vinegar products are stored in used receptacles that hold from 15,000 to 50,000 L and so the effect observed through the use of a barrique is merely indicatory.

A uniform concentration occurs in function of the size of the receptacle: it may occur after 1 to 3 weeks, or after many months. This is because the quantity of the substances extracted from the receptacle is a function of the storage time together with the ratio of surface to volume. Therefore the size of the receptacle has a very important effect on the quantity of the substances extracted from the wood. For example, a typical barrique that holds 2.25 hL has a ratio surface/volume of 90 cm^2/L. For the sake of comparison, other examples are reported as follows:

Volume of receptacle (hL)	Ratio surface/volume (cm^2/L)
2.25	90
70	30
150	23
380	16
500	15

Therefore, with respect to the parameter of surface/volume, the number of days assumes significance only if the receptacle is very small. In order to better study the importance of the effects of the ratio surface/volume, some Authors have artificially increased that ratio, as

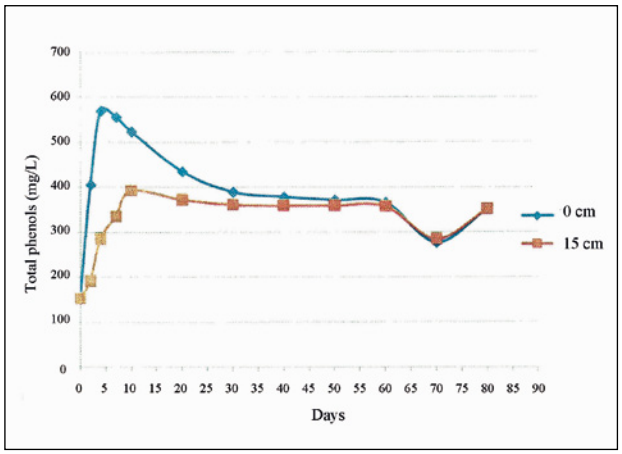


Fig. 1 - Concentration of total phenols in vinegar near the wall of a new barrique in function of days (our laboratory data).

already mentioned above, by inserting toasted wood chips into the liquid being "aged" (TESFAYE *et al.*, 2004). Using this method it is possible to reproduce in the vinegar in 15 days the same changes that are obtained with a lower ratio after some months. Data (expressed in mg/L) outlining the total quantity of phenol extracted from wood as a function of number of days was reported by TESFAYE and appears below:

American Oak	Days				
	0	15	30	60	90
Boiled and toasted	43	392	394	452	428
Toasted	43	422	423	407	409

Data elaborated by: W. Tesfaye *et al.*, "Evolution of wine vinegar composition during accelerated aging with oak chips", Analytica Chimica Acta, 513:239-245, 2004.

The proof that variations in the composition of vinegar do not depend on the number of days, but rather on the receptacle and the treatment carried out on that wood, is to be found in this work. In fact, in receptacles of wood that have only been toasted, the phenol content reaches its highest peak within 15 days and then begins to diminish steadily up to the 90th day. In contrast, in receptacles made of wood that has been boiled and toasted the highest level is reached within 15 days, but it then remains constant for a month, and then increases by 12% by the 60th day, and then begins to decline on the 90th day.

Simple phenols also show a similar trend as can be observed in Fig. 2.

The same trend can be observed in the results of a panel-test which revealed that after 15 days a marked woody aroma is perceivable; this sensory feature, as well as the other organoleptic features present, do not change significantly with time (VEI-15 = 15 days; VEI-30 = 30 days; VEI-90 = 90 days); to cite the report ver-

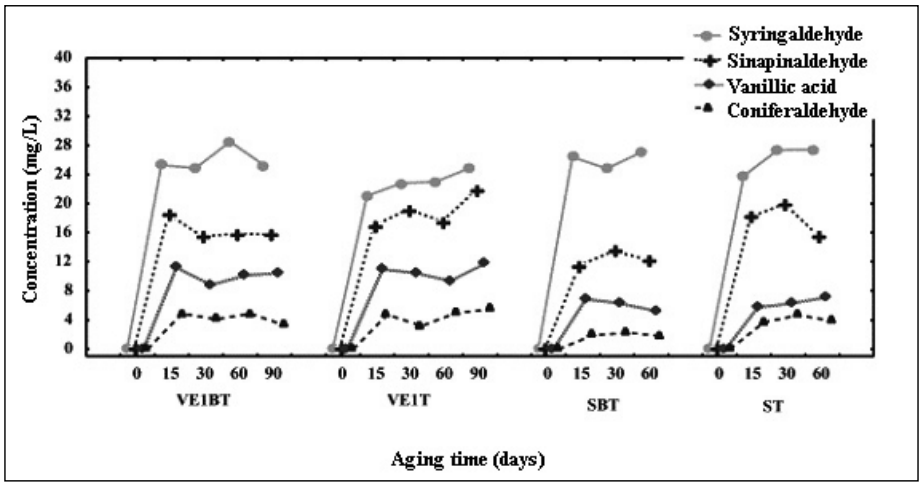


Fig. 2 - Evolution of several phenolic compounds during accelerated aging of a wine vinegar sample and a synthetic vinegar (TESFAYE *et al.*, 2004). (VE1BT = vinegar with 1% (v/v) of alcohol in oak chips boiled and toasted; VE1T = vinegar with 1% (v/v) of alcohol in oak chips toasted; SBT = synthetic vinegar with 2% (v/v) of alcohol in oak chips boiled and toasted; ST = synthetic vinegar with 2% (v/v) of alcohol in oak chips toasted). Synthetic vinegar (control) was prepared in the laboratory by dissolving different volatile and non-volatile compounds in an aqueous acetic acid solution.

batim: *"In our experiences with vinegar, at 15 days a marked woody aroma was perceived by tasters* (Fig. 3)*; this attribute and all the others do not change with time"* (TESFAYE *et al.*, 2004).

Morales *et al.* studied the volatile substances extracted from wood and observed that their quantity depended on the age of the wood (the number of cycles of use they had undergone); the quantity that can potentially be extracted is relevant when the barrels are new and, negligible when they have been used. The extraction of vanillin is most influenced by the ratio surface/volume; in fact, by increasing the contact surface, after 15 days of steeping in the wood receptacles the amount of vanillin in the vinegar reaches levels of between 12.4 and 15.4 mg/L, while vanillin content in vinegar kept in barrels that have a broader ratio, reaches levels of 1.4 to 3.4 mg/L after 90 days (MORALES *et al.*, 2004). Therefore the refining for 15 days in small, new barrels produces much better effects than a refining for 90 days in large barrels.

This demonstrates that the indication of a steeping period in wood is meaningless if the volume of the receptacle is not also specified.

In general, it has been observed that most of the compound volatiles are given off by the wood within 15 days of the vinegar's residence, and subsequently their concentration does not change until the 90th day (the time during which the experiment was conducted) (Fig. 4).

Except for 2-furfural, the equilibrium of which depends on caramel, in practice the other substances examined reach their maximum percentage after 15 days and are still at that same level after 90 days.

From all of the above cited researches, a fact clearly emerges: the indication of the capacity of a receptacle is essential in making a determination of the characteristics of the product. If the

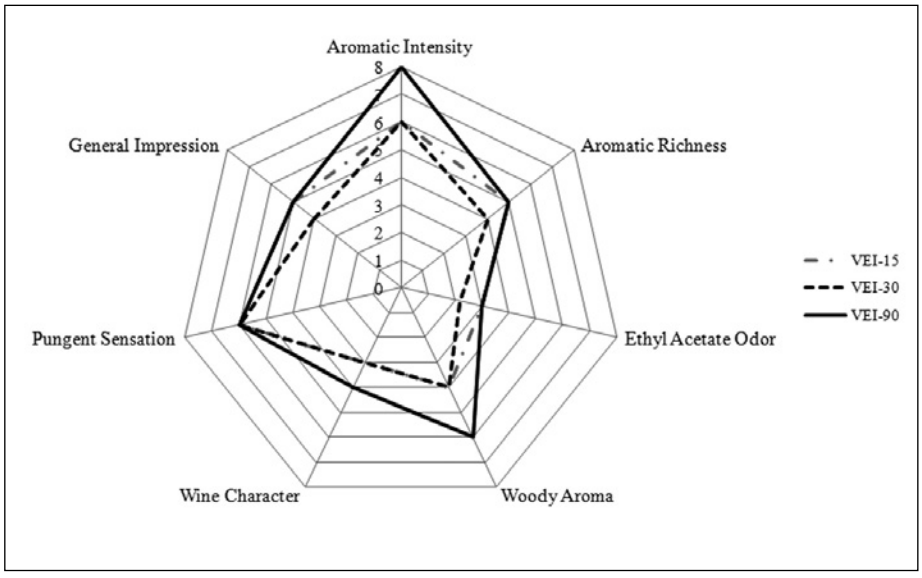


Fig. 3 - Sensory profile of vinegars with equal ethanol content (1% v/v) and different aging time (TESFAYE *et al.*, 2004).

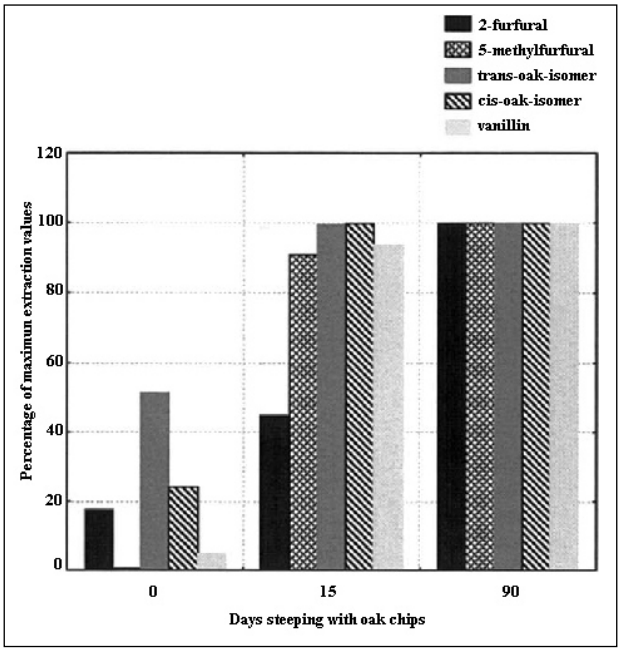


Fig. 4 - Rate of extraction of different compounds in accelerated aging. The concentrations are expressed as an average percentage. To calculate these figures to the high concentration of the compound has been assigned the value of 100%. (2-furfural = 1.46±0.02 mg/L; 5-methylfurfural = n.d.; *trans*-oak isomer = 0.102 ±0.003 mg/L; *cis*-oak isomer = 0.207±0.012 mg/L; vanillin = 0.663±0.008 mg/L).

volume of the receptacle is not indicated, the time of residence in the receptacle is irrelevant.

Most of the works which focused on the effects of the surface/volume ratio have dealt with wine rather than vinegar. These works give ample confirmation of the observations made above. Among these numerous studies, of great relevance here is that of PÉREZ-PRIETO *et al.* (2002) where it is noted that even when the volume is very small the changes in barrel size are always relevant: "The characteristics of wine matured in 220-L new barrels versus those matured in 1,000-L new barrels were very different".

A fitting conclusion to this consideration of the effects of the surface/volume ration are the words of an expert in the study of Enology, Professor Usseglio-Tomasset, who on page 305 of his treatise on Enological Chemistry said: *"the ratio between the volume of wine and the surface of contact with the wood has a great importance; the contribution of wood, perceivable in small receptacles like barrique bordolesi that hold 225 L, becomes negligible in receptacles of tens of hectoliters"* (USSEGLIO-TOMASSET, 1978).

The effects of the type of wood of which the receptacle is made on the period of refining

Most of the substances transferred to the vinegar (polyphenols, simple phenols, compound volatiles, etc.) depend on the speed of penetration of the liquid into the wood rather than on the distribution of these substances in the liquid.

The speed of penetration of the liquid into the wood varies greatly from one type of wood to another. Therefore, the level of extraction of substances depends on some characteristics of the receptacle used:

- Species and geographic origin of trees

There is a great deal of diversity among barrels owing to their various compositions, physical characteristics that depend on the species and geographical origin of the wood from which they are made. The wood most frequently used to make barrels is oak – either American oak (*Quercus alba*) or French oak (*Quercus robur* o *Q. petraea*). Their compositions have been studied in depth and it has been shown that they are very different (MILLER *et al.*, 1992; TOWEY and WHATERHOUSE, 1996a). In a study of 11 substances extracted from wood it was observed that all of the samples of American origin were distinguishable from the sample of French origin (MARCO *et al.*, 1994). In brief, the French oak gives off more phenols than the American oak; lactone compounds (which have a light aroma of coconut) are given off more by the American oak; vanillin (which has the most pronounced aroma) varies greatly from wood to wood.

Other Authors had already reported that an analysis of the contents of the group of substances called lactones is a simple way of identifying the type of wood in which the product was stored-whether American or French oak: "[...] the ratio *cis*/*trans* can be used to distinguish those wines aged in American oak barrels and those wines aged in French oak barrels" (WATERHOUSE and TOWEY, 1994). "The only generalization that can be made on this subject is that the American white oak species gives a greater quantity of *cis*-oak lactone to the wine compared to European oaks" (GARDECERDÁN and ANCÍN-AZPILICUETA, 2006).

A recent and rather exhaustive study, which compared the effects of different woods – oak, chestnut, acacia and cherry – on the composition of vinegar, was carried out by CEREZO *et al.* (2008). The quantity of phenol that the vinegar had extracted from the wood allowed for the discrimination at a 100% of the effect of the various woods studied. An acetification of 45 days led to differences in the quantity of phenol reported (mg/L) depending on the type of wood used. These differences are reported below:

Substance	Oak	Chestnut	Cherry	Acacia
Gallic acid	30	78	29	29
Vanillic acid	1.36	1.26	1.22	1.08
Syringic acid	4.95	4.4	4.5	3.0
Ellagic acid	4.44	3.86	1.54	2.8

It is to be observed that the gallic acid increases significantly when the wood is chestnut; the

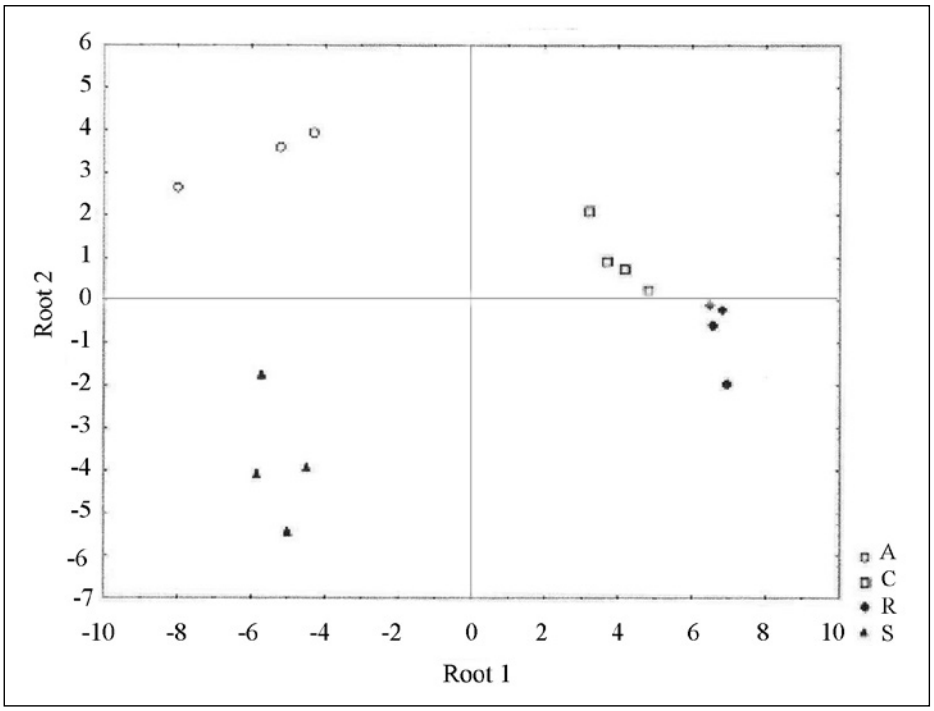


Fig. 5 - Plot of the two first roots issued from discriminant analysis for group F of samples. (A= acacia; C= cherry; R= oak; S = chestnut) (CEREZO *et al.*, 2010).

cherry and acacia woods release less substance.

In the current year two studies have been published, one regarding the differences between the woods of the American oak and the French oak (CALLEJÓN *et al.*, 2010), the other regarding the differences between the woods of the acacia, cherry, chestnut and oak (CEREZO *et al.*, 2010).

The first work clearly evidences the differences between wood that comes from the American oak and that which comes from the French oak: the porosity and the chemical composition are quantitatively different and therefore influence the final aroma of the vinegar. The porosity influences the formation of esters; greater porosity favours the evaporation of water and therefore the concentration of the substances, including the aromatic ones. To better explain this effect, a comparison is made between the composition of vinegar stored in wood and that stored in glass bottles. In the glass after a year the volatile compounds are higher than those present in the vinegar stored in wooden barrels. In the barrels there is a higher level of acetic acid and the levels of alcohols and aldehydes are significantly higher. In the glass bottles this phenomenon also takes place but with lesser intensity. The acetaldehyde is higher in the barrels because of the oxidation of alcohol and the same is true for the diacetyl that is formed from the oxidation of the acetoin. The limited contact with air that occurs in the bottle reduces the oxygen available to the microorganisms and their activity, therefore, decreases.

The second work deals with a balsamic vinegar made from Trebbiano; it demonstrates the differences between the woods based on their content of total and simple phenols. The analyses were carried out after 45 days, after 6 months and after a year. The phenol contents, proposed as wood markers (gallic acid, gallic ethyl ester, taxifolin, ellagic, vanillic and caffeic acid), made it possible to discriminate between the woods, with a significant classification at 100%, as is shown in the Fig. 5. Liquid chromatography with photodiode array detection (LC-DAD) was used for the analysis of phenolic compounds, whereas LC-MS/MS for taxifolin.

The 5-HMF does not undergo variations in the first 45 days in the barrels of cherry and acacia, but does show a modest increase of 10% in the barrels of oak and chestnut, as is shown below. For two of the woods studied, a contact time of six months has no influence, and for the other two woods, it has only a slight influence:

HMF content (mg/L) in Balsamic vinegar made with Trebbiano in receptacles of various woods and having various periods of ripening

Days	Oak	Chestnut	Cherry	Acacia
0	237	220	216	231
45	255	241	212	231
180	286	290	254	285
365	382	407	356	371

Data reported in A.B. Cerezo *et al.*, "Effect of wood on the phenolic profile and sensory properties of wine vinegars during ageing", Journal of Food Composition and Analysis, 23:175-184, 2010.

The effect of the wood on the sensorial properties of the vinegars is strong enough to be perceived by the tasters on the test panel; a sweet, woody aroma with tones of vanilla is perceived in vinegars aged in oak, while in those aged in

cherry, the aroma is that of mixed berries. The results of Cerezo's study show that the wood used in the aging of the vinegar determines both its chemical composition and its sensory properties, but these parameters are not perceivable after only brief periods of time of residency in the wood or in receptacles that are consumed after use in many processing cycles.

- Treatments undergone by the wood

These treatments include: the method used for producing the staves (ARTAJONA SERRANO, 1991), the maturation (SEFTON *et al.*, 1993) the drying, whether natural (VIVAS and GLORIES, 1993) or artificial, as for example, thermic treatment (boiling or toasting of the staves) (DEVES, 1994; FRANCIS *et al.*, 1992; ROUS and ALDERSON, 1983).

The thermic treatment of the staves provokes a disintegration of the wood's components (lignin, cellulose, hemicellulose) which generates both simple phenolic compounds and volatile compounds. Moreover, depending on the type of treatment (more or less intense) substances are formed that are later available for release in the vinegar. Thus the level of differentiation between French oak and American is, firstly, correlated to the degree of toasting and, secondly, greatly influenced by some substances (the group of lactones and eugenol) that permit a differentiation of 100% between French oak and American oak (BOZALONGO *et al.*, 2007; SARNI *et al.*, 1990).

The untoasted wood contains very small quantities of volatile phenol. Following thermic treatment – which may vary greatly in intensity, with temperatures that range from 180° to 230°C, and heating times that range from 5 to 15 minutes – the lignin generates large quantities of volatile phenol (particularly guaiacol and eugenol) which have a great influence on the organoleptic quality of the finished product. Aldehydes (among which vanillin is to be found) are also formed, and ketones, products of disintegration of fats and tannins (CHATONNET *et al.*, 1989). Even barrels that have the same origin, the same producer and that have undergone the same thermic treatment, can have varying effects on the aging of the product (TOWEY and WATERHOUSE, 1996b).

In a recent study 19 different aromatic substances were extracted from wood coming from oaks of American, French, Hungarian and Russian origin (DIAZ-MAROTO *et al.*, 2008) that had undergone varying treatments. The results are very interesting: the quantity of vanillin extracted from wood ranges from 0.03 mg/kg in untoasted Russian oak up to 3.81 mg/kg in toasted Hungarian oak (an increase of over two orders of magnitude); the furfural aldehyde ranges from 18.7 mg/kg in the untoasted French oak to 234.2 mg/kg in toasted Hungarian oak (more than one order of magnitude). Generally speaking, for all of the 19 compounds extracted, the differences in substances extracted between the untoasted and toasted woods was almost always more than an order of magnitude.

Therefore the yield of aromatic substances in toasted wood, after a week, is equivalent to the yield of the same substances for more than 10 weeks in untoasted wood.

- Number of use cycles and the amount of product that "passes through" the receptacle

Another relevant parameter of aging is the number of times, or the length of the periods in which the receptacles are used. Wood losses its capacity to yield substances in function of the number of times the receptacle is refilled, and the total quantity of product which has remained in contact with the receptacle. The number of times the barrels are used is another factor that determines the oak wood depletion. Due to the fact that the pool of the oak extractives in a barrel is finite, the quality of those compounds and their rate of extraction generally diminishes with the utilization of the barrel over successive years (TOWEY and WATERHOUSE, 1996a, GARDE-CEDÁN and ANCÍN-AZPILICUETA, 2006). Although several factors, such as vinegar composition, barrel maintenance techniques, or barrel fermentation can affect the extraction process, the age of barrels is of primary importance for the quality of vinegar because the pool of extractable compounds in wood is finite, the rate of extraction and the amounts of compounds extracted diminished as the barrel is used in successive years (TOWEY and WATERHOUSE, 1996b).

The yield of substances from wood occurs principally when the barrels being used are new and in their first year of use. In this regard, it is worth noting that a barrique, which has a very high surface/volume ratio, is no longer considered usable for product enhancement after only two or three reuses and is thereafter used only as a simple container. It has been estimated that the direct aromatizing action of a new barrique has already been exhausted around the eighth month of the products residence (MOIO *et al.*, 1999a; MOIO *et al.*, 1999b). This is also confirmed by the fact that the different sizes of the receptacles has less and less effect on the product when the receptacles are used over and over again PÉREZ-PRIETO *et al.*, 2002).

The Regulation is silent on the number of cycles for which a wood receptacle may be used, even though indicating only the time of residence (regardless of whether the wood is exhausted or not) is meaningless. When the wood of a receptacle has become exhausted, the receptacle is comparable to any other container of inert material, whether of wood or some other material (glass, ceramic, etc.).

CONCLUSIONS

The period of refining, the volume of the receptacles, the type of wood, the geographic origin of the wood used, the natural or thermic treatments carried out on the wood and the number of cycles for which the receptacle has been used are all parameters that are closely linked together and they cannot be dealt with separately.

In fact, it has been demonstrated that a refining for 15 days in small barrels produces effects that are greatly superior to those of a refining for 90 days in large barrels.

In addition, it has been shown that the amount of aromatic substances yielded from a toasted wood after only one week is equivalent to the amount of the same substances yielded after more than 10 weeks when the wood is not toasted.

The test panel found that after 15 days a marked woody aroma was perceivable. This sensorial feature, similarly to the other features, did not change significantly with the passage of time (15, 30, and 90 days).

Practically speaking, if the receptacle is small the effect is depleted in a short time (1 or 2 weeks), if it is large (70 hL or more) the effect can last for months. For small barrels a short period is sufficient, for large barrels it takes a much longer period (months and years) but, at this point the phenomena of oxidation and reduction intervene, both processes which are beyond the effects of a simple residence of two months.

Therefore, if the residence time is less than 6 months, the chemical and physical characteristics of maturation or the onset of aging are not noticeable; when the receptacle is very large not even the phenomena linked with the substances yielded by the wood are noticeable.

In addition, the yield of substances in new receptacles is over, at the latest, in a year and when the wood of the receptacle is exhausted, it is comparable to any other receptacle made of inert material (glass, fiberglass, ceramic, etc).

Since the Regulation is silent on the size of the receptacle, on the geographic origin of the wood, on the various methods of treatment which the wood may undergo and on the number of cycles for which a receptacle can be used, then it stands to reason that the time of refining, as a parameter in isolation from the others, can have no relevance whatsoever.

In conclusion, a citation from a recent review of research into the factor of quality as a function of refining in oak barrels seems appropriate: "Finally, after having looked at the studies in this area, we would conclude that it does not seem very logical to establish an ageing period in barrels through legislation, as happens in some of the traditional wine-producing countries" (GARDE-CERDÁN and ANCÍN-AZPILICUETA, 2006).

NOTES

[1] Particular types of grapes are chosen in order to obtain the analytic and organoleptic qualities listed at point 4.2 in Attachment II of the Regulation. It is, however, obvious that those same qualities can be obtained with any type of grape through the use of modern technological treatments.

[2] It should be noted here that the for a product to have Protected Designation of Origin (PDO) status all phases of its production must take place within the same geographic area (as happens, for example, with Traditional Balsamic Vinegar from Modena) while to have Protected Geographic Indication (PGI), it is necessary that at least one of the phases (i.e. production, and/or transformation, and/or processing) takes place in the geographic area itself. Among the many transformations that take place in the production of Balsamic Vinegar from Modena, conditioning is a secondary one. Indeed, it could be said that if it were to be required that even conditioning take place in the geographic zone of origin the Balsamic Vinegar from Modena would be, for all intents and purposes, a PDO, and not a PGI.

[3] Often this term is applied to the period during which the wine is conserved in glass bottles which follows its aging period in wooden casks.

REFERENCES

Antonelli A., Chinnici F. and Masini F. 2004. Heat-induced chemical modification of grape must as related to its concentration during the production of traditional balsamic vinegar: a preliminary approach. Food Chem. 88: 63.

Artajona Serrano J. 1991. Caracterizaciòn del roble segùn su origen Y grado de tostado mediante GC Y HPLC. Vitic. Enol. Profes. 14: 62

Bozalongo R., Carrillo J.D., Torroba M.A.F. and Tena M.T. 2007. Analysis of French and American oak chips with different toasting degrees by headspace solid-phase microextraction-gas chromatography-mass spectrometry. J. Chromatogr. A 1173(1-2): 10.

Callejòn R.M., Torija M.J., Mas A., Morales M.L. and Troncoso A.M. 2010. Changes of volatile compounds in wine vinegars during their elaboration in barrels made from different woods. Food Chem. 120: 561.

Cerezo A.B., Tesfaye W., Soria-Díaz M.E., Torija M.J., Mateo E., García-Parilla M.C. and Troncoso A.M. 2010. Effect of wood on the phenolic profile and sensory properties of wine vinegars during ageing. Journal of Food Composition and Analysis 23: 175.

Cerezo A.B., Tesfaye W., Torija M.J., Mateo E., García-Parilla M.C. and Troncoso A.M. 2008. The phenolic composition of red wine vinegar produced in barrels made from different woods. Food Chem. 109: 606.

Chatonnet P., Boidron J.N. and Pons M. 1989. Incidence du traitement thermique du bois de chêne sur sa composition chimique. 2e partie: Évolution de certains composes en fonction de l'intensité de brûlage. Conn. Vigne Vin. 23(4): 223.

Del Signore A. 2001a. Chemometric Analysis and Volatile Compounds of Traditional Balsamic Vinegars from Modena", JFOE 50 (2): 77.

Del Signore A. 2001b. Chemometric Analysis and Chemical and Physical Parameters of Traditional Balsamic Vinegars from Modena. J. Commodity Sci. 40 (I): 33.

Del Signore A., Stancher B. and Calabrese M. 2000. Characterisation of Balsamic Vinegars by Amino Acid Content Using a Multivariate Statistical Approach. Ital. J. Food Sci. 12 (3): 317.

Deves M. 1994. Demystifying oak. Wine Ind. Journal 9(3): 175.

Diaz-Maroto M.C., Guchu E., Castro-Vásquez L., de Torres C. and Pérez-Coello M.S. 2008. Aroma-active compounds of American, French, Hungarian and Russian oak woods, studied by GC-MS and GC-O. Flavor Fragr. J. 23(2): 93.

Francis I., Sefton M.A. and Williams P.J. 1992. A study by sensory descriptive analysis of the effects of oak origin, seasoning and heating on the aromas of oak model wine extracts. Am. J. Enol. Vitic. 43: 23.

Gàlvez M.C., Barroso C.G. and Pérez-Bustamante J.A. 1995. Influence of the origin of wine vinegar in their low molecular weight phenolic content. Acta Horticulturae 388: 269.

García Parrilla M.C., González G.A., Heredia F.J. and Troncoso A.M. 1997. Differentiation of Wine Vinegar Based on Phenolic Composition. J. Agric. Food Chem. 45: 3487.

García Parrilla M.C., Heredia F.J. and Troncoso A.M. 1999. Sherry wine vinegars: phenolic composition changes during aging. Food Research International 32: 433.

Garde-Cerdán T. and Ancín-Azpilicueta C. 2006. Review of quality factors on wine ageing in oak barrels, Trends on Food Science & Technology 17: 438.

Garoglio P.G. 1981. "La Nuova Enologia" – Enciclopedia Vitivinicola Mondiale, Ed. AEB, Brescia.

Garrotte G., Kabel M.A., Schols H.A., Falqué E., Domínguez H. and Parajó J.C. 2007. Effect of eucalyptus globules wood autohydrolysis conditions on the reaction products. J. Agric. Food Chem. 55: 9006.

Giaccio M. and Del Signore A. 1997. Il contenuto di 5-acetossimetil-2-furaldeide nell'aceto balsamico tradizionale di Modena e di Reggio Emilia. Riv. Merceol. 36 (II): 91.

Kadim D. and Mannhaim C.H. 1999. Kinetic of Phenolic Extraction during Aging of Model wine solution. Am. J. Enol. Vitic. 50(1): 33.

Marco J., Artajona J., Larrechi M.S. and Rius F.X. 1994. Relationship between geographical origin and chemical composition of wood for oak barrels. Am. J. Enol. Vitic. 45(2): 192.

Masino F., Chinnici F., Franchini G.C., Ulrici A. and Antonelli A. 2005. A study of relationships among acidity in sets of casks for Aceto Balsamico Tradizionale of Reggio Emilia by multivariate techniques. Food Chem. 92: 673.

Miller D.P., Howell G.S., Michaelis C.S. and Dickmann D.I. 1992. The content of phenolic acid and aldehyde flavor components of white oak as affected by site and species. Am. J. Enol. Vitic. 43: 333.

Moio L., Del Prete G., Diana M. and Valentino A. 1999a. Il legno di rovere impiegato nella fabbricazione delle barriques. Influenza dell'origine geografica e l'intensità della tostatura sull'aroma del vino rosso. Industrie delle Bevande 158: 615.

Moio L., Del Prete G., Diana M. and Valentino A. 1999b. Influence of origin and heat treatment of wood for oak barrels (*Quercus robur* L.) on red wine aroma. Industrie delle Bevande 159: 13.

Morales M.L., Benítez B. and Troncoso A.M. 2004. Accelerating aging of wine vinegar with oak chips: evaluation of wood flavour compounds. Food Chem. 88: 305.

Ough C.S. and Amerine M.A. 1988. "Methods for Analysis of Must and Wines", 2nd Ed., The Wiley-Interscience Pub. New York.

Pérez-Prieto L.J., López-Roca J.M., Martínez-Cutillas A., Pardo Mínguez F. and Gómez-Plaza E. 2002. Maturing Wines in Oak Barrels. Effects of Origin, Volume, and Age of the Barrel on the Wine Volatile Composition. J. Agric. Food Chem 50: 3272.

Quesada Granados J., Villalón Mir M., López Gª Serrana H. and López Martinez M.C. 1996. The influence of added caramel on furanic aldehyde content of matured brandies. Food Chem. 56: 415.

Ribéreau-Gayon J. 2007. "Trattato di Enologia", Edagricole, Bologna.

Rous C. and Alderson B. 1983. Phenol extraction curves for white wine aged in French and American oak barrels. Am. J. Enol. Vitic. 34: 211.

Sarni F., Moutounet M., Puech J.L. and Rabier P. 1990. Effect of heat treatment of oak wood extractable compounds. Holzforschung 44: 461.

Sefton M.A., Francis I.L., Pocock K.F. and Williams P.J. 1993. The influence of natural seasoning on the concentrations of eugenol, vanillin, and cis- and trans-β-methyl-γ- octalactone extracted from French and American oakwood. Sciences des Aliments 13: 629.

Singleton V.L. 1974. Some aspects of the wooden container as a factor in wine maturation. Advanced Chemical Series 137: 254.

Tesfaye W. 2002. Evolution of Phenolic compounds during an Experimental Aging in Wood of Sherry Vinegar. J. Agric. Food Chem. 50: 7053.

Tesfaye W., Morales M.L., Benítez B., García-Parilla M.C. and Troncoso A.M. 2004. Evolution of wine vinegar composition during accelerated aging with oak chips. Analytica Chimica Acta 513: 239.

Towey J.P. and Waterhouse A.L. 1996a. Barrel-to-barrel variation of volatile oak extractives in barrel-fermented Chardonnay. Am. J. Enol. Vitic. 47: 17.

Towey J.P. and Waterhouse A.L. 1996b. The extraction of volatile compounds from French and American oak barrels in Chardonnay during three successive vintages. Am. J. Enol. Vitic. 47: 163.

Usseglio-Tomasset L. 1978. "Chimica Enologica", Ed. AEB, Brescia.

Vivas N. and Glories Y. 1993. Sistema de secado de madera de roble para tonelería. Vitivinicultura 5-6: 47.

Waterhouse A.L. and Towey J.P. 1994. Oak lactone isomer ratio distinguishes between wine fermented in American and French oak barrels. J. Agric. Food Chem. 42: 1971.

Wilker K.L. and Gallander J.F. 1989. Comparison of Seyval blanc wines aged with air- and klin-dried American white oak. Am. J. Enol. Vitic. 40: 224.

Paper received October 22, 2010 Accepted January 5, 2011

PAPER

DO MONOACYLGLYCEROLS ACT AS PRO-OXIDANTS IN PURIFIED SOYBEAN OIL? EVIDENCE OF A DOSE-DEPENDENT EFFECT

F. CAPONIO, V.M. PARADISO, G. BRUNO, C. SUMMO, A. PASQUALONE and T. GOMES*
Department of Biology and Chemistry of Agro-Forestry and Environment (DIBCA),
University of Bari, Via Amendola 165/a, 70126 Bari, Italy
*Corresponding author: tommaso.gomes@agr.uniba.it

ABSTRACT

An investigation was carried out on the effect of adding increasing amounts of monoacylglycerols (MAG) – obtained from a purified soybean oil (PSO) – on the oxidation of the same PSO. PSO and the MAG/PSO solutions were subjected, during accelerated oxidation at 60°C, to routine analyses and High-Performance Size-Exclusion Chromatography of polar compounds. The addition of low amounts of MAG (5-10 g kg^{-1}) caused an increase of oxidation. Adding higher amounts of MAG, the pro-oxidant effect progressively decreased. The solution containing 30 mg kg^{-1} showed the lowest oxidative levels. Therefore, the effect of MAG in PSO oxidation is not univocal, being dose-dependent.

- Key words: accelerated oxidation test, HPSEC analysis, monoacylglycerols, oxidative stability, polar compounds, pro-oxidant effect -

INTRODUCTION

One of the most important factors for evaluating the quality of edible oils is the oxidative stability. In fact, oxidation results in rancidity and deterioration of the nutritional quality, colour, flavour and safety of edible oils (ANTOLOVICH *et al.*, 2002).

It has been proved that oxidation of edible oils takes place through a chain reaction that is essentially based on a radical mechanism involving the initiation, propagation and termination steps. Oxidation normally proceeds slowly at the initial stage and undergoes a sudden rise in its rate at the later stages (VELASCO and DOBARGANES, 2002).

Oil stability is related to its resistance to autoxidation during processing and storage, which in turn depends on the presence and amount of various minor compounds (CAPONIO *et al.*, 1999; RIETJENS *et al.*, 2007; ALBERTI *et al.*, 2009).

Several investigations were carried out on the role of the triacylglycerol hydrolysis compounds in the evolution of oxidation in edible oils. In particular, it has been shown that FFA accelerates the oxidation rate of PSO at certain concentrations (MIYASHITA and TAKAGI, 1986; MISTRY and MIN, 1987). PARADISO *et al.* (2010) investigated the effect exerted by FFA added to purified olive oil submitted to an accelerated oxidation test. The authors observed that FFA had a pro-oxidant activity on unmodified triacylglycerols at lower levels, while a catalytic effect on the decomposition of peroxides at higher levels. FREGA *et al.* (1999) studied the effect of oleic acid in virgin olive oil. The results showed a pro-oxidant action of oleic acid in the filtered oil and an opposite behaviour in the unfiltered oil. The filtered oil is less stable than cloudy oil containing suspended and dispersed materials. Apparently, due to their composition, these suspended materials play a stabilising role by acting as antioxidants and/or as a buffer against increasing acidity (LERCKER *et al.*, 1994).

Some studies regarding the pro-oxidant effect of MAG and DAG have been carried out. In particular, MISTRY and MIN (1987) showed that Sn-α-monolinolein acted as a pro-oxidant when added to soybean oil at 5 and 10 g kg^{-1}. The same authors (1988) studied the effect of 2.5 and 5.0 g kg^{-1} of monostearin, distearin, monolinolein and dilinolein on the oxidative stability of PSO by measuring headspace oxygen in the bottles. As the level of MAG and DAG increased from 2.5 to 5.0 g kg^{-1}, the headspace oxygen consumption of PSO increased significantly during the storage at 55°C compared to that of the control sample. Thus, all these MAG and DAG acted as pro-oxidants in PSO. COLAKOGLU (2006) studied the oxidation kinetics of soybean oil in the presence of 10 g kg^{-1} monolein at 55°C, by determining oxygen consumption and peroxide value, and showed that the addition of monolein to soybean oil accelerated the rate of lipid oxidation during storage.

All these studies regarding the pro-oxidant effect of MAG and DAG have been carried out using the MAG and DAG commercial standards added to purified/refined soybean oils by measuring the headspace oxygen consumption and/or hydroperoxide formation during the accelerated oxidation conditions. On the other hand, HPSEC of PC, when applied during the accelerated oxidation conditions, allows one to investigate the oxidative processes by directly monitoring the variations in the amounts of the oxidized triacylglycerols (ox-TAG, including the primary oxidation products) and of the triacylglycerol oligopolymers (TAGP, secondary oxidation products). It could provide more information than indirect, non-selective methods, such as oxygen consumption.

The aim of the present paper, therefore, was to study the effect of adding increasing amounts of MAG – obtained in purity (> 98%) via partial saponification of PSO – on the oxidative stability of the same PSO by performing the accelerated oxidation tests at 60°C in a forced-air oven and evaluating the results by both routine and the HPSEC analyses of PC.

MATERIALS AND METHODS

Preparation of purified oil

Soybean oil was purified using the method described by LEE and MIN (1990) with some slight modifications. A 50 cm × 4 cm column was packed with 75 g of silica gel with 70-230 mesh ASTM (Merck Darmstadt, Germany); 12.6 g charcoal:celite (2:1); 37.5 g sucrose powder:celite (2:1); and 75 g of silica gel. A total of 250 g of oil was subjected to column chromatography. A flow-rate of 0.20 mL min^{-1} was applied to the outlet of the column to obtain a slight depression with a vacuum pump. The resulting oil constituted PSO.

Preparation of purified MAG

Purified MAG were obtained via partial saponification of an aliquot of PSO. In particular, 10 g of PSO were added into 100 mL of a 2N NaOH solution in methanol. After 3 min, 200 mL of distilled water and 200 mL of diethyl ether were added. After mixing, the aqueous phase was removed while the ether phase was repeatedly washed with distilled water (80 mL each time), until the pH of discarded water was neutral. Then, after filtration on anhydrous sodium sulphate, the ether phase was removed in a rotary evaporator. The adopted saponification method led to the formation of pure MAG (> 98%), as confirmed by the HPSEC analysis (data not shown), while FFA were discarded as soap with the aqueous

phase. The obtained MAG were added into PSO to obtain MAG/PSO solutions at the following proportions: 5 g kg^{-1} (MAG5); 10 g kg^{-1} (MAG10); 20 g kg^{-1} (MAG20); 30 g kg^{-1} (MAG30).

Accelerated oxidation test

Five grams of PSO and of each MAG/PSO solution were weighed in 50 mL open glass vials and kept for 18 days at 60°C. Five vials were prepared for PSO and for each MAG/PSO solution. At 4, 6, 9, 14 and 18 days, a vial for PSO and each solution was taken from the oven and its content was subjected to routine and HPSEC analyses. The accelerated oxidation test was carried out twice.

Routine analyses

All the samples (unheated PSO, as well as from the PSO and each MAG/PSO solution after accelerated oxidation test) were submitted to the routine analyses as described by the International Organization for Standardization (ISO, 2007). In particular, FFA content was determined according to the ISO 660 method, peroxide value (PV) following ISO 3960 method, and UV absorption at 232 and 270 nm (conjugated dienes and trienes respectively) as described by the ISO 3656 method (ISO, 2007). The fatty acid composition of PSO and purified MAG was determined by gas-chromatographic analysis of fatty acid methyl esters, prepared according to official methods (Official Journal of the European Communities, 2002), utilizing a gas chromatograph (Fisons HRGC mega 2 series, Milan, Italy) equipped with an FID detector and an SP 2340 fused silica capillary column (0.25 mm i.d. × 60 m length and 0.20 μm film thickness, Supelco, Bellefonte, PA, USA). The temperatures of the split injector, detector and oven were 210°, 220° and 200°C, respectively. The splitting ratio was 1:100. The identification of each fatty acid was carried out by comparing the retention time with that of the corresponding standard methyl ester purchased from Sigma-Aldrich (St. Louis, MO, USA). Several analyses carried out on the same sample produced a repeatability coefficient with a percent coefficient variation (CV%) of about 5% for each determination.

Polar compounds separation and HPSEC analysis

All the samples (unheated PSO, as well as from the PSO and each MAG/PSO solution after accelerated oxidation test) were submitted to silica gel chromatography, according to the AOAC method no. 982.27 (AOAC, 2003), to separate PC. Then, the PC were analysed by HPSEC to determine the amount of TAGP (secondary oxidation products), ox-TAG (including the primary oxidation products), and DAG. The chromatographic system consisted of a Perkin-Elmer pump, (series 200, Beaconsfield, UK), a 7125S sample injector (Rheodyne, Wertheim-Mondfeld, Germany), a 50 μL injector loop and a series of three columns (0.75 cm i.d. × 30 cm in length, Perkin-Elmer Ltd., Beaconsfield, UK). The columns were packed with highly cross-linked styrene divinylbenzene copolymers with a particle diameter of 5 μm and pore diameters of 500, 500 and 100 Å, respectively. A guard column (7.5 mm i.d. × 5 cm in length, Perkin-Elmer Ltd., Beaconsfield, UK) was used. The detector was a differential refractometer (series 200A, Perkin Elmer, Beaconsfield, UK). The elution solvent used was tetrahydrofuran for HPLC at a flow rate of 1.0 mL min^{-1}. Peak identification and quantification were carried out as described elsewhere (GOMES, 1992; GOMES and CAPONIO, 1999). The precision of the HPSEC method, expressed as CV% (n = 10), was 1.9% for PC, 3.2% for TAGP, 2.3% for ox-TAG, and 2.0% for DAG.

All the determinations were carried out in duplicate.

Data analysis

Analysis of variance (ANOVA), followed by the Tukey HSD Test for multiple comparisons, was carried out on the experimental data by the XL-Stat software (Addinsoft, New York, USA).

RESULTS AND DISCUSSION

Table 1 reports the analytical characteristics of the starting PSO. It shows that this oil was almost exclusively composed of unmodified triacylglycerols and contained very low amounts of PC. The fatty acid composition of MAG (Table 2) was comparable to that of PSO used for their preparation.

Two-way ANOVA with first order interaction was performed on the analytical data obtained during the accelerated oxidation test performed at 60°C in a forced-air oven (Table 3). *Time(d), MAG(g kg^{-1}),* and the first-order interaction

Table 1 - Analytical characteristics of the purified soybean oil.

Parameters	Value
FFA (%)	0.00
PV (meq O_2 kg^{-1})	0.0
K_{232}	1.288
K_{270}	0.897
TAGP (g kg^{-1})	0.01
ox-TAG (g kg^{-1})	0.81
DAG (g kg^{-1})	0.32
PC (g kg^{-1})	1.14

FFA, free fatty acids; PV, peroxide value; TAGP, triacylglycerol oligopolymers; ox-TAG, oxidized triacylglycerol; DAG, diacylglycerols; PC, polar compounds.

Table 2 - Fatty acid composition of the purified soybean oil and of the purified monoacylglycerols.

Fatty acid (%)	Purified soybean oil	Monoacylglycerols
$C_{14:0}$	0.51	0.36
$C_{16:0}$	11.33	12.21
$C_{16:1}$	0.35	0.33
$C_{17:0}$	0.29	0.29
$C_{17:1}$	0.19	0.22
$C_{18:0}$	3.29	2.94
$C_{18:1}$	27.87	26.99
$C_{18:2}$	39.37	41.02
$C_{18:3}$	4.21	3.93
$C_{20:0}$	1.01	0.60
$C_{20:1}$	1.03	0.63

time(d)×MAG(g kg^{-1}) were the variables included in the models. The ANOVA models were significant in all cases ($p < 0.001$). The data reported in the table show that throughout the whole duration of the experiment the addition of low amounts of MAG (5 g kg^{-1}) generally determined a significant increase of the oxidation indices. A further increase, in most cases significant, was observed as the MAG reached 10 g kg^{-1}. Regarding the ox-TAG and K_{232} indices, their variations resulted significant only after 10 and 14 days, respectively. The data pointed to an evident prooxidant effect of MAG on triacylglycerols, when added to PSO in amounts up to 10 g kg^{-1}.

The MAG20 solutions showed oxidation levels

Table 3 - Mean values and results of the multiple comparisons by the Tukey HSD test.

Determinations		MAG (g kg^{-1})	Time (days)				
			4d	6d	10d	14d	18d
PV	PSO	0	63.5 n	90.4 kl	155.9 g	226.8 e	294.6 b
	MAG5	5	73.7 m	96.1 ij	158.8 g	230.7 de	264.6 c
	MAG10	10	71.4 m	98.0 i	159.5 g	234.7 d	324.1 a
	MAG20	20	56.7 o	92.6 jk	148.0 h	221.4 f	320.1 a
	MAG30	30	55.0 o	87.0 l	143.7 h	217.7 f	299.4 b
K_{232}	PSO	0	3.11 m	2.27 q	4.18 j	4.87 h	5.38 f
	MAG5	5	3.24 l	3.21 l	4.37 i	5.30 f	8.81 a
	MAG10	10	2.83 n	2.67 o	3.34 k	5.69 e	8.23 b
	MAG20	20	1.80 t	2.39 p	2.62 o	5.14 g	7.41 d
	MAG30	30	1.78 t	1.99 s	2.16 r	5.06 g	7.80 c
K_{270}	PSO	0	0.23 jk	0.30 gh	0.32 fg	0.43 d	0.68 a
	MAG5	5	0.24 ijk	0.31 fg	0.33 fg	0.55 bc	0.56 b
	MAG10	10	0.26 ij	0.30 fgh	0.34 ef	0.57 b	0.57 b
	MAG20	20	0.24 jk	0.28 hi	0.32 fg	0.45 d	0.65 a
	MAG30	30	0.23 k	0.30 gh	0.31 fg	0.37 e	0.52 c
PC	PSO	0	38.3 s	65.7 p	101.9 m	131.5 g	155.2 d
	MAG5	5	41.3 r	68.2 o	104.4 l	133.9 f	162.7 b
	MAG10	10	42.3 r	70.5 n	109.4 j	138.6 e	169.4 a
	MAG20	20	41.2 r	67.4 o	107.3 k	125.7 h	164.3 b
	MAG30	30	36.3 t	63.1 q	102.9 lm	117.0 i	157.5 c
TAGP	PSO	0	8.2 o	14.4 l	24.4 i	36.1 d	48.9 c
	MAG5	5	10.7 n	15.4 kl	25.6 h	36.1 d	51.1 b
	MAG10	10	11.1 n	16.8 j	27.2 g	37.2 d	54.3 a
	MAG20	20	10.1 n	16.1 jk	26.6 gh	32.9 e	50.6 b
	MAG30	30	6.6 p	13.1 m	24.3 i	29.6 f	48.6 c
ox-TAG	PSO	0	29.4 no	50.1 klm	76.2 j	94.0 e	104.9 c
	MAG5	5	29.8 no	51.3 kl	77.3 ij	95.3 e	109.8 b
	MAG10	10	30.2 n	51.7 k	79.9 h	99.2 d	113.0 a
	MAG20	20	29.8 no	49.9 lm	78.5 hi	90.6 f	111.3 b
	MAG30	30	28.6 o	48.7 m	77.0 ij	85.8 g	106.3 c
DAG	PSO	0	0.2 m	0.6 ijkl	0.7 ghijk	0.9 fgh	0.8 ghi
	MAG5	5	0.5 jkl	0.8 fghi	0.9 efgh	1.3 abc	0.9 defg
	MAG10	10	0.5 l	1.1 cde	1.6 a	1.5 a	1.2 bcd
	MAG20	20	0.6 ijkl	0.7 hijkl	1.4 ab	1.0 defg	1.1 cdef
	MAG30	30	0.4 lm	0.5 kl	0.9 fgh	0.9 efgh	0.8 ghij

PSO, purified soybean oil; MAG, monoacylglycerols; PV, peroxide value (meq O_2 kg^{-1}); K_{232}, specific absorption at 232 nm; K_{270}, specific absorption at 270 nm; PC, polar compounds (g kg^{-1}); TAGP, triacylglycerol oligopolymers (g kg^{-1}); ox-TAG, oxidized triacylglycerols (g kg^{-1}); DAG, diacylglycerols (g kg^{-1}). Lower case letters are used to compare the samples considering the combined effects of *MAG (%), time(d)* and *time(d)×MAG(%)* interaction: different letters mean significant difference at $p \leq 0.05$.

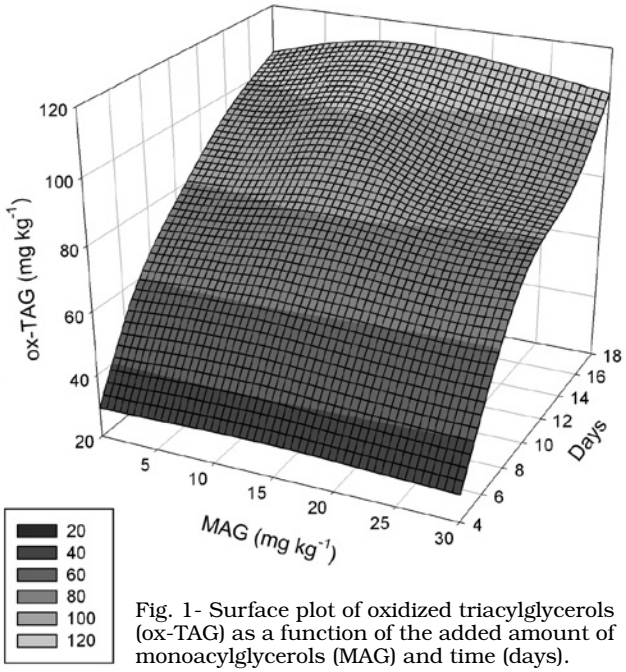


Fig. 1- Surface plot of oxidized triacylglycerols (ox-TAG) as a function of the added amount of monoacylglycerols (MAG) and time (days).

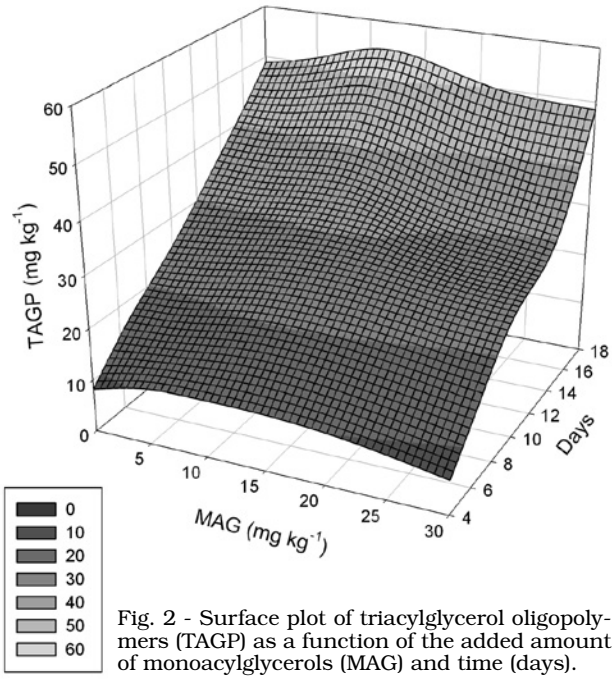


Fig. 2 - Surface plot of triacylglycerol oligopolymers (TAGP) as a function of the added amount of monoacylglycerols (MAG) and time (days).

lower than the MAG10 solutions, pointing to a dose-dependent behaviour of MAG, though the TAGP and ox-TAG indices, compared with those of PSO, showed a persistent pro-oxidative effect on triacylglycerols. The MAG30 solutions, finally, reported the lowest oxidation levels with generally significant differences with respect to PSO. The trends described for ox-TAG and TAGP can be better appreciated at a glance in the Figures 1 and 2, which graphically represent these oxidation indices as a function of both the independent variables.

This result, obtained using purified MAG having the same fatty acid composition respect to the soybean oil they were added to, confirms the findings of other authors (MISTRY and MIN, 1988; COLAKOGLU, 2006) in soybean oil with commercial standards of MAG (monostearin, monolein, monolinolein) added. On the contrary, when added in higher amounts, MAG determined lower rates of formation of ox-TAG and TAGP in soybean oil. It has been previously reported that MAG, having hydrophilic and hydrophobic groups in the same molecules, concentrate at the surface of the oil (COLAKOGLU, 2006). They decrease the surface tension of oil and increase the diffusion rate of oxygen from the headspace to the oil accelerating the oxidation processes (CHOE and MIN, 2006). This could explain the pro-oxidant effect exerted by MAG when added in lower amounts. The trend reversal observed when the amounts increased could be due to oxidation of the MAG layer at the surface, thus preventing oxygen to reach the triacylglycerols under the surface layer. This kind of barrier effect could cause a progressive decrease of the rates of oxidation involving triacylglycerols, as observed in the experimental trials.

These findings were different with respect to those of a previous study (GOMES *et al.*, 2010) carried out in purified olive oil, where MAG acted to reduce the rate of triacylglycerol oxidation, with an effect proportional to the level of MAG. Since both investigations used refined, purified oils, almost exclusively composed of unaltered triacylglycerols, free of oxidation products and anti- or pro-oxidant compounds, the differences observed should be attributed to the fatty acid composition of the oils, with special reference to the different levels of polyunsaturated fatty acids. This means that MAG could act both as pro-or anti-oxidants in purified oils according not only to the levels of addition, but also to the composition of the lipid matrix they are added to.

CONCLUSIONS

This investigation pointed out that MAG can play either a pro-oxidant role or a protective effect towards triacylglycerols when added in low or high amounts, respectively. This role is attributable to the surface layer they form in the oil, due to the presence of both hydrophilic and hydrophobic groups in their molecules. Therefore, the effect of MAG in PSO oxidation is not univocal, being dose-dependent. The comparison with previously reported results induces one to believe that the effect of MAG depends also on the fatty acid composition of the purified oils they are added to. Considering that MAG are usually used as emulsifiers, a better understanding of

the role of MAG in the oxidative processes could give useful information about the stability of the lipid fraction in foods.

ABBREVIATIONS LIST

ANOVA, Analysis of variance.

CV%, percent coefficient variation.

DAG, diacylglycerols.

FFA, free fatty acids.

HPSEC, High-Performance Size-Exclusion Chromatography.

MAG, monoacylglycerols.

ox-TAG, oxidized triacylglycerols.

PC, polar compounds.

PSO, purified soybean oil.

PV, peroxide value.

TAGP, triacylglycerol oligopolymers.

REFERENCES

Alberti A., Amorati R., Campredon M., Lucarini M., Macciantelli D. and Pedulli G.F. 2009. Antioxidant activity of some simple phenols present in olive oil. Acta Aliment. Hung. 38: 427.

Antolovich M., Prenzler P.D., Patsalides E., Mcdonald S. and Robard K. 2002. Methods for testing antioxidant activity. Analist. 127: 183.

AOAC. 2003. Official methods of analysis of Association of Official Analytical Chemists International (17th Edn.). W. Horwitz (Eds.): AOAC Press, Arlington, USA. Method no. 982.27.

Caponio F. and Alloggio V. 1999. Phenolic compounds of virgin olive oil: influence of paste preparation techniques. Food Chem. 64: 203.

Choe E. and Min D.B. 2006. Mechanism and factors for edible oil oxidation. Comp. Rev. Food Sci. Food Safety. 5: 169.

Colakoglu A.S. 2006. Oxidation kinetics of soybean oil in the presence of monoolein, stearic acid and iron. Food Chem. 101: 724.

Frega N., Mozzon M. and Lercker G. 1999. Effects of free fatty acids on oxidative stability of vegetable oil. J. Am. Oil Chem. Soc. 76: 325.

Gomes T. 1992. Oligopolymer, diglyceride and oxidized triglyceride contents as measure of olive oil quality. J. Am. Oil Chem. Soc. 69: 1219.

Gomes T. and Caponio F. 1999. Effort to improve the quantitative determination of oxidation and hydrolysis compound classes in edible vegetable oils. J. Chromatogr. A 844: 77.

Gomes T., Caponio F., Bruno G., Summo C. and Paradiso V.M. 2010. Effect of monacylglycerols on the oxidative stability of olive oil. J. Sci. Food Agric. 90: 2228.

ISO. 2007. Animal and Vegetable Fats and Oils. International Organization for Standardization, Geneva.

Lee S.H. and Min D.B. 1990. Effects, quenching mechanism and kinetics of carotenoids in chlorophyll-sensitized photooxidation of soybean oil. J. Agric. Food Chem. 38: 1630.

Lercker G., Frega N., Bocci F. and Servidio G. 1994. "Veiled" Extra-virgin olive oils: dispersion response related to oil quality. J. Am. Oil Chem. Soc. 71: 657.

Mistry B.S. and Min D.B. 1987. Effects of fatty acids on the oxidative stability of soybean oil. J. Food Sci. 52: 380.

Mistry B.S. and Min D.B. 1988. Prooxidant effects of monoglycerides and diglycerides in soybean oil. J. Food Sci. 53: 1896.

Miyashita K. and Takagi T. 1986. Study on the oxidative rate and pro-oxidant activity of free fatty acids. J. Am. Oil Chem. Soc. 63: 1380.

Official Journal of the European Communities. 2002. EC Regulation No. 796/2002. N.L 1287 of May 6th.

Paradiso V.M., Gomes T., Nasti R., Caponio F. and Summo C. 2010. Effects of free fatty acids on the oxidative processes in purified olive oil. Food Res. Int. 43: 1389.

Rietjens S.J., Bast A. and Haenen G.R.M.M. 2007. New insights into controversies on the antioxidant potential of the olive oil antioxidant hydroxytyrosol. J. Agric. Food Chem. 55: 7609.

Velasco J. and Dobarganes C. 2002. Oxidative stability of virgin olive oil. Eur. J. Lipid Sci. Technol. 104: 661.

Paper received December 23, 2010 Accepted March 18, 2011

PAPER

LC-ESI-MS/MS IDENTIFICATION OF OLEUROPEIN AS MARKER OF *OLEA EUROPAEA* L. LEAVES USED AS A BULKING AGENT IN GROUND OREGANO AND SAGE

M. BONONI and F. TATEO*
Dipartimento di Produzione Vegetale, Facoltà di Agraria, Università degli Studi di Milano,
Via Celoria 2, 20133 Milano, Italy
*Corresponding author: Tel. +39 02 50316540, Fax +39 02 50316539
email: fernando.tateo@unimi.it

ABSTRACT

The identification of the secoiridoid oleuropein by LC-ESI-MS/MS (negative) is demonstrated for the quality control directed toward confirmation of the presence of *Olea europaea* L. leaves used as bulking agent in ground oregano and sage. The described operating conditions are useful for the simple qualitative identification of this marker of *O. europaea* L. leaves. No tolerance limits are defined for the presence of the above mentioned extraneous vegetable material in ground oregano and sage, the herbs which have been identified in various cases as adulterated with *O. europaea* L. leaves. Despite the importance of quality control of aromatic herbs, only a limited number of papers have been published on the identification of phenolic compounds as markers of adulteration. The proposed method is simple and suitable for rapid routine analysis.

- Key words: aromatic herbs, adulteration, olive leaves, quality control, oleuropein, LC-ESI-MS/MS (negative) -

INTRODUCTION

While ground spices and herbs are often adulterated by the addition of bulking agents, few methods are available for screening commercial batches of some ground herbs such as oregano and sage for the presence of *O. europaea* L. leaves. In a recent paper we addressed this issue by the use of a stereo zoom microscope system to visualize the presence of the ground olive leaves (BONONI *et al.*, 2010a). However, an instrumental analytical method to obtain a specific and documentable confirmation would be useful. More recently we proposed a rapid GC/MS test based on the detection of two specific markers derived from the biophenol fraction of ground olive leaves to confirm the adulteration of ground oregano with this bulking agent (BONONI *et al.*, 2010b). In the present study, we have extended our earlier investigations adopting the phenolic compound oleuropein, a secoiridoid, as the more characteristic marker of *O. europaea* L. leaves and we have developed a method to confirm the presence of milled olive leaves in mixtures of ground oregano or sage. The secoiridoids, which have an oleosidic structure, are a specific group of coumarin-like compounds present in moderate amounts in *Oleaceae* and oleuropein in particular is an ester of 2-(3,4-dihydroxyphenyl) ethanol (known as hydroxytyrosol) and the elenolic acid glucoside (Fig. 1). In the present study, our aim is not to describe analytical parameters useful for quantitation of oleuropein, but rather to suggest and demonstrate the applicability of the simple operating conditions for the identification of this marker in the routine quality control of two very common aromatic herbs.

Oleuropein is present in the leaves of the olive tree and has been found in all constituent parts of the fruit (peel, pulp and seed) and various methods have been developed for the analysis of this compound (ANGEROSA *et al.*, 1995; JAPÓN-LUIÁN *et al.*, 2006; JAPÓN-LUIÁN and LUQUE DE CASTRO, 2008; JEMAI *et al.*, 2009; KIRITSAKIS *et al.*, 2010; LAGUERRE *et al.*, 2009; MARSILIO *et al.*, 2001; PAPOTI and TSIMIDOU, 2009; SAITTA *et al.*, 2002). The phenolic composition differs amongst different olive varieties, but oleuropein and ligstroside are the most common compounds present in the *O. europaea* L. cultivars and their concentrations are dependent on the season (BOUAZIZ and SAYADI, 2005; JEMAI *et al.*, 2009; KIRITSAKIS *et al.*, 2010; RANALLI *et al.*, 2006; RANALLI *et al.*, 2009; SOLER-RIVAS, 2000; TUCK and HAYBALL, 2002). Because of season variations in the content of these compounds and because of differences in drying conditions, their quantitative detection results would be difficult and essentially useless.

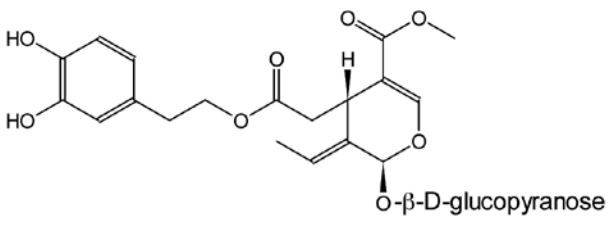


Fig. 1 - Structure of oleuropein (M.W. = 540 Da; CAS 32619-42-4), the main oleoside in Oleaceae.

Herein we show the simple identification of oleuropein by LC-ESI-MS/MS in negative ion in samples of ground oregano leaves (or in ground sage leaves) containing ground olive leaves as a contaminant. We also demonstrate the fragmentation of the parent ion of oleuropein and describe the derived ions.

MATERIALS AND METHODS

Reagents

Ethyl alcohol (99%) was purchased from Sigma-Aldrich (Milan, Italy). Filters GH Polypro (Hydrophilic Polypropylene Membrane Filter) 0.45 μm Pall were purchased from VWR (Milan, Italy). Acetonitrile, formic acid and water, all of chromatographic grade, were purchased from Merck (Darmstadt, Germany).

Samples

Authentic samples of oregano and sage were prepared from fresh plant material by drying at 40°C for ten days and crushing, following established industrial technologies. The samples were ground to a particle size diameter of 1.0 - 5.0 mm as usually found in the retail market. Other genuine samples, derived from other industrial drying procedures, have been also analyzed.

Dried leaves of *Olea europaea* L. of different origins, treated as previously described or collected in various regions of Italy and dried under both controlled and non-controlled conditions, were also studied. These leaves were ground to similar dimensions as for oregano.

Preparation of extracts

The vegetative samples (oregano or sage) subjected to the quality control analyses were treated as follows: ~ 1 g of each herb was mixed with 5 mL of ethyl alcohol at 20°-25°C. After magnetic stirring for 6 hours, the extract was centrifuged at 500 rpm for 3 min and the supernatant was filtered through a Pall 0.45 μm filter. For the LC-ESI-MS/MS (negative) analysis, an aliquot (20 μL) of the supernatant was injected in the HPLC system.

The efficiency of the extraction using ethanol/water (80/20) solutions was also evaluated, but the extraction results for oleuropein were less selective than those with pure ethanol.

HPLC/MS Equipment

The analytes were separated using a Shimadzu integrated HPLC system (Shimadzu, Milan, Italy) which consisted of a Shimadzu CBM 20 A system controller, two Shimadzu LC 20 AD XR pumps including a Shimadzu degasser DGU A3, a Shimadzu SIL 20 ACXR autosampler and a Shimadzu CTO 20 AC column oven. The analytes were detected using a triple quadrupole Applied Biosystems 2000 Mass Spectrometer with Analyst Software (Version 1.4.1) equipped with a Turbo Ion Spray Source operated in the ESI (negative ion) mode.

Chromatographic conditions

A stainless steel column Supelcosil™ 150 mm x 3 mm with 3 μm particle size and 120 Å pore diameter was used. Compounds were separated using as solvent A a mixture of water / formic acid (99.9:0.1 v/v) and as solvent B a mixture of acetonitrile / formic acid (99.9:0.1 v/v) at a flow rate of 0.4 mL/min. The elution was performed with a linear gradient from 6 to 20% B in 20 min, than isocratic 20% B for 20 min and finally returning to the initial conditions in 10 min.

MS analysis

To establish the appropriate conditions for the identification of oleuropein, an olive leaves ethanolic extract was produced. The extract was previously injected in the source of the mass spectrometer by continuous infusion at 10 μL/min.

The data acquisition was performed in the negative ion mode electrospray ionization (ESI-MS) and was achieved in Q1 mode. The same methodology was applied after fragmentation of the precursor ion (MS^2 mode). Analysis conditions to obtain the m/z 539.1 to 377.1 transition were fixed as follows: ionization voltage 4.5 kV, probe temperature 350°C, declustering potential (DP; -30 V), focusing potential (FP; -400 V), entrance potential (EP; -10V), cell entrance potential (CEP; 5 V), collision energy (CE; 16 V) and cell exit potential (CXP; 5 V). The m/z 377 ion, derived from the fragmentation of the pseudomolecular $[M-H]^-$ ion, corresponds to the oleuropein aglycone resulting from the loss of the glucose moiety.

RESULTS AND DISCUSSION

Using the analytical conditions described in Materials and Methods, various samples of oregano and sage from different origins were examined and the presence of oleuropein was not evident. On the other hand, various samples of olive leaves from Apulia and Tuscany regions of Italy were examined and in all cases oleuropein was identified as the most abundant compound.

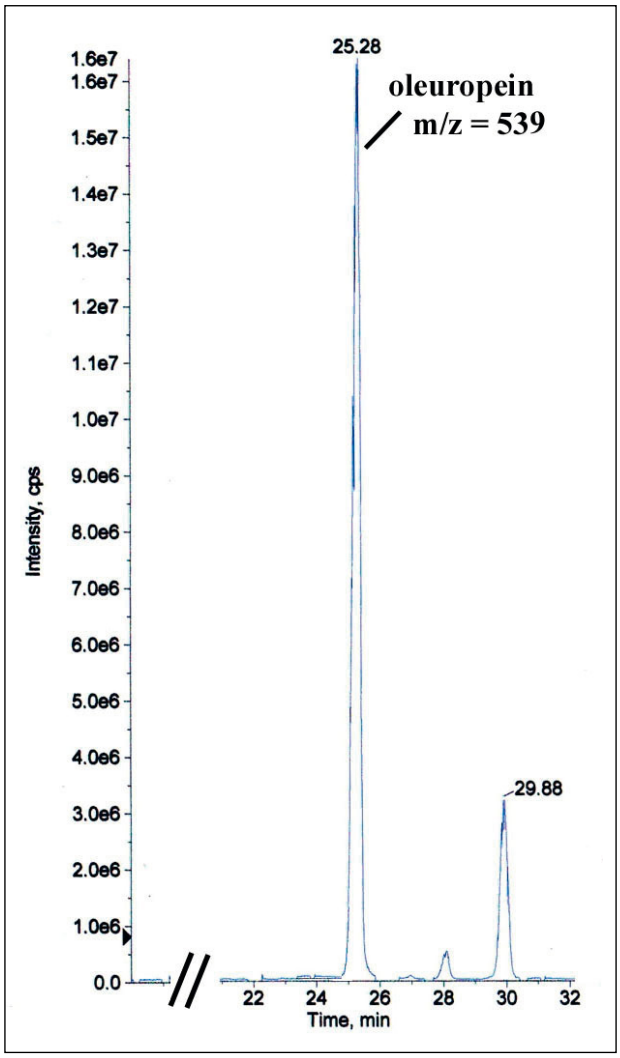


Fig. 2 - LC-ESI-MS/MS in negative ion mode chromatogram obtained from a sample of dried olive leaves ethanolic extract. The Q1 profile appearing at r.t. 25.3 min represents the precursor ion of oleuropein with m/z 539, corresponding to the pseudomolecular [M-H]- ion. The peak at r.t. 29.9 min is the isomer oleuroside, having the same molecular mass, 540 Da.

The Q1 chromatogram produced from the olive leaves ethanolic extract (Fig. 2) shows the profiles of the precursor ion (m/z = 539), which corresponds to the pseudomolecular $[M-H]^-$ ion of both oleuropein (major) and its isomer oleuroside (minor), whose molecular masses are 540 Da.

A comparison of the chromatograms of a sample of pure oregano (A) and a sample of oregano contaminated with *O. europaea* L. dried leaves (B) (Fig. 3) clearly shows that the Q1 chromatogram produced from the oregano ethanolic extract (Fig. 3A) did not contain the ion m/z = 539. The two peaks produced from oregano at r.t. < 25.0 min have m/z = 537 and their evidence is due only to the acquisition range (m/z = 539

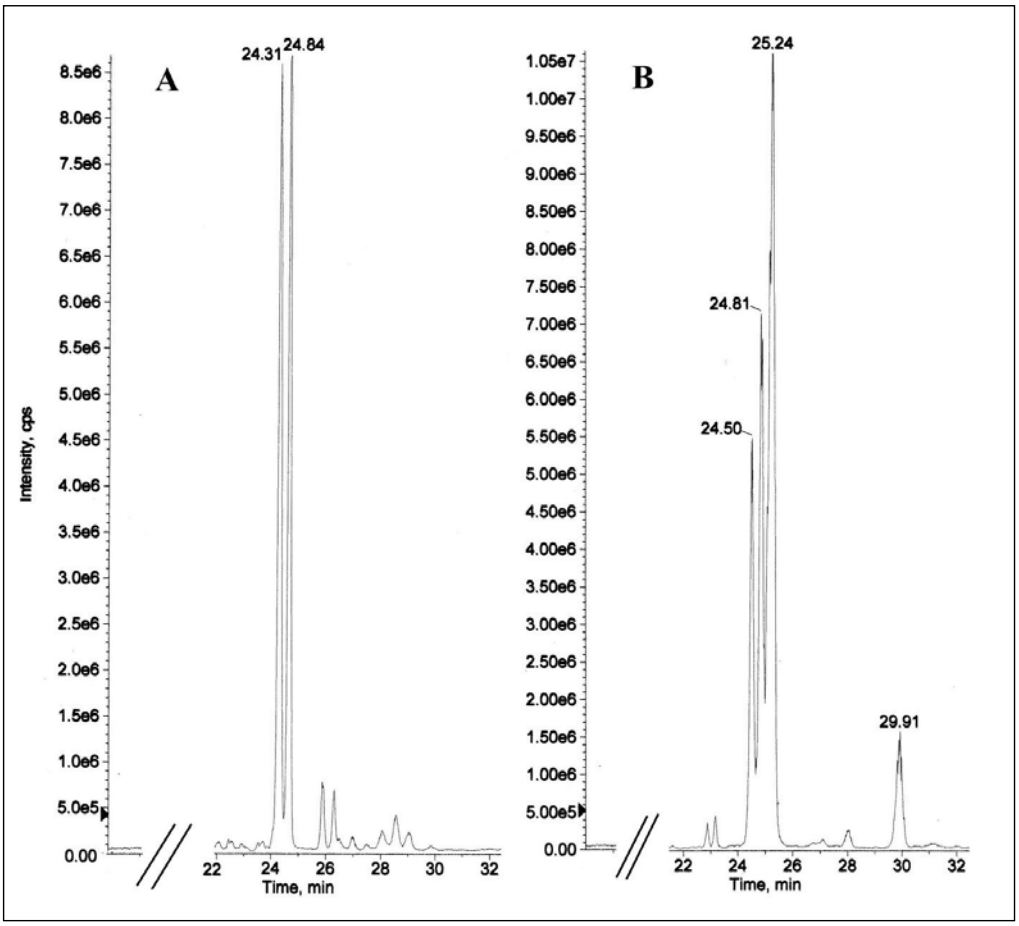


Fig. 3 - LC-ESI-MS/MS in negative ion mode chromatogram obtained from a sample of pure oregano (A) and a sample of oregano contaminated with ~ 20% of *Olea europaea* L. dried leaves (B). The peak at 25.2 min in (B) corresponds to the pseudomolecular [M-H]-ion of oleuropein (m/z = 539).

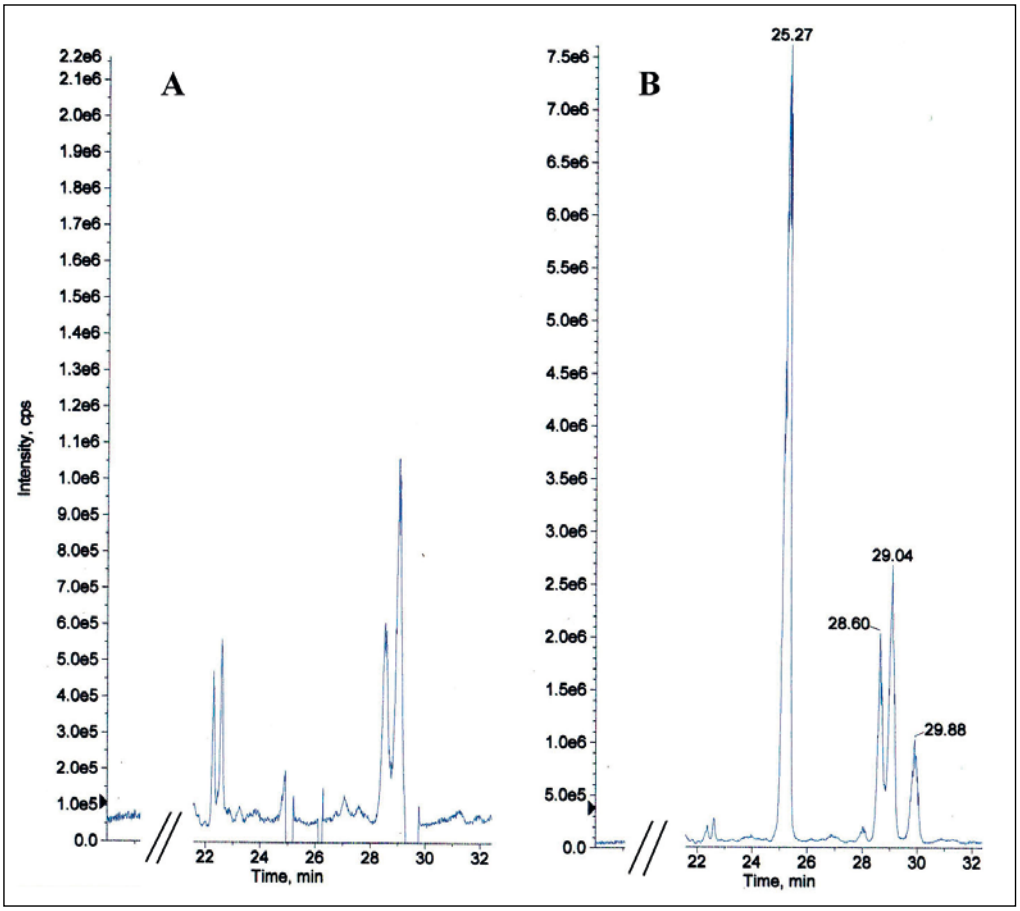


Fig. 4 - LC-ESI-MS/MS in negative ion mode chromatogram obtained from a sample of pure sage (A) and a sample of sage contaminated with ~ 10% of *Olea europaea* L. dried leaves (B). The peak at 25.3 min in (B) corresponds to the pseudomolecular [M-H]-ion of oleuropein (m/z = 539).

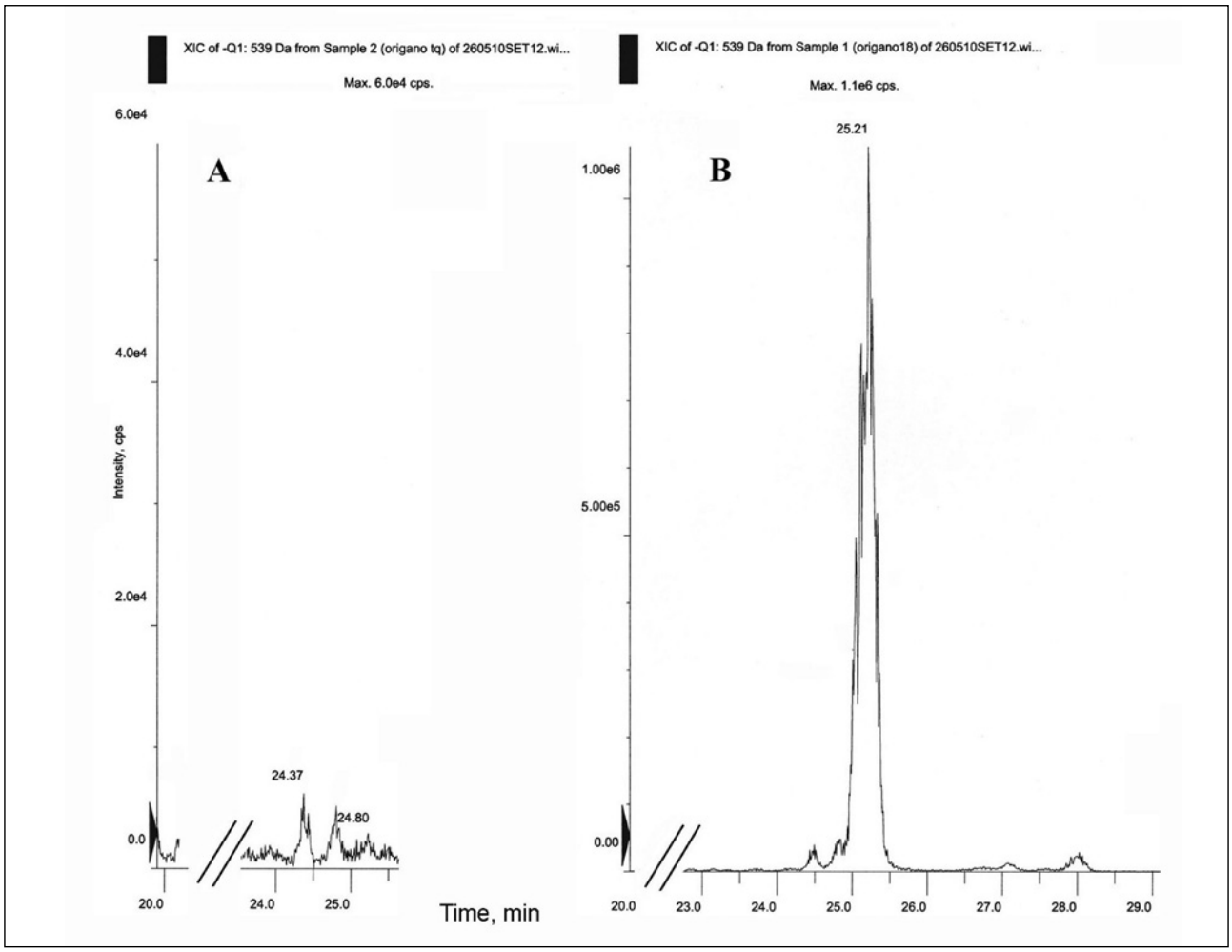


Fig. 5 - Extracted ion m/z = 539 detectable on LC-ESI-MS/MS in negative ion mode chromatogram obtained from a sample of pure oregano (A) and a sample of oregano contaminated with ~ 20% of *Olea europaea* L. dried leaves (B). The intensity values readable for pure oregano has not an analytical sense in the neighborhood of r.t. 25 min.

± 3). On the other hand, an extract of oregano containing ~ 20% of olive leaves shows a strong peak at r.t. 25.2 min having m/z = 539, i.e. the $[M-H]^-$ ion of oleuropein.

Fig. 4 compares a sample of pure sage (A) and a sample of sage contaminated with ~ 10% of olive leaves (B). The extract of contaminated sage shows, as in the case of contaminated oregano, the $[M-H]^-$ ion of oleuropein at r.t. 25.3 min.

For a better evidence Fig. 5 and 6 are reported which show, for the same samples of oregano and sage before considered, the extracted m/z = 539 ion chromatogram. The clear trace of m/z = 539 ion identified in the contaminated oregano and sage did not detectable at corresponding r.t. in the chromatograms of pure oregano and sage.

Operating in MS^2 mode the precursor ion at m/z 539 displayed the characteristic product ions at m/z 377, 307 and 275, as has been confirmed by others Authors (BAZOTI *et al.*, 2010; DEL BOCCIO *et al.*, 2003).

CONCLUSIONS

Even though the amounts of oleuropein in the collected olive leaves may vary to the age of the samples and due to genetic factors, this molecule will always be present in sufficient quantity to be identified in all dried samples derived from various origins and during various collecting periods. Therefore this secoiridoid phenolic compound may serve as a "marker" of the presence of ground and dried olive leaves in some herbs used in retail and industrial markets. In this paper, two examples (for ground oregano and sage) of the application of this LC-MS/MS method are shown for quality control. Considering that the olive leaves cannot be considered as a common extraneous vegetable material tolerable in aromatic herbs, no limits of their presence have been defined at the present time. Consequently, there is no finite quantity of olive leaves that can be tolerable in ground oregano or sage, which are the most common aromatic herbs used for food preparation. The quantita-

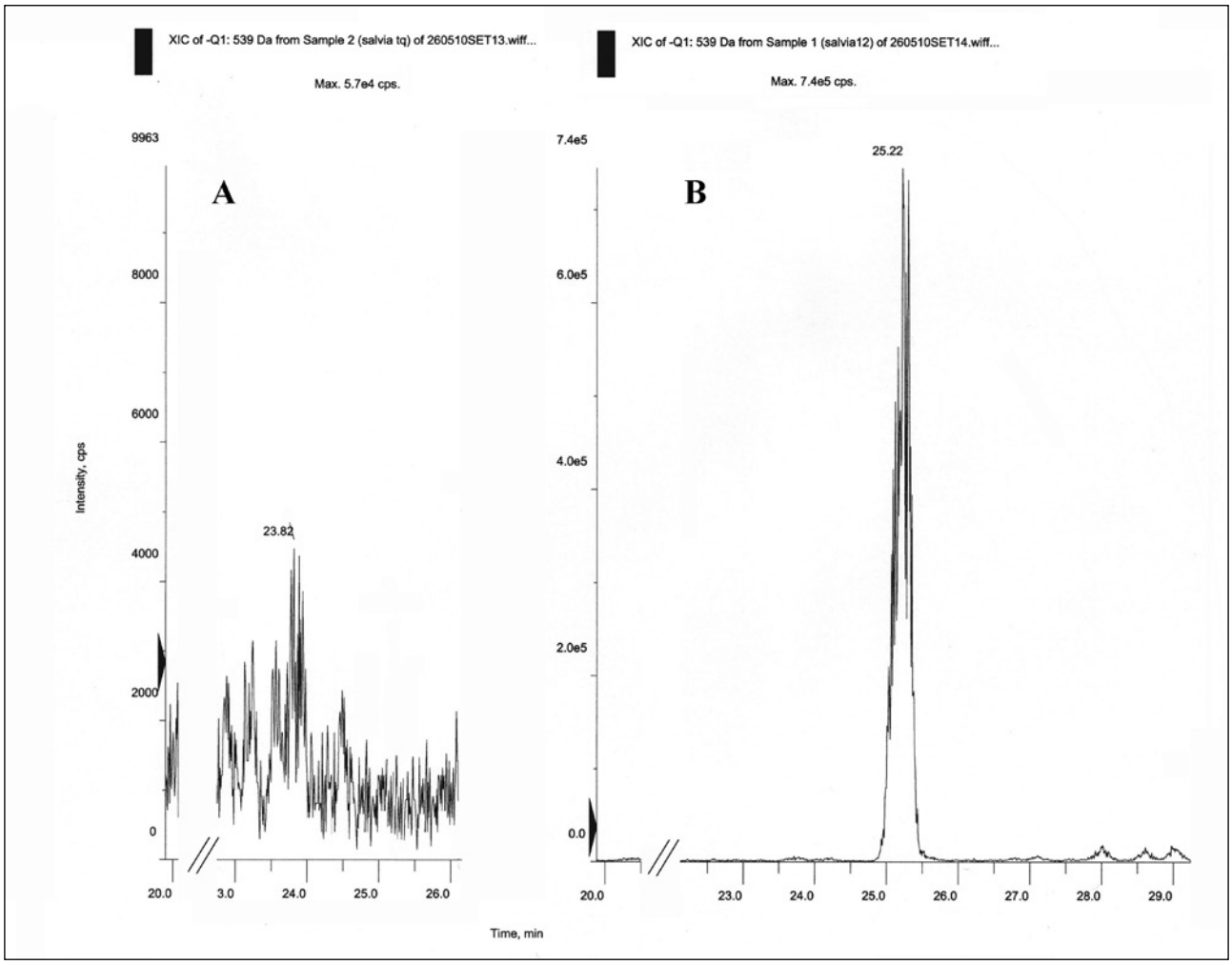


Fig. 6 - Extracted ion m/z = 539 detectable on LC-ESI-MS/MS in negative ion mode chromatogram obtained from a sample of pure sage (A) and a sample of sage contaminated with ~ 10% of *Olea europaea* L. dried leaves (B). The intensity values readable for pure sage has not an analytical sense in the neighborhood of r.t. 25 min.

tive evaluation of the presence of olive leaves, on the other hand, is not useful and is practically impossible, due to the highly variable content of oleuropein in dried olive ground leaves. The criterion for olive leaves identification presented in this paper is evidently useful for qualitative measurements, as specific confirmation of the adulteration evaluated by stereo zoom microscopy.

Therefore the suggested method has for the first time permitted a real identification of olive leaves for routine quality control and it has been adopted in the Analytical Research Laboratories (Di.Pro.Ve. - University of Milan, Italy).

REFERENCES

Angerosa F., d'Alessandro N., Konstantinou P. and Di Giacinto L. 1995. GC-MS evaluation of phenolic compounds in virgin olive oil. J. Agric. Food Chem., 43: 1802.

Bazoti F.N., Gikas E. and Tsarbopoulos A. 2010. Simultaneous quantification of oleuropein and its metabolites in rat plasma by liquid chromatography electrospray ionization tandem mass spectrometry. Biomed. Chromatogr. 24: 506.

Bononi M., Fiordaliso I. and Tateo F. 2010b. Rapid GC/MS Test for Identification of *Olea europaea* L. Leaves in Ground Oregano. Ital. J. Food Sci. 22 (4): 479.

Bononi M., Fiordaliso I. and Tateo F. 2010a. Introduzione alla tecnica di stereo-microscopia per l'identificazione di foglie di *Olea europaea* L. in origano contuso. Ingredienti Alimentari 50: 6.

Bouaziz M. and Sayadi S. 2005. Isolation and evaluation of antioxidants from leaves of a Tunisian cultivar olive tree. Eur. J. Lipid Sci. Technol. 107: 497.

Del Boccio P., Di Deo A., De Curtis A., Celli N., Iacoviello L. and Rotilio D. 2003. Liquid chromatography-tandem mass spectrometry analysis of oleuropein and its metabolite hydroxytyrosol in rat plasma and urine after oral administration. J. Chromatogr. B 785: 47.

Japòn-Lujàn R. and Luque De Castro M.D. 2008. Liquid-Liquid Extraction for the Enrichment of Edible Oils with Phenols from Olive Leaf Extracts. J. Agric. Food Chem. 56: 2505.

Japón-Luján R., Luque-Rodriguez J.M. and Luque de Castro M.D. 2006. Multivariate optimization of the microwave-assisted extraction of oleuropein and related biophenols from olive leaves. Anal. Bioanal. Chem. 385: 753.

Jemai H., Bouaziz M. and Sayadi S. 2009. Phenolic Composition, Sugar Contents and Antioxidant Activity of Tuni-

sian Sweet Olive Cultivar with Regard to Fruit Ripening. J. Agric. Food Chem. 57: 2961.

Kiritsakis K., Kontominas M.G., Kontogiorgis C., Hadjipavlou-Litina D., Moustakas A. and Kiritsakis A. 2010. Composition and Antioxidant Activity of Olive Leaf Extracts from Greek Olive Cultivars. J. Am. Oil Chem. Soc. 87: 369.

Laguerre M., López Giraldo L.J., Piombo G., Figueroa-Espinoza M.C., Pina M., Benaissa M., Combe A., Rossignol Castera A., Lecomte J. and Villeneuve P. (2009). Characterization of olive-leaf phenolics by ESI-MS and evaluation of their antioxidant capacities by the CAT assay. J. Am. Oil Chem. Soc. 86: 1215.

Marsilio V., Campestre C. and Lanza B. 2001. Phenolic compounds change during California-style ripe olive processing. Food Chem. 74: 55.

Papoti V.T. and Tsimidou M.Z. (2009). Impact of sampling parameters on the radical scavenging potential of olive (*Olea europaea* L.) leaves. J. Agric. Food Chem. 57: 3470.

Ranalli A., Contento S., Lucera L., Di Febo M., Marchegiani D. and Di Fonzo V. 2006. Factors Affecting the Contents of Iridoid Oleuropein in Olive Leaves (*Olea europaea* L.). J. Agric. Food Chem. 54: 434.

Ranalli A., Marchegiani D., Contento S., Girardi F., Nicolosi M.P. and Brullo M.D. 2009. Variations of iridoid oleuropein in Italian olive varieties during growth and maturation. Eur. J. Lipid Sci. Technol. 111: 678.

Saitta M., Lo Curto S., Salvo F., Di Bella G. and Dugo G. 2002. Gas chromatographic-tandem mass spectrometric identification of phenolic compounds in Sicilian olive oils. Anal. Chem. Acta 466: 335.

Soler-Rivas C., Espin J.C. and Wichers H.J. 2000. Review Oleuropein and related compounds. J. Sci. Food Agric. 80: 1013.

Tuck K.L. and Hayball P.J. 2002. Major phenolic compounds in olive oil: metabolism and health effects. J. Nutr. Biochem. 13: 636.

Paper received November 8, 2010 Accepted March 30, 2011

PAPER

"TOTAL FLAVONOID" CONTENT ASSESSMENT VIA ALUMINUM [AL(III)] COMPLEXATION REACTIONS. WHAT WE REALLY MEASURE?

V.T. PAPOTI, S. XYSTOURIS, G. PAPAGIANNI and M.Z. TSIMIDOU*
Aristotle University of Thessaloniki, Department of Chemistry,
Laboratory of Food Chemistry and Technology, 541 24, Thessaloniki, Greece
*Corresponding author: Tel. +30 2310 997796, Fax +30 2310 997779,
email: tsimidou@chem.auth.gr

ABSTRACT

Frequently applied protocols for "total flavonoid" determination are examined with respect to limitations of Al(III)-flavonoid complexation reactions employed. Flavonoids and other phenolics tested were selected on the basis of their structure. The validity of observations and suggestions was then examined for various olive leaf extracts. Al-complexation reaction in the presence of $NaNO_2$ seems to be nonselective for flavonoid assessment as codetermination of o-diphenols strongly affects the results. The latter were found to correlate well with the respective Folin-Ciocalteau data (r^2 = 0.76). Under acidic conditions complexation with catecholic groups is not favored so that such protocols are considered more appropriate for flavonoid content estimation. The expression "total flavonoid" content is not always fully representative of what is really measured. Researchers should acknowledge analytical limitations of each protocol when they interpret their results.

- Key words: aluminium-flavonoid complexation, flavonoids, *Oleaceae,* olive (*Olea europaea* L.) leaves, plant extracts -

INTRODUCTION

Flavonoids, compounds found in all plant tissues (seeds, fruit skin or peel, bark, flowers, leaves, etc.) have been appraised for numerous biological and pharmacological effects since the antiquity (HAVSTEEN, 2002). Currently, many of the health-related effects of flavonoids are attributed to their antioxidant properties, whereas a wide variety of products containing them are commercially available (BENAVANTE-GARCÍA *et al.*, 1999; HAVSTEEN, 2002; MALEŠEV and KUNTIĆ, 2007; KIOKAS *et al.*, 2008).

Flavonoid analysis in a particular plant extract, as well as analysis of other phenolic compounds, includes not only identification of individual or groups of compounds using either simple or sophisticated separation techniques coupled to selective detection means but also estimation of their total content. Thus, except for the well-known Folin-Ciocalteu assay for the estimation of the "total phenol" content colorimetric assays can also be found in literature for the assessment of "total flavonoid" content.

Flavonoids due to their structure (Fig. 1), easily chelate metal ions and form complex compounds. It seems that metal-flavonoid complexation reactions are particularly appropriate for analytical objectives as formed complexes bear exceptional spectrophotometric characteristics. The respective colorimetric protocols, mainly those involving Al(III), are simple, rapid, and inexpensive and have met wide application even as official methods (MALEŠEV and KUNTIĆ, 2007). Nevertheless, current analytical demands that require validation of all analytical steps so that a method can be accepted for use led us to reconsider applicability of some of these protocols for the "total flavonoid" assessment.

Al(III) is likely to bind with 1, 2 or 3 molecules of bidentate ligands to form 1:1, 1:2, and less often 1:3, complex compounds, respectively. In the case of flavonoids, those that can act as ligands bear 5 or 3-hydroxy-4-keto and/ or o-dihydroxy groups (Fig. 1). The 3', 4'-dixydroxy group of B ring (cateholic moiety) seems to be less important though in a few studies it appears to be the basic complexation site (BODINI *et al.*, 1999; CORNARD *et al.*, 2001; CORNARD and MERLIN, 2003). Besides the above factors, reaction environment (pH, solvent) also influences analytical data (PORTER and MARKHAM, 1970; CORNARD *et al.*, 2001; MALEŠEV and KUNTIĆ, 2007). Methanol is reported to be preferable than ethanol when used in mixture with water (PORTER and MARKHAM, 1970). Last but not least, aluminum forms acid stable complexes with the 5 or 3-hydroxy-4-keto groups of flavonoids and acid labile ones with any o-dihydroxy group present (MARKHAM and MARBY 1968; VOIRIN, 1983).

In the present study the Al(III)-flavonoid complexation as an analytical procedure was examined with respect to reaction environment. Flavonoids tested have been (or can be) used as reference compounds. Other phenolics were also examined to show method limitations. The validity of observations and suggestions was then tested for selected plant extracts.

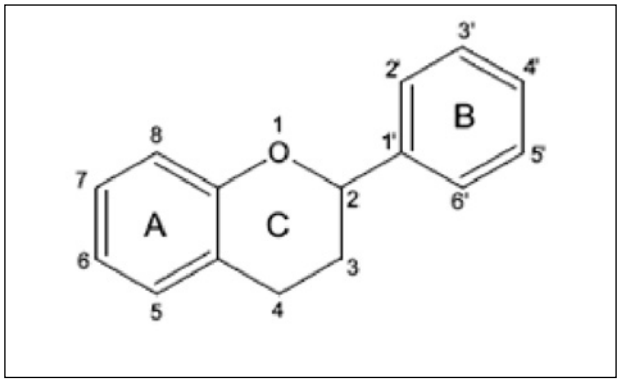


Fig. 1 - Basic flavonoid structure and main subclasses.

bond C_2-C_3	C_3 substitution	C_4 substitution	subclass
double	-	keto	flavone
simple	hydroxy	-	flavanonol
double	hydroxy	keto	flavonol
simple	-	keto	flavanone

MATERIALS AND METHODS

Chemicals

Oleuropein (98%) was purchased from Extrasynthèse (Genay, France); tyrosol (98%) was from Sigma-Aldrich (Steinheim, Germany); morin, quercetin, catechin hydrate minimum (98%), caffeic acid, ferulic acid and p-coumaric acid were from Sigma Chemical Co. (St. Louis, MO). DPPH$^{\bullet}$ radical (1,1-diphenyl-2-picrylhydrazyl, 90%) was also from Sigma Chemical Co. (St. Louis, MO, USA). Folin-Ciocalteu (F-C) reagent and sodium carbonate anhydrous were purchased from Panreac Quimica (Barcelona, Spain). Aluminum chloride anhydrous was from Fluka Chemie (Buchs, Switzerland). MeOH (99.7%) was from J. T. Baker (Baker Analyzed, Holland). Sodium hydroxide, sodium nitrite and acetic acid were from Merck (Darmstadt, Germany). All other common reagents and solvents were of the appropriate purity from various suppliers.

Leaf sampling

A representative olive leaf sample was used. The latter resulted from pooling together new and mature leaves collected on different dates (June 2006-February 2008) from trees belonging to various cultivars of an olive orchard (Agricultural Research Station, Agios Mamas, Chalkidiki, Greece). Detailed information on sampling parameters and postharvest treatment of

the plant material are described in PAPOTI and TSIMIDOU (2009).

Leaf extracts preparation

Decoction: A suitable aliquot of distilled water was boiled in an Erlenmeyer flask. Then, an appropriate amount of the lyophilized plant material (1 g dry sample/100 mL H_2O) was added and the mixture was further boiled for 10 min.

Infusion A: A suitable aliquot of boiled distilled water was immediately transferred to an Erlenmeyer flask, which contained an appropriate amount of the lyophilized plant material (1 g dry sample/100 mL H_2O). The mixture was left to stand at 75°C for 10 min.

Infusion B: A suitable aliquot of boiled distilled water was immediately transferred to an Erlenmeyer flask, which contained an appropriate amount of the lyophilized plant material (1 g dry sample/100 mL H_2O). The mixture was left to stand at room temperature for 5 min.

Tincture: A suitable amount of the lyophilized plant material (1 g dry sample/50 mL solvent) was extracted either in ethanol or in aqueous ethanol (50%, v/v) for 24 h at 25°-30°C under shaking (150 rpm).

Extract by sonication: Lyophilized plant material (1/40 w/v) was treated in an ultrasonic bath at room temperature for 5 min. Extraction solvent was either methanol or aqueous methanol (50%, v/v).

Determination of total polar phenol (TPP) content

TPP content of extracts was estimated using the F-C assay. Analytical details for the applied protocol are given in (PAPOTI and TSIMIDOU, 2009). Oleuropein was used as the external standard.

Determination of flavonoid content (FL)

The flavonoid content was determined according to two widely applied aluminium chloride colorimetric methods based on the formation of aluminium-flavonoid complexes. Protocols applied were based on previously described methodologies (MARKHAM and MARBY, 1968; ZHISHEN and JIANMING, 1999; CVEK *et al.*, 2007) that were modified in consistence to experimental requirements. The flow diagram of the employed protocols (protocol A and B) is given in Fig. 2 while details are presented below.

Protocol A is subdivided in protocols A_1 and A_2 as follows:

Protocol A_1: Flavonoid content was estimated via a modified protocol based on that employed by CVEK *et al.* (2007). Briefly, an aliquot (0.1 mL) of an aluminium chloride solution (2% w/v aluminium chloride in methanol) was added to 1 mL of the test solution (standard or extract 1,500 ppm as dry extract) and subsequently 1.4 mL of methanol was added. The mixture was left for 30 min in the darkness at room temperature and thereafter the absorbance was measured at 415 nm against a control. Absorbance measurements were corrected by subtracting initial sample absorbance at 415 nm.

Protocol A_2: Flavonoid content was estimated via the validated protocol of CVEK *et al.* (2007) applying the same procedure as in protocol A_1 in the presence of acid. Therefore, the aluminium chloride solutions added to the test solution, as well as the subsequently added methanol were acidified (5% v/v acetic acid).

The repeatability of measurement calculated for a morin standard solution and an extract was found satisfactory (CV% = 1 for both, $n = 5$). All determinations were carried out in triplicate.

The complexation activity of the tested standards with Al(III) was expressed via y = bx + a equations with y to represent the absorbance and x the final concentration (mM) tested. The respective concentration ranges tested for protocols A_1 and A_2 were: quercetin (0.01-0.09 mM and 0.006-0.059 mM); catechin (0-14 mM for both); morin (0.01-0.10 mM for both); oleuropein (0.6-4.5 mM for both); caffeic (0.22-0.78 mM for both); tyrosol (0-29 mM for both); *p*-coumaric acid (0-7 mM for both); ferulic acid (0-15 mM for both).

Results for the extracts were expressed as μg FL/g dry leaf through a morin calibration curve.

Protocol B: Aliquots of the test solution (standard or extract 1,500 ppm as dry extract) were appropriately diluted with doubly distilled water to achieve final volume of 1.775 mL. Then, at zero time (t=0) 75 μL $NaNO_2$ (5%) were added to the mixture. Subsequently, at 6 and 11 min 150

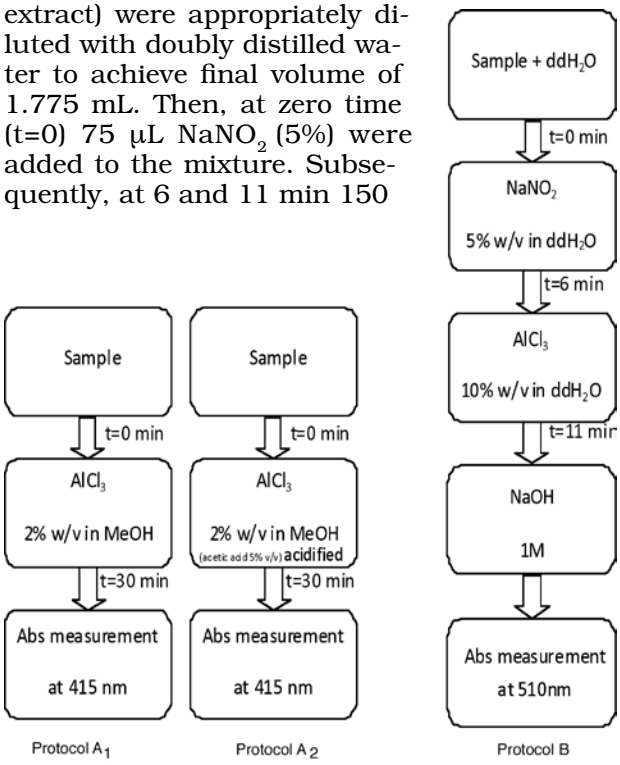


Fig. 2 - Diagrammatic presentation of Al-flavonoid complexation procedures (protocols A_1, A_2 and B) widely applied for flavonoid content determinations.

μL $AlCl_3$ (10%) and 0.5 mL NαOH 1M were added respectively to the mixture. Reactions took place in the darkness at room temperature. Absorbance was then measured at 510 nm against a control. The repeatability of measurement calculated for a catechin standard solution and an extract was found satisfactory (CV% < 5 for both, $n = 9$). Absorbance measurements were corrected by subtracting initial sample absorbance at 510 nm. All determinations were carried out in triplicate. The complexation activity of the tested standards with Al(III) was expressed via y = bx + a equations with y to represent the absorbance and x the final concentration (mM) tested. Concentration ranges for quercetin, catechin, morin, oleuropein, caffeic, tyrosol, p-coumaric and ferulic acids were 0.05-0.57 mM, 0.03-0.14 mM, 0.1-1.6 mM, 0.02-0.11 mM, 0.02-0.13 mM, up to 29 mM, up to 7 mM, up to 15 mM, respectively.

Results for the extracts were expressed as μg FL/g dry leaf through a catechin calibration curve.

RESULTS AND DISCUSSION

Careful search in literature indicated variation in experimental conditions upon application of Al(III)-flavonoid complexation reaction. It seems that critical issues such as the co-presence of compounds structurally related to flavonoids (e.g. o-diphenolic compounds) and chemistry of the protocols applied are not always taken into consideration or justified in the experimental design or results discussion. In the majority of relevant published papers (Table 1) the protocols applied are those examined in our study.

Protocol A involves addition of methanolic solution of $AlCl_3$ to a sample tested and can be applied in the presence or absence of acid. The presence of acid does not allow complexation with catecholic groups (VOIRIN, 1983). Literature search for "total" flavonoid determination in plant material indicated that version A_1 is almost exclusively applied (Table 1). Version A_2 is met mainly in publications aiming at structure elucidation and detection of o-dihydroxyl in combination with A_1 (MARKHAM and MARBY, 1968; VOIRIN, 1983). Similar protocols have been used for account fluorescent chelates formed among aluminum and certain flavonoids (flavonols that contain a free 3-hydroxyl and 4-keto oxygen binding site) (HOLLMAN *et al.*, 1996).

Solubility limitations of aluminum salts in various solvents have been reported in the past (HOLLMAN *et al.*, 1996). By trial and error 99.7% v/v aqueous methanol was found preferable in the case of protocol A. Additionally, as reaction time interval varied in literature, trials on the kinetics of reaction (0-30 min) were performed for an indicative morin solution and findings showed the importance of this parameter (data not shown). For the rest of the analysis measurements were obtained after 30 min of the addition of $AlCl_3$.

Complexation reaction in the presence of $NaNO_2$ (protocol B) is met equally often for "total flavonoid" determination as that of A_1. However, reaction conditions of protocol B are close to those applied in the past for the determination of o-diphenols (BARNUM, 1977). In that case, according to BARNUM (1977), the addition of so-

Table 1 - A representative overview of literature studies employing aluminum complexation reaction protocols for "total flavonoid" content determination in plant extracts.

Reference	Material	Protocol	Standard	Expression
ZHINSEN and JIANMING, 1999	Mulberry leaves	(B)	Rutin	Flavonoids
KIM *et al*., 2003	Plums	(B)	Catechin	Total flavonoids
MARINOVA *et al*., 2005	Fruits	(B)	Catechin	Total Flavonoids
MEDA *et al*., 2005	Honey	(A_1)	Quercetin	Total flavonoids
GALVEZ *et al*., 2005	Plantago species	(A_1)	Luteolin 7-*O*-glucoside	Total flavonoids
RATHEE *et al*., 2006	*Mammea longifolia* bud	(B)	Epicatechin	Total flavonoids
ORDONEZ *et al*., 2006	*Sechium edule* (Jacq.) Swartz tissues	(A_1)	Quercetin	Total flavonoids
MERSCH-SUNDERMANN *et al*., 2006	Mauritian plant	(A)	Quercetin	Total flavonoids
TSAI *et al*., 2007	Rosemary leaves	(B)	Catechin	Total flavonoids
LIN and TANG, 2007	Fruits & vegetables	(A) modification	Quercetin	Total flavonoid content
CHEN *et al*., 2007	Herbs	(B)	Catechin	Total flavonoids
MAKRIS *et al*., 2007	Agri food solid wastes	(B)	Catechin	Total flavonoids
VINA *et al*., 2007	Brussel sprouts	(B)	Catechin	Total flavonoids
CVEK *et al*., 2007	Croatian propolis	(A_2)	Galangin	Flavones & flavonols
TSAI *et al*., 2008	Herbs	(B)	Catechin	Total flavonoids
CONFORTI *et al*., 2008	Mediterranean edible plants	(A_1)	Quercetin	Total flavonoids
SIMIRGIOTIS *et al*., 2008	Asian fruits	(B)	Quercetin	Total flavonoid content
BARREIRA *et al*., 2008	Chestnut tissues	(B)	Catechin	Flavonoid content
EBRAHIMZADEH *et al*., 2008	Iranian medicinal plants	(A) modification	Quercetin	Flavonoid content
WANG *et al*., 2008	Citrus peels	(B) modification	Rutin	Total flavonoids
AL *et al*., 2009	Honey	(B)	Quercetin	Total flavonoids

Table 2 - Complexation activity of selected flavonoids and other phenolic compounds with Al(III) under different experimental conditions.

Compound	Protocol A_1	Protocol A_2	Protocol B
Quercetin	y =8.92x+0.06 R^2=0.9857	y =11.33x-0.04 R^2=0.9759	y =1.94x+0.01 R^2=0.9834
Catechin	- [a]	- [b]	y =10.76x+0.05 R^2=0.9818
Morin	y =10.61x-0.03 R^2=0.9932	y=10.76x+0.01 R^2=0.9984	y =0.44x+0.10 R^2=0.9805
Oleuropein	y =0.19x+0.05 R^2=0.9885	-	y =10.02x-0.01 R^2=0.9971
Caffeic acid	y =0.89+0.18 R^2=0.9828	-	y =8.59x-0.03 R^2=0.9906
p-coumaric acid	-	-	-
Tyrosol	-	-	-
Ferulic acid	-	-	? [c]

[a] slight absorbance (<0.05 Abs) at max concentration tested for protocol B (14 mM).
[b] slight absorbance (<0.02 Abs) at max concentration tested for protocol B (14 mM); Negative in excess of acid 10% v/v.
[c] Same results for 2, 10 and 15 mM tested.

dium nitrite results in nitration of any aromatic ring bearing a catechol group with its 3 or 4 positions unsubstituted or sterically unblocked. In order to better understand chemistry of reaction, reagents were gradually added and spectra were recorded. The latter showed that in cases of reaction a complex was immediately formed after $AlCl_3$ addition, which resulted in a yellow color formation that was then turned to red immediately after NaOH addition. Kinetics showed that reactions are almost immediately completed after $NaNO_2$ and $AlCl_3$ addition; however time intervals of 5 min were included in accordance to literature practices.

In the majority of studies employing protocols A and B for "total flavonoid" determination in plant extracts quercetin and catechin (see Table 1) are the standards of choice for result expression. The flavonol quercetin (Fig. 3a) is rather used upon application of protocol A and the flavanonol catechin (Fig. 3b) seems to be preferred in protocol B. To our knowledge no documentation appears for these preferences in the respective papers. Nevertheless, it is questionable whether all flavonoids present in the samples examined can be determined via these experimental conditions or the selected standards are adopted through validation procedure.

In our study apart from these two widely employed reference flavonoids (quercetin and catechin) some other phenolics were selectively tested on the basis of structural characteristics. The chosen compounds were the flavonoid morin and the phenolic compounds oleuropein, caffeic acid, tyrosol, p-coumaric and ferulic acids (Fig. 3c-h). Morin, a 2' hydroxyflavonol, was selected because it possesses the 5 and 3-hydroxy-4-keto groups of interest and lacks the catecholic moiety in B ring (Fig. 3c). Other characteristic flavonoids e.g. luteolin (flavone), apigenin (flavone) and respective glucosides as rutin (flavonol glucoside) were not included in the study. This was not only due to their high cost but also because they bear the 5 -hydroxy-4-keto group that is expected to be less potent for complexation in comparison to the 3 -hydroxy-4-keto one under neutral, acidic or alkaline environment (CORNARD *et al.*, 2001). Besides, HOLLMAN *et al.*, (1996) that studied the required structural properties of flavonols employing an aluminum complexating protocol, similar to that of protocol A, have stressed the importance of the presence of a free 3-hydroxyl group. Oleuropein (Fig. 3d), present in a great number of *Oleaceae* plants (NENADIS and TSIMIDOU, 2009), along with caffeic acid (Fig. 3e) were tested to examine whether, as catechol derivatives, could interfere with the total flavonoid complexation process. Furthermore, tyrosol (Fig. 3f), p-coumaric acid (Fig. 3g) and ferulic acid (Fig. 3h) were tested to complement discussion about possible sources of interference. Results for the above compounds under different reaction conditions are presented in Table 2.

Obviously, the presence of monophenols (tyrosol and p-coumaric acid; Table 2) does not influence quantitative data in both protocols. The presence of a guaicol moiety (ferulic acid; Table 2) was of some importance only under the conditions of protocol B whereas that of a catecholic group became crucial for complexation, irrespectively of the class of the molecule (flavonoid or not). Thus, slope values of oleuropein and caffeic acid indicated a high complexation

activity of these phenolics, comparable to that of catechin. However, in the presence of 5 and/or 3-hydroxy-4-keto groups (quercetin; Table 2) complexation via a catecholic moiety seems to diminish under reaction conditions of protocol B. The fact that complexation through 5 and 3-hydroxy-4-keto sites (characteristic moieties of many flavones, flavonols, flavanononones, isoflavones) is not favored under conditions of protocol B, initially noted for quercetin, was also verified for morin.

Protocol A was found incompatible with catechin in line with sited references (Table 1) that avoid it as a reference compound. It seems that its catecholic moiety (ring B) does not act as a site of complexation under acidic conditions. Protocol A was found appropriate for the determination of quercetin and morin as 5 and 3-hydroxy-4-keto moieties seem to be the main sites of complexation under these experimental conditions. To our view morin has to replace quercetin as an external standard in relevant studies because it lacks catecholic groups that may interfere; quercetin is more appropriate than rutin because the latter lacks a free 3-hydroxyl group and escapes fluorescence (HOLLMAN *et al.*, 1996). Our data drawn for morin (Table 2) were as expected since the absence or presence of acid in the reaction medium was not crucial.

The influence of potent interfering compounds indicates that protocol B is not that selective for flavonoid determination in the presence of compounds bearing catecholic moieties. However, most plant extracts contain different classes of phenolic compounds, each one of importance for their overall functional properties. In the case of such extracts as most of non flavonoids bear catecholic moieties, determination of "total flavonoids" in the presence of sodium nitrite is nonspecific. This limitation is not always considered (references for protocol B in Table 1). In addition co-determination of other groups of phenolics (e.g. secoiridoids, simple phenols) via protocol A_1 is negligible in comparison to findings upon application of protocol B. Using protocol A_2 complex formation via o-dihydroxy moieties is totally eliminated. This was also verified for catechin, as the slight absorbance observed under protocol A_2 conditions disappeared in excess of acid (10% v/v instead of 5% v/v). It can be argued that the three protocols examined give biased results and the expression "total flavonoids" is rather illusive. It should not be forgotten that such methods are usually applied before chemical characterization of an extract that possibly contains other types of phenolic compounds except for flavonoids. This is next exemplified for the case of olive leaf extracts.

Case study: Assessment of "total flavonoid" content of various olive leaf extracts

The applicability of the above mentioned aluminum-flavonoid complexation protocols was examined for various extracts of a representative olive leaf sample (Table 3). Olive leaf is a source rich in simple phenols (tyrosol, hydroxytyrosol and derivatives, phenolic acids: i.e. caffeic, chlorogenic, gallic, protocatechic, homovanillic, ferulic), secoiridoids (oleuropein, oleuroside, oleuropein aglycone, 3,4-DHPEA-EDA, demethyloleuropein), flavonoids (luteolin, apigenin, and their derivatives, quercetin, quercitrin, rutin) and other relevant compounds (e.g. verbas-

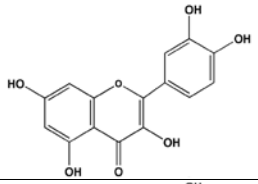
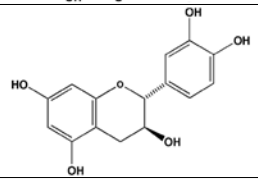
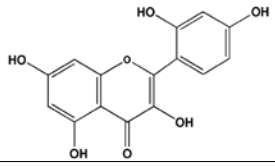
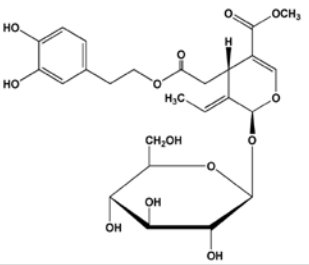
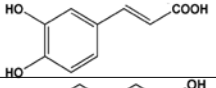
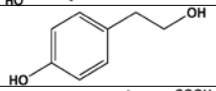
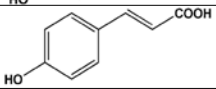
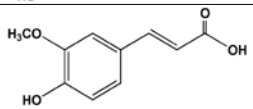
Structure	Compound/ class
Flavonoids	
	(a) Quercetin/ flavonol
	(b) Catechin/ flavanonol
	(c) Morin/ 2'hydroxyflavonol
Phenolics	
	(d) Oleuropein/ secoiridoid
	(e) Caffeic acid/ phenolic acid
	(f) Tyrosol/ simple phenol
	(g) p-coumaric acid/ phenolic acid
	(h) Ferulic acid/ phenolic acid

Fig. 3 - Structural characteristics of compounds studied.

Table 3 - Flavonoid content and TPP values of various types of olive leaf polar extracts; extracts are classified in ascending order according to their total polar phenol content.

Extraction parameters[a]	TPP Content	Flavonoid Content		
	mg oleuropein/g dry leaf	µg morin/g dry leaf		µg catechin/g dry leaf
	F-C[b]	A_1[c]	A_2[c]	B[c]
Tincture, 50% aq. EtOH, 1 day, 1/50 w/v, 30°C	48	6044±117	3337±95	8677±959
Extract [d], MeOH, 5 min, 1/40 w/v, AT[e]	62	7899±56	6874±133	18464±379
Tincture, EtOH, 1 day, 1/50 w/v, 30°C	64	10378±118	7830±54	18529±485
Extract [d], 50% aq. MeOH, 5 min, 1/40 w/v, AT[e]	65	5240±63	3105±84	24560±1483
Infusion B, H_2O, 5 min, 1/100 w/v, AT[e]	85	4354±31	2401±58	46398±689
Infusion A, H_2O, 10 min, 1/100 w/v, 75°C	104	5259±240	2788±42	35413±1818
Decoction, H_2O, 10 min, 1/100 w/v, 100°C	114	7872±46	4729±201	44553±1163

[a] Extraction parameters: extract type, solvent, time, leaf weight/solvent volume, temperature.
[b] Results are given as the mean value of two measurements.
[c] Results are given as the mean value ± standard deviation (n = 3).
[d] prepared by sonication.
[e] AT: ambient temperature.

coside) (RYAN *et al.*, 2002; TSIMIDOU and PAPOTI, 2010). For the same extracts assessment of total polar phenol content was also carried out via the F-C assay.

As expected (PAPOTI and TSIMIDOU, 2009; GOULAS *et al.*, 2010) all of the extracts were identical qualitatively with regard to RP- HPLC phenol and flavonoid profile but varied in terms of quantitative data. Nevertheless, an interesting correlation was found between F-C data and protocol B ones (r^2= 0.76) whereas the respective correlations for F-C and A protocol versions were not significant [r^2= 0.33 (A_1), r^2= 0.27 (A_2)]. The above finding is strong evidence on the unsuitability of protocol B for "total flavonoid" content assessment. Taking into consideration the structural characteristics of flavonoids as well as of other phenolics expected in the studied extracts, protocol B can not be considered specific and selective for their determination. Many of olive leaf non flavonoid components (hydroxytyrosol and derivatives, oleuropein and derivatives, verbascoside, caffeic, chlorogenic, gallic, protocatechic acids) bearing catecholic moieties must influence quantitative data upon application of this protocol (HRNCIRIC and FRITSCHE, 2004).

The relatively low correlations for F-C and A_1 and A_2 protocols indicated that groups other than the catecholic moieties contribute to the final quantitative data drawn upon application of protocol A versions. Between A_1 and A_2 protocols the second one seems more specific for flavonoid determination. In this case "total flavonoid" content should mainly correspond to quercetin levels of an olive leaf extract. Other flavonoids, which lack a free 3 hydroxyl group, are expected to contribute less to complex formation reaction. The magnitude of respective results between A_1 and A_2 varied for the same extract and also among extracts. Alcoholic ones contained higher amounts of flavonoids in comparison to aqueous or aqueous alcoholic extracts as expected (Table 3). Moreover, in relevance to the protocol used even the order of samples in terms of their flavonoid content alters.

As an epilogue it can be said that protocol A_2 is preferable for the assessment of the presence of flavonoids that can form complexes with Al(III). The expression "total flavonoid" content is not fully representative of what is really measured in a plant extract as the method is dependent on the structure of the individual flavonoids present. Researchers should acknowledge this analytical limitation when they interpret their results.

REFERENCES

Al M.L., Daniel D., Moise A., Bobis O., Laslo L. and Bogdanov S. 2009. Physico-chemical and bioactive properties of different floral origin honeys from Romania. Food Chem. 112: 863.

Barnum D.W. 1977. Spectrophotometric determination of catechol, epinephrine, dopa, dopamine and other aromatic vic-diols. Anal. Chim. Acta 89: 157.

Barreira J.C.M., Ferreira I.C.F.R., Oliveira M.B.P.P. and Pereira J.A. 2008. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. Food Chem. 107: 1106.

Benavente-García O., Castillo J., Marin F.R., Ortunõ A. and Del Rìo J.A. 1997. Uses and properties of Citrus flavonoids. J. Agric. Food Chem. 45: 4505.

Bodini M.E., Copia G., Tapia R., Leighton F. and Herrera L. 1999. Iron complexes of quercetin in aprotic medium. Redox chemistry and interaction with superoxide anion radical. Polyhedron 18: 2233.

Chen H.-Y., Lin Y.-C. and Hsieh C.-L. 2007. Evaluation of antioxidant activity of aqueous extract of some selected nutraceutical herbs. Food Chem. 104:1418.

Conforti F., Sosa S., Marrelli M., Menichini F., Statti G.A., Uzunov D., Tubaro A., Menichini F. and Della Loggia R. 2008. *In vivo* anti-inflammatory and *in vitro* antioxidant activities of Mediterranean dietary plants. J. Ethnopharmacol. 116: 144.

Cornard J.P. and Merlin J.C. 2003. Comparison of the chelating power of hydroxyflavones. J. of Mol. Struct. 651-653: 381.

Cornard J. P., Boudet A.C. and Merlin J.C. 2001. Complexes of Al(III) with 3%4%-dihydroxy-flavone: characterization, theoretical and spectroscopic study. Spectrochim. Acta A 57: 591.

Cvek J., Medić-Šarić M., Jasprica I., Zubčić S., Vitali D., Mornar A., Verdina-Dragojević I. and Tomić S. 2007. Optimisation of an extraction procedure and chemical characterisation of Croatian propolis tinctures. Phytochem. Anal. 18: 451.

Ebrahimzadeh M.A., Pourmorad F. and Bekhradnia A.R. 2008. Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. Afr. J. Biotechnol. 7: 3188.

Gälvez M., Martín-Cordero C., Houghton P.J. and Ayuso M.J. 2005. Antioxidant activity of methanol extracts obtained from plantago species. J. Agric. Food Chem. 53: 1927.

Goulas V., Papoti T.V., Exarchou V., Tsimidou Z.M. and Gerothanasis I. 2010. Contribution of flavonoids to the overall radical scavenging activity of olive (*Olea europaea* L.) leaf polar extracts. J. Agric. Food Chem. 58: 3303.

Havsteen B.H. 2002. The biochemistry and medical significance of the flavonoids. Pharmacology & Therapeutics 96: 67.

Hollman P.C.H., van Trijp J.M.P. and Buysman M.N.C.P. 1996. Fluorescence detection of flavonols in HPLC by postcolumn chelation with aluminium. Anal. Chem. 68: 3511.

Hrnciric K. and Fritsche S. 2004. Comparability and reliability of different techniques for the determination of phenolic compounds in VOO. Eur. J. Lipid Sci. Technol. 10: 540.

Kim D.-O., Chun O.K., Kim Y.J., Moon H.-Y. and Lee C.Y. 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J. Agric. Food Chem. 51: 6509.

Kiokias S., Varzakas T. and Oreopoulou V. 2008. *In vitro* activity of vitamins, flavonoids, and natural phenolic antioxidants against the oxidative deterioration of oil-based systems. Crit. Rev. Food Sci. 48: 78.

Lin J.-Y. and Tang C.-Y. 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem. 101: 140.

Makris D.P., Boskou G. and Andrikopoulos N.K. 2007. Polyphenolic content and *in vitro* antioxidant characteristics of wine industry and other agri-food solid waste extracts. J. Food Comp. Anal. 20: 125.

Malešev D. and Kuntić V. 2007. Investigation of metal-flavonoid chelates and the determination of flavonoids via metal-flavonoid complexing reactions. J. Serb. Chem. Soc. 72: 921.

Marinova D., Ribarova F. and Atanassova M. 2005. Total phenolics and total flavonoids in bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy 40: 255.

Markham K.R. and Mabry T.J. 1968. A procedure for the ultraviolet spectral detection of ortho-dihydroxyl groups in flavonoids. Phytochemistry 7: 1197.

Meda A., Lamien C.E., Romito M., Millogo J. and Nacoulma O.G. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 91: 571.

Mersch-Sundermann V., Bahorun T., Stahl T., Neergheen V.S., Soobrattee M.A., Wohlfarth R., Sobel R., Brunn H.E., Schmeiser T., Lamy E. and Aruoma O.I. 2006. Assessment of the DNA damaging potency and chemopreventive effects towards BaP-induced genotoxicity in human derived cells by *Monimiastrum globosum*, an endemic mauritian plant. Toxicol. in Vitro, 20: 1427.

Nenadis N. and Tsimidou M.Z. 2009. Oleuropein and related secoiridoids. Antioxidant activity and sources other than *Olea europaea* L. (olive tree). Recent Prog. Med. Plants 25: 53.

Ordonez A.A.L., Gomez J.D. Vattuone M.A. and lsla M.I. 2006. Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. Food Chem. 97: 452.

Papoti V.T. and Tsimidou M.Z. 2009. Impact of sampling parameters to the radical scavenging potential of olive (*Olea europaea* L.) leaves. J. Agric. Food Chem. 57: 3470.

Porter L.J. and Markham K.R. 1970. The unsuitability of ethanol as a solvent for the spectroscopic detection of functional groups in hydroxyflavones with aluminum chloride. Phytochemistry 9: 1363.

Rathee J.S., Hassarajani S.A. and Chattopadhyay S. 2006. Antioxidant activity of Mammea longifolia bud extracts. Food Chem. 99: 436.

Ryan D., Antolovich M., Prenzler P., Robards K. and Lavee S. 2002. Biotransformations of phenolic compounds in *Olea europaea* L. Sci. Hort. 92: 147.

Simirgiotis M.J., Adachi S., To S., Yang H., Reynertson K.A., Basile M.J., Gil R.R., Weinstein I.B. and Kennelly E.J. 2008. Cytotoxic chalcones and antioxidants from the fruits of Syzygium samarangense (Wax Jambu). Food Chem. 107: 813.

Tsai P.-J., Tsai T.-H. and Ho S.-C. 2007. *In vitro* inhibitory effects of rosemary extracts on growth and glucosyltransferase activity of Streptococcus sobrinus. Food Chem. 105: 311.

Tsai T.-H., Tsai T.-H., Chien Y.-C., Lee C.-W. and Tsai P-J. 2008. *In vitro* antimicrobial activities against cariogenic streptococci and their antioxidant capacities: A comparative study of green tea versus different herbs. Food Chem. 110: 859.

Tsimidou M.Z. and Papoti V.T. 2010. Bioactive ingredients in olive leaves. p. 351-358. In: "Olives and olive oil in health and disease prevention". V.P. Preedy and R.R. Watson (Ed.), Elsevier Inc.

Viña S.Z., Mugridge A., García M.A., Ferreyra R.M., Martino M. N., Chaves A.R. and Zaritzky N.E. 2007. Effects of polyvinylchloride films and edible starch coatings on quality aspects of refrigerated Brussels sprouts. Food Chem.103: 701.

Voirin B. 1983. UV spectral differentiation of 5-hydroxy- and 5-hydroxy-3- methoxyflavones with mono-(4';), di-(3', 4') or tri-(3', 4', 5') - substituted B rings. Phyrochemistry 22: 2107.

Wang Y.-C., Chuang Y.-C. and Hsu H.-W. 2008. The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. Food Chem. 106: 277.

Zhishen J.M.T. and Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64: 555.

Paper received October 25, 2010 Accepted February 1, 2011

PAPER

IRRADIATION OF READY-TO-EAT SAUSAGES CONTAINING LYCOPENE

M.C. GÁMEZ[1], M.L. GARCÍA[1], M.D. SELGAS[1] and M.M. CALVO[2]*
[1]Departamento de Nutrición, Bromatología y Tecnología de los Alimentos,
Facultad de Veterinaria, Universidad Complutense 28040, Madrid, Spain
[2]Instituto de Ciencia y Tecnología de Alimentos y Nutrición. CSIC. C/ Juan de la Cierva 3,
28006 Madrid, Spain
*Corresponding author: Tel. +34 91 5622900,
e-mail: mmcalvo@ifi.csic.es

ABSTRACT

The objective was to obtain a new ready-to-eat (RTE) dry fermented sausage enriched with lycopene by the addition of dry tomato peel as font of this carotene; the product was submitted to irradiation and stored. The effect of these treatments on microbiota, lycopene concentration, physico-chemical and sensory properties were studied. Levels of total viable bacteria, lactic acid bacteria and *Micrococcaceae* decreased as a result of irradiation. Lycopene content was not affected by irradiation and storage. The *b** and the hue angle showed the largest changes of colour parameters. The tomato peel modified slightly the texture parameters. Sensory quality was not acceptable in 4 kGy irradiated samples. A new RTE product enriched with lycopene was obtained.

- Key words: dry fermented sausages, dry tomato peel, irradiation, lycopene, ready-to-eat -

INTRODUCTION

Consumers demand from meat products high quality, convenience, innovation, safety, natural flavour and taste, high or novel nutritional value, and extended shelf life (AYMERICH *et al.*, 2008). In fact, demand is growing for foods that are easy to manufacture and distribute, such as ready-to-eat (RTE) products, as well as products that are fast and easy to cook, such as ready-to-cook (RTC) products. Shelf-life and hygienic quality can be compromised by inadequate management (slicing, packaging) that favours the growth of foodborne pathogens, some of which can grow at refrigeration temperatures, such as *Listeria monocytogenes* and *Escherichia coli* O157:H7 (FARKAS and ANDRASSY 1998; SAMELIS *et al.*, 2005).

Thus, companies have begun to treat these products at the post-packaging stage using so-called "non-thermal technologies", as the high pressure and the irradiation, which are proving to be more than sufficient for guaranteeing the security of RTE foods (ROSS *et al.*, 2003). Irradiation is an effective way to eliminate pathogens in foods such as meat products (ZHU *et al.*, 2005; CABEZA *et al.*, 2007, 2009). It is clear that the response of any given pathogen or spoilage organism to irradiation depends on a numerous of factors; some of them have been described by O'BRYAN *et al.* (2008). However, irradiation causes physico-chemical and biochemical changes that may affect the nutritional value and the sensory characteristics of irradiated food (ALFAIA *et al.*, 2007; O' BRYAN *et al.*, 2008).

Functional foods cannot long remain immune to the RTE trend; for that, it is very important to know the effect of the non-thermal technologies on the general quality of the products and the viability of the functional ingredients. Scarce works have been performed in this sense.

In recent years the interest in foods with compounds that can have health benefits, such as those containing carotenoids like lycopene, has increased. There is mounting evidence to support the health benefits derived from this carotenoid; they are based on its apparent ability to provide protection against many chronic diseases due to its antioxidant properties (OMONI and ALUKO, 2005; ERDMAN *et al.*, 2009). The beneficial health effects of this carotenoid have been reviewed (RAO, 2006; CHOSKI and JOSHI, 2007; KAVANAUGH, *et al.*, 2007). Tomato and tomato products are rich in lycopene.

Tomato industries generate large amounts of waste and by-products (seeds and peel) containing highly biologically active compounds as lycopene, compound responsible of the characteristic red colour of this vegetable. In previous works we have proposed the direct addition of dry tomato peel as source of lycopene to fresh and dry-fermented meat products with successfully results (CALVO *et al.*, 2007b; CALVO *et al.*, 2008; GARCÍA *et al.*, 2009). KIM *et al.* (2009 a,b) also reported on the addition of tomato powder or lycopene to pork products.

This work deals with the manufacture of ready-to-eat (RTE) dry fermented sausages enriched with lycopene. For this purpose, dry-fermented sausages enriched with dry tomato peel (CALVO *et al.*, 2008) were manufactured, sliced, packaged under vacuum and treated with E-beam radiation as non-thermal technology. Doses of 2 and 4 kGy were applied due to demonstrated effectiveness against foodborne pathogens as *Listeria* and *Salmonella spp* (LIM *et al.*, 2008; CABEZA *et al.*, 2007, 2009). The effect of this treatment on both the lycopene content and their physico-chemical and sensory properties of these meat products have been studied.

MATERIAL AND METHODS

Preparation of dry tomato peel (DTP)

Tomato peels were obtained from a local factory and freeze-dried in a lyophiliser (FM12XL, Virtis). Dry tomato peel (DTP) were ground with a mill (particle size, 0.025-0.05 mm) and stored at –30°C until use.

Sausage manufacture

The sausages were manufactured according to a traditional formula for Spanish *salchichón*: lean pork meat (85%) and pork backfat (15%), mixed with 5% of a commercial mixture of salt and spices (Anvisa, Arganda del Rey, Madrid). The mixture contained salt, dextrose, dextrin, spices, milk protein, flavour enhancer, emulsifiers, antioxidants, nitrites and ascorbic acid.

The meats were obtained from a local abattoir and maintained frozen (–18°C). Before use, the meat was maintained for 18 h at 4°C, and then it was chopped up and mixed with the spices. Two batches (5 kg each one) were manufactured; to the first one DTP (2%) (w/w) was added while the other was manufactured without it (control batch).

After mixing, the ingredients were stuffed into artificial casings with a diameter of 50 mm that had previously been moistened with lukewarm water. Ripening was carried out in a laboratory ripening cabinet (G-28, Ibercex, San Fernando de Henares, Madrid, Spain) programmed under the following conditions: 48 h at 22°C and a relative humidity (RH) of 92%, 12 h at 18°C/85% RH, 24 h at 18°C/89% RH and at 12°C/85% RH until the end of the experiment (21 days). The batches were manufactured in triplicate.

When the ripening was complete, the sausages were sliced: 2 mm thick for physico-chemical, colour and sensory analysis and 1 cm thick for the texture analysis. They were vacuum-packed to reach about 20 kPa into 10 x 10 cm laminated film bags (polyamide and polyethylene, film

thickness: 90 μm) of low gas permeability (oxygen transmission rate 35 cm^3/ 24 h m^2 bar and carbon dioxide 150 cm^3/ 24 h m^2 bar). Packed samples were maintained at 4°C for less than 24 hours before irradiation.

Irradiation treatment

Sausages were transported under refrigeration (< 4°C) to the irradiation plant IONISOS Iberica (Tarancón, Cuenca, España), which houses a linear electron accelerator, E-beam irradiation, (Rhodotron TT200, IBA Industrial, Belgium). Doses of 2 and 4 kGy were delivered using a 10-MeV electron beam; these doses have been described as effective for reducing the level of pathogenic microorganisms as *Listeria* or *Salmonella* (SAMELIS *et al.*, 2005; GOMES and DA SILVA 2006; O'BRYAN *et al.*, 2008; CABEZA *et al.*, 2007, 2009). The actual dose absorbed was determined by cellulose triacetate dosimeters placed on top of each sample during irradiation treatment. Sample temperature was controlled by indicator labels and never exceeding 2°C. After the treatment, sausages were transported under refrigeration to the laboratory. Samples were stored at 4°C and analyzed after 0, 30, 60 and 90 days of storage.

Microbiological analyses

Microbiological analyses were performed aseptically using 10 g of the sausages. The samples were homogenised with 90 mL of 1% peptone water, serially diluted and plated in duplicate. Total viable counts (TVC) were determined on Plate Count Agar (PCA), *Micrococcaceae* spp on Mannitol Salt Agar (MSA) and LAB on double-layer Man Rogosa Sharpe agar (MRS) acidify to pH 5.6 with 0.14% sorbic acid according to the instructions of the manufacturer. PCA, MSA and MRS plates were incubated at 32°C for 48 h. Culture media were purchased by Oxoid (Unipath Ltd., Basingstoke, Hampshire, UK). Microbial counts of the DTP were also performed on PCA.

pH determination

The pH was measured in a homogenate prepared with 1 g of sausage and 9 mL of distilled water, using a Crison 2001 pH-meter. All determinations were made in triplicate.

Lycopene quantification

The lycopene extraction of both dry tomato peel and fermented sausages was performed after saponification with KOH-methanol and separation with petroleum ether according to the method of CALVO *et al.* (2008). After extraction the samples were dissolved in 500 μL of a mixture of methanol/methyl-*tert*-butyl-ether (MTBE) 25/75 (v/v), then filtered through a 0.45 μm filter.

Identification and quantification of lycopene was performed by HPLC as described by CALVO, *et al.* (2007a). A Beckman System Gold binary delivery system, module 126, (Beckman Instruments, Fullerton, CA, USA) equipped with a UV–vis photodiode array detector, model 1689 was used. Analytical separation was carried out on a stainless steel (250 x 6.6 mm i.d., 5 μm particle size) Develosil UG C_{30} column (Nomura Chemical, Sojo, Japan) with a guard cartridge (Phenomenex, Macclesfield, UK) packed with ODS C_{18}. Sample injection was performed by means of a valve (Rheodyne, Cotati, CA) with a 20 μl sample loop.

The linear mobile phase gradient was methanol (4% H_2O)/MTBE from 83:17 to 33:67 over 60 min at a flow rate of 1 mL/min. The column was thermostated at 22°C in a column oven (Shimadzu CO-10AS, Columbia, MD). The Gold Nouveau software data system (Beckman Instruments) was used to evaluate peak areas. All the extractions and quantifications were performed in triplicate.

Colour analysis

Colour was measured on the surface of the sausage slices. The colour was measured with a Chroma Meter CR-300 colorimeter (Minolta Corporation, Osaka, Japan) using the Space colour CIE L^* a^* b^* system and calibrated with a pink tile (L^* 44.88, a^* 25.99, b^* 6.67) using a D-65 illumination source. The colour was measured on the surface of twenty-five slices (2 mm thick) and at room temperature from each sausage.

Textural analysis

The textural properties of dry fermented sausages were evaluated using the Stable Micro System Mod. TA.XT 2i/25 texturometer. The textural profile analysis (TPA) test (BOURNE, 1978) was performed using the central cores of five slices of each sample (height, 1 cm; diameter, 2.5 cm) which were compressed twice to 50% of their original height. The following parameters were determined: hardness (N), referring to the maximum force required to compress the sample; cohesiveness (ratio), the extent to which the sample can be deformed prior to rupture; springiness (cm), the ability of the sample to recover its original form after the deforming force is removed; and chewiness (N cm), the work required to masticate the sample for swallowing. The crosshead speed was 2 mm/s and at least five replicates were measured for each sample.

Sensory analysis

Samples were evaluated by 25 semi-trained assessors selected according to their eating habits, their familiarity with the sausages to be analysed, and the reproducibility of their evalua-

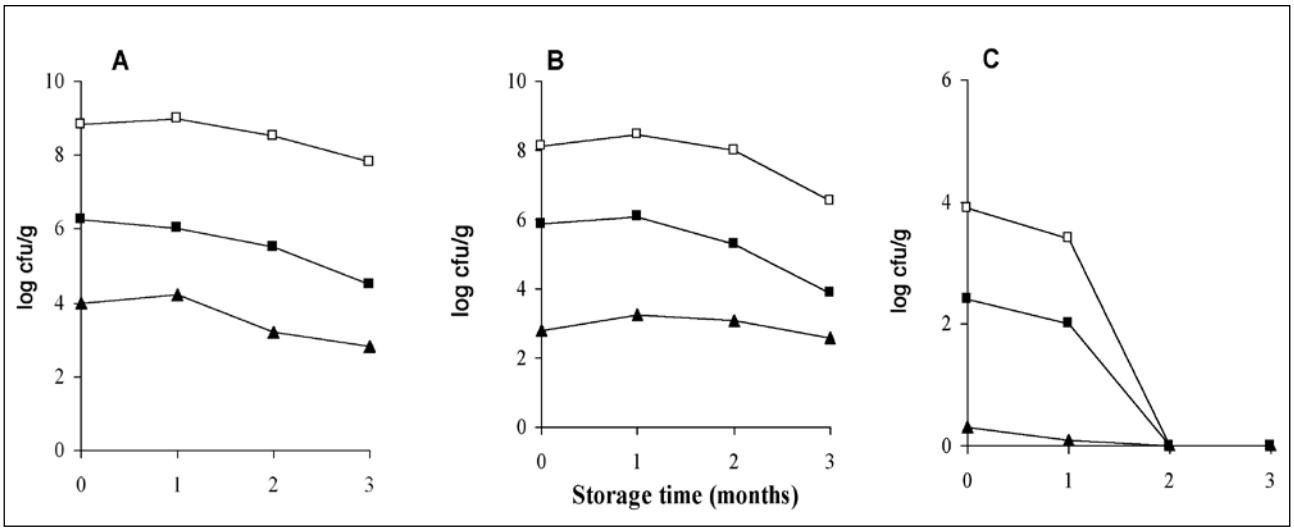


Fig. 1 - Total viable counts (A), lactic acid bacteria (B) and *Micrococcaceae* (C) of non-irradiated (□) and irradiated with 2 kGy (■) and 4 kGy (▲) dry fermented sausages during storage under refrigeration.

tions. The analysis was performed in individual booths constructed according to the specifications of the International Standards Organisation ISO (1985) under white fluorescent lights. Two slices of each sample were served to the assessors. Unsalted crackers and room-temperature water were also provided to clean the palate between samples.

Acceptability level was asked; a hedonic test was performed during storage. In each session two series of samples were served: one series of sausages without DTP, including a non-irradiated sample, a sample irradiated with 2 kGy, and a sample irradiated with 4 kGy; and the other series with the three corresponding samples but containing DTP. For each series, the panelists evaluated the overall sensory acceptability on a 5-point hedonic scale (1-extremely poor, 2-poor, 3-acceptable, 4- good and 5-excellent). The final score was the sum of the values given to each sample, divided by the number of panellists. The limit of acceptability was fixed in 3.

Statistical methods

The physico-chemical properties, lycopene concentration, colour, textural and sensory properties were analysed using a one-way analysis of variance (ANOVA) using the Statgraphics Plus program (Version 5.0).

RESULTS AND DISCUSSION

Microbial counts

Microbiota counts in experimental sausages during storage are presented in Fig. 1. At the end of the ripening, TVC and LAB in non-irradiated samples ranged between 8-9 log cfu/g, while *Micrococcaceae* reached counts of 4 log cfu/g. These values were similar to those described by several authors for these meat products (ORDOÑEZ *et al.*, 1999; MENDOZA *et al.*, 2001; FLORES *et al.*, 2005; SALAZAR *et al.*, 2009).

After ionizing treatment, TVC and LAB levels decreased 2-3 logarithmic cycles at doses of 2 kGy and 5 cycles at treatments of 4 kGy. *Micrococcaceae* counts decreased 1.5 logarithmic cycles at 2 kGy and close of 4 cycles at 4 kGy. During storage, the TVC and LAB maintained the same values or slightly decreased (1-2 log cycles) but *Micrococcaceae* decreased during the first month of storage, and were non-detectable after 2 months. Storage under vacuum conditions induces a drastic decrease of aerobic microorganism (*Micrococcaceae*), whereas the lower reduction of LAB count is due to its microaerophilic metabolism.

This microbiota behaviour is similar to that described previously in several types of meats products irradiated at similar doses (SATIN 2002; JAVANMARD *et al.*, 2006; CABEZA *et al.*, 2007). It is also consistent with approved procedures for irradiating uncooked chilled meat (SMITH and PILLAR, 2004).

Microbial counts were similar in the sausages added with DTP (data not shown) due to the low microbial count detected in the DTP (< 2 log cfu/g).

Changes in pH

After ripening, the pH values of control ranged between 5.06±0.08 and 4.95±0.05, values reported by several authors for this type of meat product (FLORES *et al.*, 2005; CABEZA *et al.*, 2009; SALAZAR *et al.*, 2009). Batches with DTP showed average values of 4.71±0.15; this can

be due to the low pH of DTP (4.02±0.06). A decrease in pH by the tomato addition was also observed by CANDOGAN (2002) and DEDA *et al.* (2007) when they added tomato paste to beef patties and frankfurters, respectively.

No significant ($p>0.05$) influence of irradiation and storage time on pH were observed. This is in agreement with SAMELIS *et al.* (2005) and CHOULIARA *et al.* (2006) who also reported no significant differences in pH between irradiated and non-irradiated dry fermented sausages, either during fermentation or ripening steps.

Lycopene concentration

The lycopene content found in non-irradiated sausages before storage was 1.2±0.21 mg/100 g (Table 1). This value was lower than that expected because the lycopene content of dry tomato peel was 132±0.18 mg/100 g DTP. A possible cause of this decrease is the reactions between lycopene and free radicals or other compounds formed during ripening yielding a decrease of the initial lycopene concentration. It is also possible that the saponification occurring during the extraction interfere with the complete extraction of carotenes from the lipidic phase. The use of saponification during carotenoid extraction making it difficult to get good separation among lipidic and aqueous phases inhibiting the complete extraction of carotenes, as lycopene, from the lipidic phase. OLIVER *et al.* (1998) reported that saponification produces an underestimation of β-carotene in "sobrassada", a fat cured crude sausage.

No effect of storage time was observed. However, differences of lycopene content between non irradiated batch and DTP added batches were observed at 4 kGy. It is possible that the irradiation treatment improves the extraction and recovery of lycopene due to this carotene becomes more bioaccesible when the cell walls break down. In this sense, SHI and LE MAGUER (2000) related as the extraction of lycopene can be modified by the food processing.

In a previous work performed in our laboratory (SELGAS *et al.*, 2009) we found that irradiation at 4 kGy induced a significant decrease in lycopene concentration in hamburger containing 6% of DTP; however no influence of irradiation was detected when lower amounts were added.

According to the Regulation 1924/2006 of the European Parliament (2006), the lycopene content of these sausages would be sufficient to consider them a *source* of this carotene that could contribute to its daily intake. In this sense, RAO and SHEN (2002) reported that a daily intake of 5-7 mg of lycopene by healthy humans may be sufficient to combat oxidative stress.

Table 1 - Lycopene content (mg/100 g) on non irradiated and irradiated dry fermented sausages after 0 and 3 months of storage.

	Storage time (months)	
Irradiation dosage (kGy)	**0**	**3**
0	1.20±0.21a	1.22±0.08a
2	1.14±0.14a	1.07±0.16a
4	1.49±0.07b	1.38±0.06b

Different letters in the same column indicate statistically significant difference ($p<0.05$).

Colour properties

The effect of the irradiation treatment and the storage time in the colour parameters of control and DTP added sausages, is shown in Tables 2 and 3.

Lightness (L^*) was the most stable colour parameter; it was very similar in all the sausages independent of the irradiation doses and the time of storage. L^* values of control samples at 0 days of storage (Table 2) were 49.10±1.86 for non-irradiated samples and 49.91±2.13 for samples irradiated with 4 kGy; respective values after 3 months of storage were 49.90±3.67 and 45.33±2.51. Similar values were found in DTP samples (Table 3).

a^* parameter changed in the presence of DTP due to the colorant power of the lycopene. The sausages with this ingredient showed higher values than did the control sausages; these changes in the colour were also reported by CALVO *et al.* (2008). The a^* parameter was not affected by the storage time or the irradiation treatment in control sausages (Table 2) but in DTP batches, a significant decrease was observed after the first month of storage (Table 3).

The greatest differences were observed in yellowness; the values for batches with DTP were a two-fold higher than for the controls. The increase in b^* parameter correlates with an increase in orangeness; it is possible that the lycopene degradation occurring during ripening leads to a loss of the characteristic red colour of this carotenoid, yielding an orange tonality. This change in the colour was previously observed by CALVO *et al.* (2008). In general terms, no significant influence of storage and irradiation treatment was found in control samples, whereas some influence of storage and irradiation dosage was found in DTP sausages.

The behaviour of a^* and mainly b^* parameters are reflected in the hue angle. Hue angle represent the tone of colour (tonality) being 0° red colour, 90° yellow colour, 180° green colour and 270°, blue colour (ARTÉS *et al.*, 2002). In our case, the hue angle values were higher in all samples containing DTP (orange tones) than in control sausages which showed red tones. Irradiation treatment scarcely affects the tonality

Table 2 - Influence of irradiation and storage time in colour parameters of control sausages.

Parameter	Time (months)	Irradiation dosage		
		0 kGy	2 kGy	4 kGy
L	0	49.10±1.86aA	46.62±2.86aA	49.91±2.13aA
	1	49.10±3.36aA	49.10±2.21bA	49.45±1.89aB
	2	48.70±2.88aA	48.82±2.23bA	45.62±2.38aB
	3	49.90±3.67aA	48.89±3.40bA	45.33±2.51aB
*a**	0	13.07±1.76aA	12.47±1.22aA	13.27±1.14aA
	1	13.28±1.01aA	11.65±1.67aA	12.35±0.97aA
	2	12.71±1.46aA	11.36±1.32aA	12.80±1.08aA
	3	12.63±1.60aA	12.50±1.20aA	12.37±1.54aA
*b**	0	5.32±0.67aA	5.49±0.44aA	5.74±0.68aA
	1	5.91±0.64aA	6.05±0.98aA	6.08±0.53aA
	2	6.32±0.39aA	5.98±1.07abA	5.92±0.77aA
	3	6.64±0.67aA	6.15±0.51aA	5.82±0.97aB
Hue angle	0	22.50±4.77bA	23.81±2.12aA	23.84±2.77aA
	1	24.02±3.00bA	27.56±4.45aA	26.23±2.73aA
	2	26.64±3.49abA	27.76±4.92aA	24.85±3.62aA
	3	27.96 ±4.38aA	26.41±3.61aA	25.25±3.86aA

Significant differences among storage times to each studied parameters are shown by minor letters in the same column ($p<0.05$).
Significant differences among irradiation dosages at each storage time are shown by mayor letters in the same row ($p<0.05$).

during storage. Similarly, LEE and AHN (2005) reported that irradiation and storage had no effect on colour parameters *L**, *a** or *b** of turkey breast rolls irradiated at 3 kGy.

However, these data contradict with those of CABEZA *et al.* (2009), who reported an influence of irradiation (1-3 kGy) on *L** and *a** values of commercial dry fermented sausages. This different behaviour may be related to the components of the mixture of spices used

Table 3 - Influence of irradiation and storage time in colour parameters of sausages containing dry tomato peel.

Parameter	Time (months)	Irradiation dosage		
		0 kGy	2 kGy	4 kGy
L	0	48.24±3.24aA	46.48±1.98aA	47.74±2.57aA
	1	47.24±4.66aA	47.74±3.10aA	46.18±3.75aA
	2	46.81±2.57aA	46.82±2.70aA	45.74±2.60aA
	3	45.84±4.13aA	46.05±3.67aA	47.01±4.33aA
*a**	0	14.59±1.47aB	15.97±1.27aAB	15.53±3.01aAB
	1	14.99±1.51aAB	13.91±1.63bA	15.14±1.63aA
	2	13.63±1.38bA	13.59±1.30bA	13.41±1.30bA
	3	13.03±1.60bA	12.85±2.66bA	12.79±2.66bA
*b**	0	11.49±1.53bB	11.18±2.21abB	13.62±1.84aA
	1	13.80±1.52aA	12.62±2.66aAB	12.27±1.52bB
	2	13.62±2.15aA	12.40±1.86abAB	11.48±2.15bB
	3	12.36±1.48bA	11.07±2.33bA	11.90±1.48bA
Hue angle	0	26.33±5.34bA	23.38±3.77bA	24.03±5.46bA
	1	30.67±4.24abA	34.81±4.80aA	33.50±2.75aA
	2	31.99±2.22aA	31.01±8.73abA	33.26±4.41aA
	3	28.95±3.41abA	36.52±6.27aA	35.80±4.10aA

Significant differences among storage times to each studied parameters are shown by minor letters in the same column ($p<0.05$).
Significant differences among irradiation dosages at each storage time are shown by mayor letters in the same row ($p<0.05$).

in the manufacture of sausages; it is possible that the commercial sausages studied by those authors were manufactured with colorants that can be affected by the irradiation. In our case, the mixture of spices used did not contain colorants.

As this difference in results highlights, there is scarce and in several cases contradictory information about the colour changes occurring in irradiated meats and meat products (AHN *et al.*, 1998; NAM and AHN, 2003; SELGAS *et al.*, 2009), and the data published show a great variation in the colour parameters of dry fermented sausages, as several authors have reported (PÉREZ-ALVAREZ *et al.*, 1999; GIMENO *et al.*, 2001; JAKOBSEN and BERTELSEN, 2002). In our case it is also probable that the colorant power of the lycopene masks the possible colour changes due to the irradiation, as has been indicated in previous studies performed in our laboratory (SELGAS *et al.*, 2009).

Textural properties

Tables 4 and 5 show the results of the TPA in experimental sausages without and with DTP, respectively. In the control batches (Table 4), the hardness values were similar in all batches ($p>0.05$) and in DTP sausages only increased significantly after the first month of storage (Table 5). No significant effect of irradiation was observed.

The increase in hardness, when DTP is added, has been previously reported by CALVO *et al.* (2008) and it has been attributed to the presence of fibre in the DTP. Along these lines, KNOBLISCH *et al.* (2005) reported that the content of fibre of tomato peel is close to 30 g/100 g of dry matter and it is composed mainly of insoluble fibre (cellulose and lignin). GARCÍA *et al.* (2002) reported a similar increase in hardness of dry fermented sausages supplemented with several types of soluble and insoluble fibres and they observed as the hardening of meat products was related to the fibre amount added.

Cohesiveness of the control samples did not show significant influence of irradiation treatment (Table 4). In contrast, cohesiveness of DTP sausages increased significantly ($p<0.05$) only after 3 months of storage (Table 5). Irradiation also affected this parameter; samples treated with 4 kGy showed the highest values. It has been reported that irradiation can break the high-molecular-weight carbohydrates into small units; consequently, an increase in the numbers of double bonds and carboxyl and carbonyl groups is produced, and these can react with other components of the food as proteins, yielding a more compact matrix that could increase the cohesion of the product (D'AMOUR *et al.*, 1993; KORKMAZ and POLAT, 2005; CHOI *et al.*, 2009).

Springiness showed slight changes during storage and irradiation treatment. Chewiness behaved similarly to the hardness, this is expected, because chewiness is a secondary parameter dependent on hardness, springiness and cohesiveness (BOURNE, 1978).

Table 4 - Influence of irradiation and storage time in texture parameters of control sausages.

Parameter	Time (months)	Irradiation dosage		
		0 kGy	2 kGy	4 kGy
Hardness (N)	0	109.94±6.41bAB	114.77±15.62bA	97.53±10.92bB
	1	121.19±13.9abAB	133.16±2.10aA	111.86±13.99aB
	2	120.44±4.78aA	121.80±4.78abA	114.87±12.42aA
	3	111.35±6.74bA	121.78±10.61abA	109.93±8.75aA
Cohesiveness (ratio)	0	0.56±0.06aA	0.56±0.08aA	0.58±0.05bA
	1	0.55±0.01aB	0.58±0.04aAB	0.61±0.01abA
	2	0.60±0.01aA	0.55±0.03aB	0.63±0.04aA
	3	0.58±0.03aAB	0.57±0.02aB	0.60±0.02abA
Springiness (cm)	0	0.43±0.05aA	0.37±0.01cB	0.45±0.03bA
	1	0.46±0.02aA	0.45±0.04bB	0.49±0.03abA
	2	0.44±0.02aA	0.47±0.07abA	0.46±0.05bA
	3	0.47±0.03aB	0.52±0.06aA	0.54±0.02aA
Chewiness (N cm)	0	26.33±5.34 bA	23.38±3.77bA	24.03±5.46bA
	1	30.67±4.24abA	34.81±4.80aA	33.50±2.75aA
	2	31.99±2.22aA	31.01±8.73abA	33.26±4.41aA
	3	28.95 ±3.41abA	36.52±6.27aA	35.80±4.10aA

Significant differences among storage times to each studied parameters are shown by minor letters in the same column ($p<0.05$).
Significant differences among irradiation dosages at each storage time are shown by mayor letters in the same row ($p<0.05$).

Table 5 - Influence of irradiation and storage time in texture parameters of sausages containing dry tomato peel.

Parameter	Time (months)	Irradiation dosage		
		0 kGy	2 kGy	4 kGy
Hardness (N)	0	113.52±6.07bAB	118.15±15.62bA	106.84±8.98bB
	1	131.98±6.37aA	137.00±6.66aA	126.8±16.25aA
	2	131.97±7.98aA	138.67±7.20aA	132.50±15.86aA
	3	134.40±9.19aA	135.49±4.07abA	133.82±14.05aA
Cohesiveness (ratio)	0	0.51±0.01bC	0.58±0.05bA	0.57±0.03cA
	1	0.55±0.04bB	0.59±0.034abB	0.60±0.03bcA
	2	0.53±0.04bC	0.62±0.04abB	0.68±0.09aA
	3	0.62±0.06aA	0.63±0.01aA	0.65±0.06abA
Springiness (cm)	0	0.42±0.04bcA	0.37±0.04aB	0.35±0.03bB
	1	0.48±0.05aA	0.37±0.01aB	0.35±0.03bB
	2	0.43±0.04bA	0.34±0.04aB	0.41±0.01aA
	3	0.39±0.05cA	0.34±0.01aB	0.36±0.06bAB
Chewiness (N cm)	0	24.64±3.79bA	25.31±3.17aA	21.36±2.84cA
	1	35.01±7.57aA	30.03±0.79aAB	26.44±4.09bcB
	2	30.48±4.29abA	29.18±4.39aA	36.67±9.23aA
	3	33.09±4.08aA	29.09±5.75aA	32.07±6.41abA

Significant differences among storage times to each studied parameters are shown by minor letters in the same column ($p < 0.05$).
Significant differences among irradiation dosages at each storage time are shown by mayor letters in the same row ($p < 0.05$).

Sensory analysis

In general terms, the sausages added with DTP showed lower punctuations than the control ones (Fig. 2). Significant difference ($p<0.05$) between irradiated and non-irradiated samples were observed independently of the time of storage or DTP content. The samples treated with 4 kGy always ranked in the last place, the panellist indicated that these samples presented off-flavours. Samples treated at 2 kGy showed lower score than non-irradiated samples throughout the storage period; however the values were always higher than 3 (acceptable).

These results are consistent with previous reports which indicate that the application of irradiation to these and other meat products induce the development of off-flavours commonly described as rancid, pungent or "puckery feel" (CABEZA *et al.*, 2009; BREWER, 2009; AHN and LEE, 2006). These flavours can explain the low scores given by the members of the panel to the samples irradiated to 4 kGy.

CONCLUSION

Doses of 2 kGy are adequate to obtain safe and potentially functional RTE dry fermented sausages enriched with lycopene. These doses no induce relevant physico-chemical or sensory changes, even after 90 days of storage. The final concentration of this carotenoid is sufficient to

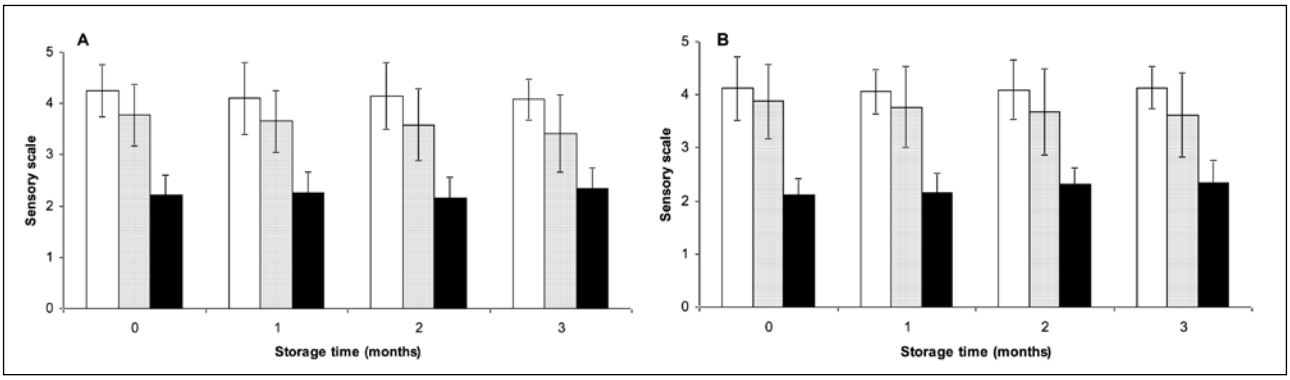


Fig. 2 - Effect of storage time on the overall acceptability of dry fermented sausages containing 0 (A) or 2% (w/w) of dry tomato peel (B), irradiated at 0 (□), 2 (▒) or 4 (■) kGy. Hedonic scale (1-extremely poor, 5-excellent).

that 100 g assure the 24% of the intake recommended as healthy.

ACKNOWLEDGEMENTS

We wish to thank María José Rodríguez for technical support. This work has been funded by the Spanish Science and Technology Commission (Comisión Interministerial de Ciencia y Tecnología, projects AGL2007-6366; Consolider-Ingenio2010 CSD2007-00016), UCM-Santander (Ref. GR58/08 Grupo 920276).

REFERENCES

Ahn D.U. and Lee E.J. 2006. Mechanisms and prevention of quality changes in meat by irradiation. In: "Food irradiation research and technology". C.H. Sommers and X. Fan (Eds.), p. 127. IFT Press, Blackwell Publishing, Iowa: USA.

Ahn D.U., Olson D.G., Jo C., Chen X., Wu C., and Lee J.I. 1998. Effect of muscle type, packaging and irradiation on lipid oxidation volatile production and color in raw pork patties. Meat Sci. 49: 27.

Alfaia C.M.M., Ribeiro P.J.L.C., Trigo M.J.P., Alfada A.J.I., Castro M.L.F., Fontes C.M.G.A., Bessa R.J.B., and Prates J.A.M. 2007. Irradiation effect on fatty acid composition and conjugated linoleic acid isomers in frozen lamb meat. Meat Sci. 77: 689.

Artés F., Mínguez M.I. and Hornero D. 2002. Fruit pigments. In: "Colour in foods". B. Douglas (Ed.), p. 248. MacDougall Woodhead Publishing Ltd.,Cambridge, England.

Aymerich T., Picouet P.A. and Monfort J.M. 2008. Decontamination technologies for meat products. Meat Sci. 78: 114.

Bourne M.C. 1978. Texture profile analysis. Food Technol. 32: 62.

Brewer M.S. 2009. Irradiation effects on meat flavour: A review. Meat Sci. 81: 1.

Cabeza M.C., Cambero I., Hoz de la L. and Ordóñez J.A. 2007. Optimization of E-bean irradiation treatment to eliminate *Listeria monocytogenes* from ready-to-eat (RTE) cooked ham. Innov. Food Sci. Emerg. Technol. 8: 299.

Cabeza M.C., de la Hoz L., Velasco R., Cambero M.I. and Ordóñez J.A. 2009. Safety and quality of ready-to-eat dry fermented sausages subjected to E-beam radiation. Meat Sci. 83: 320.

Calvo M.M., Dado D. and Santa-María G. 2007a. Influence of extraction with ethanol or ethyl acetate on the yield of lycopene, β-carotene, phytoene and phytofluene from tomato peel powder. Eur. J. Food Res. Technol. 224: 567.

Calvo M.M., García M.L. and Selgas M.D. 2008. Dry fermented sausages enriched with lycopene from tomato peel. Meat Sci. 80: 167.

Calvo M.M., Rodríguez M.J., Santa-María G., Selgas M.D. and García M.L. 2007b. Productos cárnicos y de la pesca enriquecidos en licopeno mediante la adición de piel de tomate. Patent ES 200701670.

Candogan K. 2002. The effect of tomato paste on some quality characteristics of beef patties during refrigerated storage. Eur. J. Food Res. Technol. 215: 305.

Choi J., Kim H.J., Kim J.H., Byun M.W., Chun B.S., Ahn D.H., Hwang Y.J., Kim D.J., Kim G.H. and Lee J.W. 2009. Application of gamma irradiation for the enhanced physiological properties of polysaccharides from seaweeds. Appl. Radiat. Isotopes 67: 1277.

Choski P.M. and Joshi V.Y. 2007. A review on lycopene - Extraction purification, stability and applications. Int. J. Food Prop. 10: 289.

Chouliara I., Samelis J., Kakouir A., Badeka A., Savvaidis I.N., Rigankos K. and Kontominas M.G. 2006. Effect of irradiation of frozen meat/fat trimmings on microbiological and physico-chemical quality attributes of dry fermented sausage. Meat Sci. 74: 303.

D'Amour J., Gosselin C., Arul J., Castaigne F. and Willemont C. 1993. Gamma radiation affects cell wall composition of strawberries. J. Food Sci. 58: 182.

Deda M.S., Bloukas J.G. and Fista G.A. 2007. Effect of tomato paste and nitrite level on processing and quality characteristics of frankfurters. Meat Sci. 76: 501.

Erdman J.W., Ford N.A. and Lindshield B.L. 2009. Are the health attributes of lycopene related to its antioxidant function? Arch. Biochem. Biophys. 483: 229.

Farkas J. and Andrassy E. 1998. Interaction of ionizing radiation with other physical and chemical factors in improving the safety and storage stability of foods. In: "Combination Processes for Food Irradiation". p. 15. Vienna International Atomic Energy Agency The Editorial Staff of the IAEA Panel Processing Series.

Flores M., Nieto P., Ferrer J.M. and Flores J. 2005. Effect of calcium chloride on the volatile pattern and sensory acceptance of dry fermented sausages. Eur. J. Food Res. Technol., 221: 624.

García M.L., Calvo M.M. and Selgas M.D. 2009. Dry tomato peel as font of lycopene for hamburgers. Meat Sci. 83: 43.

García M.L., Domínguez R., Gálvez, M.D., Casas C. and Selgas, M.D. 2002. Utilization of cereal and fruit fibres in low fat fermented sausages. Meat Sci. 60: 227.

Gimeno O., Astiasarán I. and Bello J. 2001. Calcium ascorbate as a potencial partial substitute for NaCl in dry fermented sausages: effect on colour, texture and hygienic quality at different concentrations. Meat Sci. 57: 23.

Jakobsen M. and Bertelsen G. 2002. Modelling colour stability. In: "Colour in foods". B. Douglas (Ed.) p. 23, MacDougall Woodhead Publishing Ltd. Cambridge, England.

Javanmard M., Rokni N., Bokai S. and Shahhosseini G. 2006. Effects of gamma irradiation and frozen storage on microbial, chemical and sensory quality of chicken meat in Iran. Food Control 17: 469.

Kavanaugh C.J., Trumbo P.R. and Ellwood K.C. 2007. The US food and drug administration's evidence-based review for qualified health claims: Tomato, lycopene and cancer. J. Nat. Cancer Inst. 99: 1074.

Kim I.S., Jin S.K, Kang S.N., Hur I.C. and Choi S.Y. 2009a. Effect of olive-oil prepared tomato powder (OPTP) and refining lycopene on the physicochemical and sensory characteristics of seasoned raw pork during storage. Korean J. Food Sci. Animal Resour. 29: 334.

Kim I.S., Jin S.K., Hur I.C., Choi S.Y., Jung H.J., Lee J.K., Kang S.H., Woo G.M. and Kang S.N. 2009b. Effect of tomato powder on meat patties as nitrite alternatives. Korean J. Food Sci. Animal Resour. 29: 382.

Knoblisch M., Anderson B. and Latshaw D. 2005. Analyses of tomato peel and seed byproducts and their use as a source of carotenoids. J. Sci. Food. Agric. 85: 1166.

Korkmaz M. and Polat M. 2005. Irradiation of fresh fruit and vegetable. In: "Improving the safety of fresh fruit and vegetables, Part III. Preservation Techniques". Wim J. (Ed.), p. 387. Woodhead Publishing Ltd. Cambridge, England.

Lee E.J. and Ahn D.U. 2005. Quality characteristics of irradiated turkey breast rolls formulated with plum extract. Meat Sci. 71: 300.

Lim D.G., Seol K.H., Jeon H.J., Jo C. and Lee M. 2008. Application of electron-beam irradiation combined with antioxidants for fermented sausage and its quality characteristics. Radiation Physics Chem. 77: 818.

Mendoza E., García M.L., Casas C. and Selgas M.D. 2001. Inulin as fat substitute in low fat, dry fermented sausages. Meat Sci. 57: 387.

Nam K.C. and Ahn D.U. 2003. Effects of ascorbic acid and antioxidants on the color of irradiated ground beef. J. Food Sci. 68: 1686.

O'Bryan C.A., Crandall P.G., Ricke S.C. and Olson D.G. 2008. Impact of irradiation on the safety and quality of poultry and meat products: a review. Crit. Rev. Food Sci. Nut. 48: 442.

Oliver J., Palou A. and Pons A. 1998. Semi-quantification of carotenoids by high-performance liquid chromatography: Saponification induced losses in fatty foods. J. Chrom. A, 829: 393.

Omoni A.O. and Aluko R.E. 2005. The anti-carcionogenesis

and anti-atherogenic effects of lycopene: A review. Trends Food Sci. Technol. 16: 344.

Ordóñez J.A., Hierro E.M., Bruna J.M. and De la Hoz L. 1999. Changes in the components of dry-fermented sausages during ripening. Crit. Rev. Food Sci. Nutr. 39*:* 329.

Pérez-Alvarez J.A., Sayas-Barberá M.E., Fernández-López J. and Aranda-Catalá A. 1999. Physicochemical characteristics of spanish-type dry-cured sausage. Food Res. Int. 32: 599.

Rao A.V. (Ed.) 2006. "Tomatoes, lycopene and human health". Ed. Scotland: Caledonian Science Press.

Rao A.V. and Shen H.L. 2002. Effect of low dose lycopene intake on lycopene bioavailability and oxidative stress. Nutr. Res. 22: 1125.

Ross A.I.V., Griffiths M.W., Mittal G.S. and Deeth H.C. 2003. Conbining nonthermal technologies to control foodborne microorganisms. Int. J. Food Microbiol. 89: 125.

Rubio B., Martínez B., García-Cachán M.D., Rovira J. and Jaime I. (2008). Effect of packaging method and the storage time on lipid oxidation and colour stability of dry fermented sausage salchichón manufactured with raw material with a high level of mono and polyunsaturated fatty acids. Meat Sci. 80: 1182.

Salazar P., García M.L. and Selgas M.D. 2009. Short-chain fructooligosaccharides as potential functional ingredient in dry fermented sausages with different fat levels. Int. J. Food Sci. Technol. 44: 1100.

Samelis J., Kakouri A., Savvaidis I.N., Riganakos K. and Kontominas M.G. 2005. Use of ionizing radiation doses of 2 and 4 kGy to control *Listeria* ssp. and *Escherichia coli* O157:H7 on frozen meat trimmings used for dry fermented sausage production. Meat Sci. 70: 189.

Satin M. 2002. Use of irradiation for microbial decontamination of meat: situation and perspectives. Meat Sci. 62: 277.

Selgas M.D., García M.L. and Calvo M.M. 2009. Effect of irradiation and storage on the physico-chemical and sensory properties of hamburgers enriched with lycopene. Int. J. Food Sci. Technol. 44*:* 1983.

Shi, J. and Le Maguer, M. 2000. Lycopene in tomatoes: Chemical and physical properties affected by food processing. Crit. Rev. Food Sci. Technol. 40: 1.

Smith J.S. and Pillar S. 2004. Irradiation and food safety. Food Technol. 58: 48.

Zhu M., Du M., Cordray J. and Ahn D.U. 2005. Control of *Listeria monocytogenes* contamination in ready-to-eat meat products. Comp. Rev. Food Sci. Food Saf. 4: 34.

Paper received October 15, 2010 Accepted January 18, 2011

PAPER

CONTENT OF SELECTED NUTRIENTS IN SOUR CHERRIES, BLACKCURRANTS AND APPLES OSMODEHYDRATED IN REUSED FRUCTOOLIGOSACCHARIDE CONCENTRATE

M. UCZCIWEK[A], E. PIASECKA[A], R. KLEWICKI[A]*, D. KONOPACKA[B], M. MIESZCZAKOWSKA-FRĄC[B] and C. BONAZZI[C]

[A] Institute of Chemical Technology of Food, Technical University of Łódź, 4/10 Stefanowskiego Street 90- 924 Łódź, Poland

[B] Research Institute of Horticulture, 18 Pomologiczna Street, 96-100 Skierniewice, Poland

[C] INRA, UMR 1145 Ingenierie Procedes Aliments, 1 Avenue des Olympiades, F-91300 Massy, France

*Corresponding author: Tel. +48 42 6313454, Fax +48 42 6367488, email: robert.klewicki@p.lodz.pl

ABSTRACT

The objective of the study was to examine the possibility of reusing an osmotic solution for fruit dehydration and its impact on fruit composition. Sour cherries, blackcurrants and apples were dehydrated at 40°C for 60 min with 60°Bx fructooligosaccharide concentrates. The solutions were used 10 times. The levels of saccharides, polyphenols, vitamin C and acidity were determined. The present study shows that fructooligosaccharide syrups can be successfully reused for fruit dehydration. The successive batches of osmodehydrated fruit were similar as regards the content and profile of carbohydrates and polyphenols. The only difference was the increasing acidity of osmodehydrated sour cherries and blackcurrants. The average content of prebiotic fructooligosaccharides amounted to 135 g kg^{-1} in apples, 99 g kg^{-1} in sour cherries and 104 g kg^{-1} in blackcurrants. The level of total phenols was: 0.80, 3.20 and 5.10 g kg^{-1}, respectively.

- Key words: acidity, ascorbic acid, carbohydrates, osmotic dehydration, polyphenols, syrup reutilization -

INTRODUCTION

New, better technologies for food production and preservation are constantly being sought after. An important objective of the food industry is to preserve food in such a way as to minimize changes to the chemical, physical, and biological characteristics of the product (RASTOGI *et al.*, 2002). It is also very important to obtain products with desirable sensory qualities. The removal of water, which enhances the durability of food products, is one of the most important issues in food technology. Osmotic dehydration is a method of dewatering without any phase changes in hypertonic solutions (containing sugars, sodium chloride, sorbitol, glycerol, etc.) under mild conditions, which makes it possible to obtain high quality products. Thus obtained fruits are characterized by attractive sensory qualities while preserving the majority of the nutritional properties of fresh fruits, *i.e.* vitamins and minerals (SAPATA *et al.*, 2009). Additionally, the application of osmotic dehydration may improve the health-promoting properties of the products as a result of migration of valuable substances from hypertonic solutions to fruit tissue. The use of fructooligosaccharide concentrates makes it possible for fruit to gain desirable prebiotic properties as oligosaccharides promote growth of beneficial lactic acid bacteria in the gut (SANGEETA *et al.*, 2005). Moreover, fructooligosaccharides (FOSs) are characterized by lower energy value [approx 10 kJ g^{-1}] compared to saccharose [17 kJ g^{-1}] (LINDEN and LORIENT, 1999), which makes it possible to obtain lower-calorie products (AACHARY and PRAPULLA, 2009). On the other hand, the susceptibility of FOSs to hydrolysis is an important limitation for the use of these carbohydrates in osmotic dehydration. They are especially labile at low pH and high temperature (L'HOMME *et al.*, 2003). Long storage also contributes to the lowering of FOS levels (BENKEBLIA *et al.*, 2007). However, dehydration under mild conditions may result in considerable reduction of FOS degradation (KLEWICKI and UCZCIWEK, 2008). Osmodehydrated products may be used for direct consumption, or, if further dried, may constitute a flavor ingredient in a variety of food products, *e.g.* cakes, confectionery filling, dairy products, ice-cream, etc. (RASTOGI *et al.*, 2002). Over the past several years, many studies have been published on osmotic dehydration. Substantial knowledge has been acquired about the impact of numerous factors such as temperature, the concentration and nature of the osmotic solution, process duration and pressure, the size, geometry and structure of the material to be dehydrated, and their influence on mass transfer (TORTOE *et al.*, 2007). But the large-scale application of osmotic dehydration is still limited by the continuing problem of optimal osmotic solution management. For both environmental and economic reasons it is necessary to reuse the dehydrating solution as much as possible in order to reduce the ratio of osmotic solution volume to the mass of the dehydrated fruit (MOYANO and ZÚÑIGA, 2003).

Once the osmotic solution has been used in the process, it is usually treated as industrial waste and rarely reused. This significantly increases the cost of the process. Furthermore, the disposal of the osmotic solution may lead to loss of some valuable natural substances such as vitamins and minerals, which are partially leached from the fruits into the solution during dehydration. The possibility to recycle the osmotic solution remains a crucial economic and ecological problem (GARCIA-MARTINEZ *et al.*, 2002; MORAGA *et al.*, 2011).

The sanitary aspects of reusing solutions have been studied (DALLA ROSA and GIROUX, 2001), but the influence of the repeated use of the syrup on the nutritional quality of the products is also an important question. Data on the effect of multiple use of a hypertonic solution on nutrients and other bioactive substances present in different fruits are rather limited (VALDES-FRAGOSO *et al.*, 2002; RUIZ *et al.*, 2005; PEIRO *et al.*, 2006; MORAGA *et al.*, 2011). The objective of this work was to examine the influence of repeated use of a hypertonic fructooligosaccharide (FOS) solution on saccharides, polyphenols, vitamin C, and fruit acidity.

MATERIALS AND METHODS

Plant materials

Sour cherries ("English Morello"), blackcurrants ("Tiben"), and apples ("Idared") were chosen as the experimental material. The fruits were picked at commercial harvest maturity at the Experimental Orchard of the Research Institute of Pomology and Floriculture in Dąbrowice, Poland. Fresh sour cherries were washed, pitted, and frozen at -25°C until processing. Blackcurrants were frozen without pre-treatment, but on the day of processing frozen berries were abraded in the carborundum drum of a kitchen food processor (Talent 881.0; Zelmer, Rzeszów, Poland). Apples were stored in a cold room (0°C; 90% RH, normal atmosphere). Before processing, apples were warmed to 20°C and cut into 1 cm cubes. The quality characteristics of raw material are shown in Table 1.

Chemicals and standards

HPLC acetonitrile (gradient grade) and formic acid were purchased from J.T. Baker (Witko, Poland). Ultrapure water (Millipore System) was used to prepare all solutions. Methanol for the extraction of polyphenols was purchased from J.T. Baker (Witko, Poland). Standards of cyanidin-3-rutinoside, myricetin, and kaempferol-3-

glucoside were purchased from Extrasynthese (Genay, France); quercetin, kaempferol, quercetin-3-rutinoside, and (-)-epicatechin were purchased from Sigma-Aldrich. The ascorbic acid standard and sugar standards were purchased from Sigma.

Osmotic dehydration

Osmotic dehydration was conducted with the use of 60°Bx fructooligosaccharide concentrates containing 333 g kg^{-1} of fructooligosaccharides, 84 g kg^{-1} of saccharose, 156 g kg^{-1} of glucose and 9 g kg^{-1} of fructose. Each time a 150 g sample of fruit was dewatered with a 1:4 ratio of fruit/osmotic solution (w/w). Dehydration was performed for 60 min in beakers placed in a shaking water bath (Julabo SW 22, Julabo Labortechnik GmbH, Seelbach, Germany) at a frequency of 140 rpm, an amplitude of 20 mm and a temperature of 40°C. The solutions were used 10 times, and some fresh 74°Bx solution was added to the remaining syrup before each subsequent dehydration cycle in order to maintain a constant concentration of 60°Bx. The amounts of used and fresh syrup which have to be mixed in order to obtain 400 g of enriched solution were calculated according to the following formulas:

$$X = \frac{400(B-60)}{B-A} \quad [g]$$

$$Y = \frac{400(60-A)}{B-A} \quad [g]$$

where:

A - concentration of used syrup measured after fruit drainage, °Brix;

B - concentration of fresh concentrate, °Brix (70°Brix);

X - amount of used syrup to be added to a new batch (of concentration A);

Y - amount of fresh concentrate to be added to a new batch (of concentration B).

The average amount of syrup (53÷54°Bx) taken for the subsequent apple dehydration cycles (batches 2-10) was 427±8 g (± standard deviation), while the average amount of fresh FOS concentrate was 179±8 g. The corresponding values for sour cherries were 407±7 g and 198±7g, respectively. In the case of blackcurrants, the amounts were as follows: 436±14 g and 167±14 g, respectively.

After removing from the solution, the fruits were placed on a sieve, dipped twice into water, and, finally, blotted on absorbent paper.

The experiment was repeated. Saccharide, polyphenol, and vitamin C content as well as acidity were determined in osmodehydrated fruit.

Carbohydrate determination

The content of carbohydrates was determined by the HPLC method using a cation exchange calcium column. The extraction was carried out as follows. Twenty-five mL of water and 1 g of $CaCO_3$ were added to 2.5 g of the ground fruit. The suspension was heated to the boiling point and maintained at this temperature for 5 min. The solution was then cooled, transferred quantitatively by means of water to a 50-mL volumetric flask, filled up to the graduation mark, kept at room temperature for 15 min, and filtered. Then, the filtrate was cleaned on a SPE column containing cation exchanger resin Amberlite IR120 and anion exchanger resin Amberlite IRA67 at a ratio of 1:2 (v/v). Subsequently, the samples were centrifuged for 5 min at 5,000 rpm, and injected into the HPLC column. The content of sugars in the samples was determined with the use of an Aminex HPX87C column from Bio-Rad (0.78 × 30 cm^2, mobile phase: water, flow: 0.5 mL/min, at 85°C). Standards of saccharose, glucose and fructose were used for the identification and quantification of the substances mentioned. An RI detector (Knauer K-2301) and a Knauer integrating system were used. The results are expressed as grams of an individual sugar per 1 kg of fruit (g/kg).

Extraction of polyphenols

Polyphenol compounds were extracted with a methanol-water-formic acid solution (50:48:2 (v/v/v)). Half a gram of ground fruit was placed in a 7-mL test tube, 5 mL of the solvent was poured on it, then it was vortexed and sonified for 5 min. After the sonication, the solution was left in the dark for 24 h for extraction, and subsequently centrifuged and poured into a flask. The above procedure was repeated four times, but the next extractions were carried out for 15 min. The combined extracts were poured into a 25-mL volumetric flask.

Total phenols determination

Total phenols were measured by the method described by Singleton and Rossi (1965) with some modification. Phenol extract (0.25 mL) was placed in 25-mL flasks, and 0.25 mL of Folin-Ciocalteau reagent and 2.5 mL of 20% Na_2CO_3 were added, filled up with water to the graduation mark and stirred. The incubation was carried out at room temperature for 1 h. The absorbance of the solutions was measured at 720 nm. The results were expressed in grams of (-)-epicatechin equivalent per kilogram of fruit (g CAE/kg). All samples were analyzed in duplicate.

HPLC analysis of polyphenols

Before analysis, the extracts were centrifuged at 5,000 rpm. Anthocyanins and other phenols

were analyzed using a KNAUER Smartline chromatograph (Germany). Compounds in the phenol extracts were separated on a 150 mm × 4.6 mm i.d., 5 μm Gemini 5u C18 110A column (Phenomenex, Torrance, CA) using gradient elution with 10% aqueous formic acid (v/v) (A) and acetonitrile-water-formic acid (50:40:10 (v/v/v)) (B). The column temperature was set to 40°C. The flow rate was 1 mL/min and the gradient program was as follows: 0-0.6 min, 12% B; 0.6-16 min 12-30% B; 16-20.5 min 30-100% B; 20.5-22 min 100% B; 22-25 min 100-12% B, 25-35 min 12% B. The injection volume was 20 μL. Data were collected by the Eurochrom 2000 program. Quercetin and myricetin compounds were assayed at 360 nm, while anthocyanins were assayed at 520 nm. Standard curves made from cyanidine-3-rutinoside, rutin, quercetin, myricetin, and kaempferol were used. Cyanidin-3-rutinoside was used to assay anthocyanins, whereas rutin was used to assay quercetin and myricetin glycosides. Isorhamnetin was assayed on the basis of the quercetin standard.

Ascorbic acid determination

One to 5 g of ground fruits were subjected to extraction using a 6% metaphosphoric acid solution (v/v) at 1°-4°C. After 2-min homogenization, the sample was transferred into a volumetric flask, filled up with metaphosphoric acid, and filtered. After dilution and repeated filtration, the content of vitamin C was determined by using the HPLC method (Agilent HP 1100 chromatograph with a DAD detector, 244 nm; two interlinked columns Supelco LC-18 [250 x 4 mm]; 30°C; mobile phase: 1% phosphoric buffer [pH = 2.5], flow rate: 0.8 mL/min). Analyses were done in duplicate.

Titratable acidity

Titratable acidity of samples was determined according to the AOAC official method 942.15. Results were expressed in g kg^{-1} of fruit, as anhydrous malic acid.

Statistics

The statistical study was carried out using one-way analysis of variance ANOVA, taking the cycle factor into account. Differences were considered significant at $p \leq 0.05$.

RESULTS

All batches of dehydrated apples were similar as regards their carbohydrate profiles (Fig. 1). The average content of carbohydrates amounted to 135±13 g kg^{-1} for fructooligosaccharides, 64±5 g kg^{-1} for saccharose, 72±6 g kg^{-1} for glucose, and 41±4 g kg^{-1} for fructose. The ANOVA test did not reveal any statistically significant differences in the content of particular saccharides between the batches of osmotically dehydrated apples. Osmotic dehydration of apples (with the osmotic syrup reused 20 times) was also investigated by VALDES-FRAGOSO *et al.* (2002). They used a 60°Bx saccharose solution for 90 min with a 5:1 osmotic solution/fruit ratio. The variance analysis of water loss and solids gain did not show any statistically significant differences between the batches, either.

Similar results were obtained for sour cherries. Fruits from all batches were similar with respect to the content of carbohydrates (Fig. 2): fructooligosaccharides (99±15 g kg^{-1}), saccharose (9±2 g kg^{-1}), glucose (115±6 g kg^{-1}), and fructose (63±4 g kg^{-1}). No statistically significant differences in the content of particular saccharides between the batches of osmotically dehydrated sour cherries were observed.

Greater variation in saccharide content was found in blackcurrant products (Fig. 3): fructooligosaccharides (104±16 g kg^{-1}), saccharose (35±6 g kg^{-1}), glucose (67±10 g kg^{-1}), and fructose (35±8 g kg^{-1}). The ANOVA test showed that the differences in the content of the various saccharides in the dehydrated blackcurrants were statistically significant ($\alpha \leq 0.05$), but no marked upward or downward trends were observed in the series of dehydration cycles. These differ-

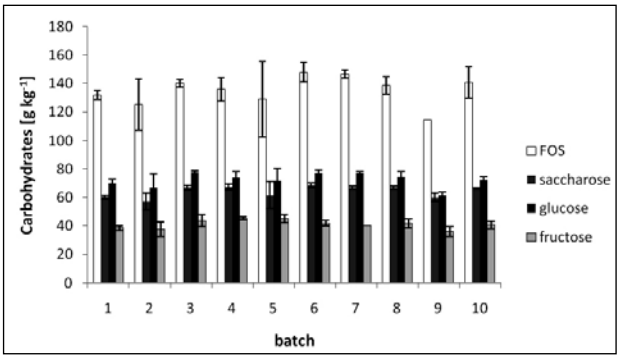


Fig. 1 - Carbohydrate content (average±standard deviation) in apples dehydrated in reused FOS syrup. ANOVA test ($\alpha \leq 0.05$): FOS P=0.24, saccharose P=0.12, glucose P=0.10, fructose P=0.12.

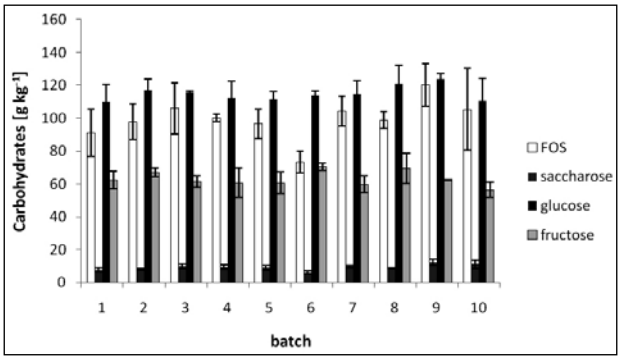


Fig. 2 - Carbohydrate content (average±standard deviation) in sour cherries dehydrated in reused FOS syrup. ANOVA test ($\alpha \leq 0.05$): FOS P=0.18, saccharose P=0.06, glucose P=0.77, fructose P=0.34.

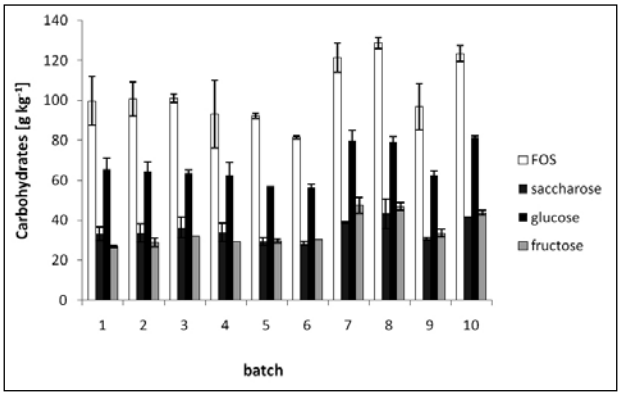


Fig. 3 - Carbohydrate content (average±standard deviation) in blackcurrants dehydrated in reused FOS syrup. ANOVA test ($\alpha \leq 0.05$): FOS P=0.003, saccharose P=0.03, glucose P=0.0002, fructose P=0.000001.

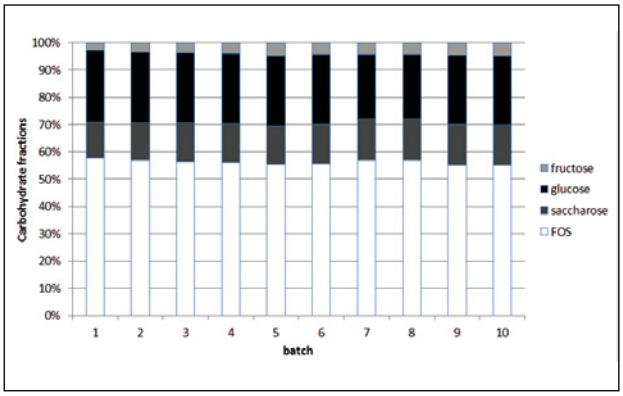


Fig. 4 - Fractions of fructooligosaccharides, saccharose, glucose and fructose in the total sugars of reused FOS syrup after dehydration of apples.

ences may have been caused by the pretreatment of the material used in the batches. Before dehydration, the blackcurrants were subjected to abrasion and the differences in the amount of skin (which blocks saccharide migration) removed are likely to have influenced saccharide content in the final product.

The content of individual carbohydrates in the raw material (Table 1) and the carbohydrate profile of the fructooligosaccharide concentrate (see *Osmotic dehydration* in MATERIALS AND METHODS) influenced the saccharide composition of the osmo-dehydrated fruits. The above-mentioned results indicate that in the processed apples and blackcurrants fructooligosaccharides were the major fraction of soluble carbohydrates. In the case of sour cherries, only the content of glucose was higher than that of FOSs. This is due to the fact that fructooligosaccharides were the predominant component of the hypertonic solution used for dehydration. A high glucose level in the hypertonic solution also resulted in the large amount of this sugar in the osmo-dehydrated material. The highest increase in glucose content in dry matter was observed for apples: from 72 g kg^{-1} in the fresh fruits to 199 g kg^{-1} in the processed ones. The corresponding values for blackcurrants amounted to 105 and 207 g kg^{-1}, respectively. In sour cherries (containing the highest amounts of glucose in the raw material) only a slight increase occurred: from 325 g kg^{-1} in the fresh fruits to 342 g kg^{-1} in the processed ones. On the other hand, as the hypertonic solution was poor in fructose, its content (in dry matter) was lower than that in the fresh fruits. The average content of fructose in the dry matter of the processed blackcurrants, apples and sour cherries amounted to: 108, 113 and 188 g kg^{-1}, respectively, while its content in the fresh fruits was: 151, 377 and 254 g kg^{-1}, respectively.

The results show that despite the relatively high acidity of blackcurrants, fructooligosaccharides are stable under the process conditions (Fig. 6). The comparable content of carbohydrates in the two previously mentioned products (dry apples and sour cherries) also indicates that no significant hydrolysis of fructooligosaccharides occurred during dehydration (Figg. 4-5). This is important because fructooligosaccharide hydrolysis may occur if the process is carried out in an acidic environment at higher temperatures. BOLIN *et al.* (1983) investigated changes in a saccharose solution which was used five times for dehydrating fruits. They claimed that the observed saccharose inversion was due to acid hydrolysis. The inversion was enhanced by acid leaching out of the fruits. Another cause of hydrolysis may be the presence of invertase. Its influence was observed by VIBERG *et al.* (1998) on dehydrating strawberries in a saccharose solution.

Table 1 - Characteristics of raw material.

	Sour cherries "English Morello"	Blackcurrants "Tiben"	Apples "Idared"
Dry matter [g kg^{-1}]	143	193	161
Saccharose [g kg^{-1}]	2.80	10.8	49.4
Glucose [g kg^{-1}]	46.5	20.3	11.6
Fructose [g kg^{-1}]	35.0	29.1	60.7
Acidity [g kg^{-1}]	10.6	40.4	5.60
Ascorbic acid [g kg^{-1}]	0.003	1.01	0.24
Total polyphenols [g kg^{-1}]	3.64	4.36	2.82

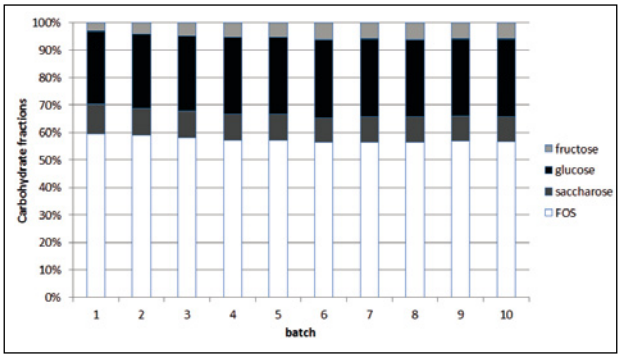


Fig. 5 - Fractions of fructooligosaccharides, saccharose, glucose and fructose in the total sugars of reused FOS syrup after dehydration of sour cherries.

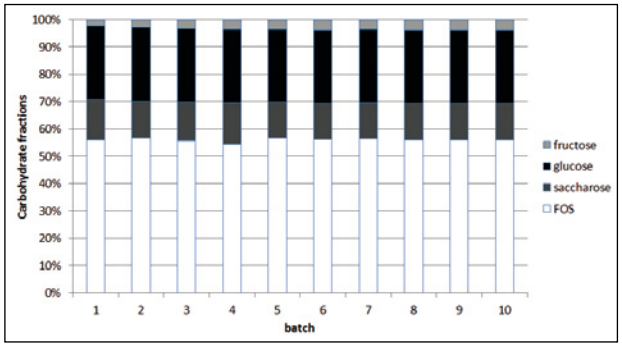


Fig. 6 - Fractions of fructooligosaccharides, saccharose, glucose and fructose in the total sugars of reused FOS syrup after dehydration of blackcurrants.

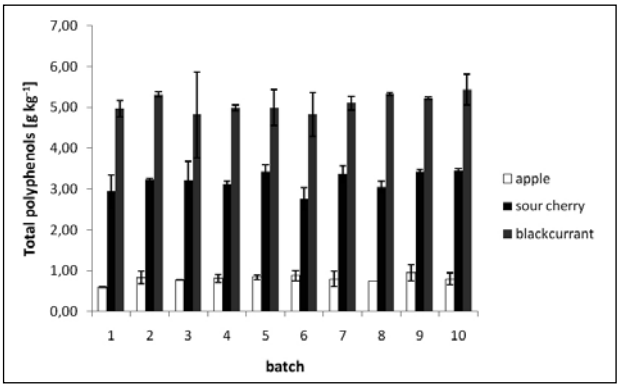


Fig. 7 - Polyphenol content (average±standard deviation) in apples, sour cherries and blackcurrants dehydrated in reused FOS syrup. ANOVA test ($\alpha \leq 0.05$): apples P=0.40, sour cherries P=0.15, blackcurrants P=0.83.

Saccharose stability during the osmotic dehydration of pineapples in reused solution was observed by RUIZ *et al.* (2005). In their work on reusing the dehydrating solution five times, they found that glucose and fructose content in the solution before using it and after using it for five times remained nearly constant (14.5 g L^{-1} ÷ 15.8 g L^{-1} and 6.5 g L^{-1} ÷ 7.8 g L^{-1}, respectively). These results indicate that the saccharose present in the solution does not decompose.

Our results proved that fructooligosaccharide syrup can be successfully used a number of times for the dehydration of apples, sour cherries and blackcurrants.

Osmo-dehydrated apples from all batches were characterized by a comparable polyphenol content amounting to 0.60 ÷ 0.95 g kg^{-1} (Fig. 7), with the average value of 0.80±0.09 g kg^{-1}.

In the case of sour cherries, polyphenol levels were significantly higher, from 2.77 g kg^{-1} to 3.45 g kg^{-1} (no direct relationship between batch number and polyphenol content was observed). The average content was 3.20±0.23 g kg^{-1}.

Blackcurrants contained the largest amounts of polyphenols. Their content ranged from 4.82 g kg^{-1} to 5.43 g kg^{-1} (with the average being 5.10±0.22 g kg^{-1}). Products from all the batches were characterized by a similar level of polyphenolic compounds. For all the above-mentioned fruits, no statistically significant differences were found in total polyphenol content between the batches of osmotically dehydrated material.

Total polyphenol levels decreased by 71% in apples as compared to the fresh material, dropped by 12% in sour cherries, but rose by 17% in blackcurrants (in this case, solids gain overweighed the effect of polyphenols "leaching" into the syrup).

There is not much literature on the subject of osmotic dehydration of sour cherries and blackcurrants. The available publications tend to present studies pertaining to the dehydration of apples and other fruit. BLANDA *et al.* (2008) found a 22% drop in polyphenol content in osmodehydrated Stark Delicious apples as compared to the input material. They conducted the process for 30 min at 100 mbar and 25°C, with an 11:1 solution/fruit ratio (v/w), the solution containing 37.9% dextrose, 15.2% saccharose, 1.0% ascorbic acid, 0.25% calcium chloride, and 0.25% sodium chloride. KOPERA *et al.* (2007) osmotically dehydrated pears in a 65°Bx saccharose solution at 25°C and at a 4:1 solution/fruit ratio (v/w). After 6 hours of dehydration they found a 50% decrease in polyphenol content as compared to fresh fruit.

HPLC analyses were performed to find out if the batches of dried fruit were comparable with regard to their polyphenol profile. Materials rich in polyphenols (dried sour cherries and blackcurrants) were tested.

As it can be seen in Tables 2 and 3, all the batches contained similar amounts of particular polyphenols.

In the case of blackcurrants, delfinidin-3-rutinoside occurred in the largest amounts (average value: 956.0±100.0 mg kg^{-1}). The other components of the main polyphenol fraction were: quercetin glucoside + quercetin rutinoside (390.0±25.0 mg kg^{-1}), delfinidin-3-glucoside (417.0±37.0 mg kg^{-1}), cyanidin-3-rutinoside (443.0±43.0 mg kg^{-1}), cyanidin-3-glucoside (125.0±11.0 mg kg^{-1}) and myricetin glycosides (127.0±14.0 mg kg^{-1}). No statistically significant difference in the content of particular polyphe-

Table 2 - Polyphenols assayed by the HPLC method in blackcurrants dehydrated in reused FOS syrup (average±standard deviation) [mg kg^{-1}].

Fraction	Batch				
	01	02	03	04	05
Myricetin glycosides[b]	120.2±8.0	119.6±13.0	105.7±7.0	122.6±9.0	135.0±7.0
Quercetin galactoside[b]	5.1±0.1	5.3±0.1	5.0±0.1	5.8±0.1	5.6±0.1
Quercetin (glucoside + rutinoside)[b]	377.4±47.0	373.0±37.0	354.0±26.0	410.7±10.0	390.9±27.0
Myricetin	7.8±0.1	7.2±0.1	8.2±2.0	6.7±1.0	9.0±1.0
Kaempferol-3-glucoside	18.7±2.0	20.8±1.0	16.9±2.0	22.1±2.0	18.6±1.0
Delphinidin-3-glucoside	414.5±26.0	397.2±46.0	356.3±34.0	409.5±17.0	414.2±1.0
Delphinidin-3-rutinoside[c]	909.1±61.0	892.5±71.0	797.2±57.0	922.8±65.0	1003.4±12.0
Cyanidin-3-glucoside[c]	127.2±7.0	119.1±21.0	108.2±14.0	125.7±4.0	121.7±4.0
Cyanidin-3-rutinoside[c]	432.7±24.0	415.9±55.0	376.0±37.0	433.5±24.0	452.0±8.0
Other anthocyanins[c]	37.6±3.0	37.4±8.0	32.6±4.0	38.8±2.0	39.3±1.0

Fraction	Batch					P
	06	07	08	09	10	
Myricetin glycosides[b]	123.6±22.0	132.0±23.0	143.2±2.0	130.3±5.0	135.1±2.0	0.25
Quercetin galactoside[b]	5.6±0.1	5.4±0.1	5.9±1.0	6.0±0.1	5.6±0.1	0.21
Quercetin (glucoside + rutinoside)[b]	397.0±28.0	413.1±8.0	384.3±14.0	405.1±15.0	394.6±7.0	0.46
Myricetin	8.1±2.0	9.1±0.1	8.7±1.0	9.4±1.0	8.1±1.0	0.43
Kaempferol-3-glucoside	19.4±3.0	19.9±5.0	19.2±0.1	18.8±0.1	20.9±1.0	0.60
Delphinidin-3-glucoside	392.0±47.0	448.1±62.0	455.5±6.0	437.5±14.0	444.4±8.0	0.20
Delphinidin-3-rutinoside[c]	897.2±34.0	1014±85.0	1071±4.0	1018±26.0	1034±40.0	0.14
Cyanidin-3-glucoside[c]	117.6±18.0	134.5±11.0	131.5±2.0	129.3±8.0	132.3±2.0	0.45
Cyanidin-3-rutinoside[c]	420.5±70.0	467.7±61.0	480.7±1.0	471.6±1.0	478.3±10.0	0.25
Other anthocyanins[c]	40.1±3.0	40.6±5.0	43.5±3.0	40.9±0.1	44.3±4.0	0.27

[b] - Expressed as quercetin-3-rutinoside; [c] - Expressed as cyanidin-3-rutinoside.
P - ANOVA test ($\alpha \leq 0.05$).

nols between the batches of osmotically dehydrated blackcurrants was observed.

In the case of sour cherries, cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside were the main polyphenol fractions (Table 3). A slightly lower level of the above-mentioned substances was observed in the dry fruit after the first dewatering (307.7 and 164.3 mg kg^{-1}, respectively). The concentrations of these polyphenols in the subsequent batches of dry sour cherries were comparable, and amounted to 397.0±32.0 mg kg^{-1} and 212.0±16.0 mg kg^{-1}, respectively. The products were marked by similar polyphenol content and the absence of statistically significant differences in the proportion of particular polyphenols between successive batches of osmotically dehydrated sour cherries.

The acidity of successive batches of osmotically dehydrated apples, sour cherries and blackcurrants are shown in Fig. 9.

Dried apple acidity (malic acid) amounted to 3.7 ÷ 4.2 g kg^{-1} and was by 40% lower than that of the fresh material (6.7 g kg^{-1}). Repeated use of the osmotic solution did not have a statistically significant influence on the acidity of the dehydrated apples.

There was a significant increase in the acidity of successive batches of dehydrated sour cherries (from 5.7 to 6.9 g kg^{-1}). The acid levels of the products were 40-50% lower than that of the fresh material (11.0 g kg^{-1}).

Dehydrated blackcurrants also revealed a statistically significant change in acidity, which increased with successive use of the osmotic solution (from 26.8 to 34.3 g kg^{-1}). This resulted from the accumulation of acids in the reused syrup. In previous work, PEIRO *et al.* (2006) observed gradual enrichment of the osmotic syrup in citric acid and ascorbic acid. The content of ascorbic acid rose from 13.0±0.4 mg per 1 kg of syrup in the first cycle to 43.3±0.4 mg kg^{-1} in the eighth cycle, and the content of citric acid increased from 0.72±0.4 g kg^{-1} in the first cycle to 4.14±0.8 g kg^{-1} in the last one. On the other hand, the greatest change in syrup pH was observed during the first cycle (from 6.7±0.2 to 3.50±0.01). After the third cycle, syrup pH equaled that of the fruit (pH = 3.2) and remained at that level in the subsequent cycles. GARCIA-MARTINEZ *et al.* (2002) observed the beneficial effect of decreased pH on inhibiting microorganism growth in the osmotic solution. The presence of acids in the solution may also help to reduce enzymatic browning (VALDEZ-FRAGOSO *et al.*, 1998).

The acidity of the blackcurrant products was 15-30% lower than that of the fresh material.

Table 3 - Polyphenols assayed by the HPLC method in sour cherries dehydrated in reused FOS syrup (average±standard deviation) [mg kg^{-1}].

Fraction	Batch				
	01	02	03	04	05
P-coumaric acid derivative 1[a]	52.3±15.0	55.2±7.0	55.5±5.0	56.1±7.0	60.3±9.0
P-coumaric acid derivative 2[a]	4.0±0.1	4.1±0.1	4.3±0.1	4.8±1.0	5.3±0.1
Neochlorogenic acid[b]	68.1±20.0	71.1±8.0	80.8±9.0	75.1±3.0	78.6±3.0
Chlorogenic acid[b]	42.4±9.0	44.8±8.0	57.4±1.0	53.1±11.0	59.8±13.0
Quercetin glucoside[c]	71.3±9.0	76.8±9.0	73.2±0.1	76.8±5.0	74.6±8.0
Isorhamnetin-3-rutinoside[c]	27.1±3.0	28.6±1.0	28.0±4.0	31.0±3.0	29.8±0.1
Kaempferol-3-rutinoside[d]	12.7±1.0	14.5±1.0	11.0±0.1	14.6±0.1	11.4±2.0
Cyanidin-3-sophoroside[e]	42.3±11.0	49.8±3.0	56.6±3.0	55.3±13.0	64.9±7.0
Cyanidin-3- glucosylrutinoside[e]	307.7±57.0	350.5±20.0	377.7±39.0	362.5±66.0	425.7±9.0
Cyanidin-3-glucoside[e]	12.1±4.0	17.7±3.0	19.4±0.1	17.0±3.0	20.7±3.1
Cyanidin-3- arabinosylrutinoside[e]	10.3±3.0	13.2±1.1	14.0±2.0	13.1±2.0	15.5±1.1
Cyanidin-3-rutinoside[e]	164.3±43.0	197.9±26.0	209.3±2.1	192.6±31.0	234.9±21.1
Peonidin-3-rutinoside[e]	10.8±1.0	12.6±1.1	13.7±1.0	13.0±0.1	15.2±1.0

Fraction	Batch					P
	06	07	08	09	10	
P-coumaric acid derivative 1[a]	57.5±0.1	59.1±4.0	59.5±6.1	54.4±11.0	58.0±2.0	0.98
P-coumaric acid derivative 2[a]	5.1±0.1	4.3±0.1	5.2±0.1	4.6 ±1.1	4.6±1.0	0.09
Neochlorogenic acid[b]	74.1±8.0	71.7±4.0	72.1±2.1	65.0±15.0	70.2±5.2	0.85
Chlorogenic acid[b]	50.4±6.0	49.6±4.2	57.1±5.0	39.9±13.0	53.6±10.1	0.41
Quercetin glucoside[c]	73.4±2.0	68.9±7.0	79.5±2.0	66.9±15.1	70.3±8.2	0.84
Isorhamnetin-3-rutinoside[c]	27.2±7.0	26.2±5.1	29.1±1.0	22.1±2.0	24.4±0.1	0.34
Kaempferol-3-rutinoside[d]	11.9±2.0	11.1±0.0	12.5±0.1	9.4±2.0	9.6±1.2	0.06
Cyanidin-3-sophoroside[e]	64.4±20.0	54.1±6.0	72.4±14.1	65.4±6.0	65.2±14.0	0.33
Cyanidin-3-glucosylrutinoside[e]	415.1±47.0	370.5±41.1	439.8±6.4	411.4±9.9	416.1±64.1	0.29
Cyanidin-3-glucoside[e]	19.3±6.0	17.2±2.0	22.5±4.1	19.4±2.2	19.8±3.0	0.32
Cyanidin-3-arabinosylrutinoside[e]	15.1±2.0	13.7±2.1	17.1±3.2	15.3±1.1	15.2±2.0	0.18
Cyanidin-3-rutinoside[e]	208.9±38.0	194.1±31.0	233.8±9.9	213.0±8.7	218.8±26.0	0.46
Peonidin-3-rutinoside[e]	13.2±1.1	12.2±1.0	14.5±1.2	12.6±1.0	13.1±1.0	0.06

[a] - Expressed as p-coumaric acid; [b] - Expressed as chlorogenic acid; [c] - Expressed as quercetin rutinoside; [d] - Expressed as kaempferol-3-glucoside; [e] - Expressed as cyanidin-3-rutinoside.
P - ANOVA test ($\alpha \leq 0.05$).

In the case of blackcurrants, products from the successive batches contained comparable amounts of vitamin C (Fig. 8). The content of ascorbic acid in dehydrated blackcurrants varied from 0.44 to 0.61 g kg^{-1}. No statistically significant differences were found in the content of vitamin C between the successive batches of osmotically dehydrated blackcurrants.

The results regarding ascorbic acid were better than those obtained by MORAGA *et al.* (2011)

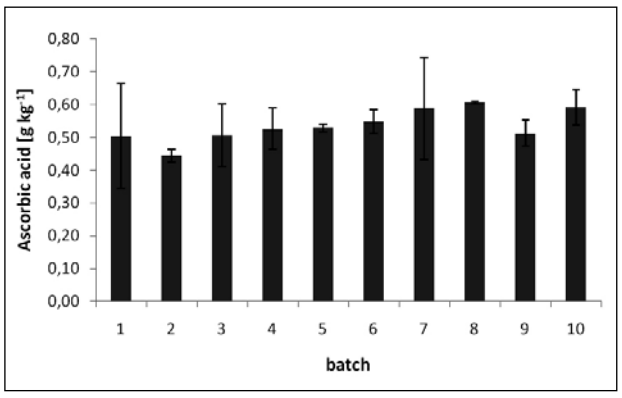


Fig. 8 - Ascorbic acid content (average±standard deviation) in blackcurrants dehydrated in reused FOS syrup. ANOVA test ($\alpha \leq 0.05$): P=0.40.

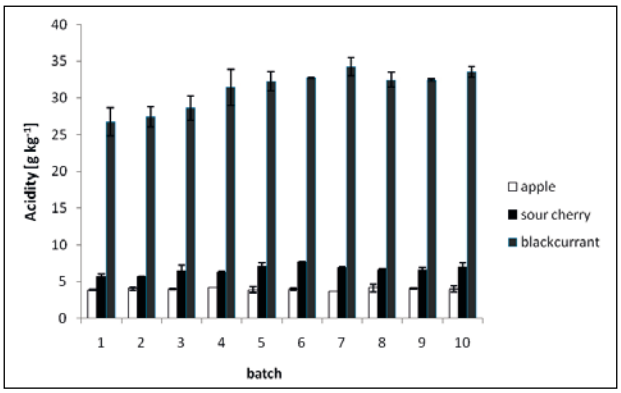


Fig. 9 - Acidity (average±standard deviation) of apples, sour cherries and blackcurrants dehydrated in reused FOS syrup. ANOVA test ($\alpha \leq 0.05$): apples P=0.76, sour cherries P=0.007, blackcurrants P=0.002.

for dehydrated grapefruit. They observed significant losses in vitamin C content, ranging from 24 to 43%.

No ascorbic acid was detected in dried apples and sour cherries, which was due to the low vitamin C content in the raw material.

CONCLUSIONS

Fructooligosaccharide syrups can be successfully reused for fruit dehydration. Ten successive batches of osmo-dehydrated fruit were similar as regards the content and profile of carbohydrates and polyphenols. The dried fruits were also comparable as regards their polyphenol and carbohydrate profiles. The only difference was the increasing acidity of osmo-dehydrated sour cherries and blackcurrants.

ACKNOWLEDGMENTS

This work is part of the ISAFRUIT Project funded by the European Commission under Thematic Priority 5-Food Quality and Safety of the 6th Framework Programme of RTD (Contract no. FP6-FOOD–CT-2006-016279).

DISCLAIMER

The views and opinions expressed in this publication are purely those of the writers and may not in any circumstances be regarded as stating an official position of the European Commission.

REFERENCES

Aachary A.A. and Prapulla S.G. 2009. Value addition to spent osmotic sugar solution (SOS) by enzymatic conversion to fructooligosaccharides (FOS), a low calorie prebiotic. Innovative Food Science and Emerging Technologies 10: 284.

AOAC. 1995. "Official method of analysis" 16th Ed. Association of Official Analytical Chemists, Washington, DC.

Benkeblia N., Shiomi N. and Osaki M. 2007. Kinetics and hydrolysis parameters of total fructooligosaccharides of onion bulbs: effects of temperature regimes and cultivars. Journal of Food Biochemistry 31: 14.

Blanda G., Cerretani L., Bendini A., Cardinali A., Scarpellini A. and Lercker G. 2008. Effect of vacuum impregnation on the phenolic content of Granny Smith and Stark Delicious frozen apple cvv. European Food Research and Technology 226: 1229.

Bolin H.R., Huxsoll C.C., Jackson R. and Ng K.C. 1983. Effect of osmotic agents and concentration on fruit quality. Journal of Food Science 48: 202.

Dalla Rosa M. and Giroux F. 2001. Osmotic treatments (OT) and problems related to the solution management. Journal of Food Engineering 49: 223.

Garcia-Martinez E., Martinez-Monzo J., Camacho M.M. and Martinez-Navarrete N. 2002. Characterisation of reused osmolic solution as ingredient in new product formulation. Food Research International 35: 307.

Klewicki R. and Uczciwek M. 2008. Effect of osmotic dehydration in fructose, saccharose and fructooligosaccharide solutions on the content of saccharides in plums and apples and their energy value. Agricultural and Food Science 17: 367

Kopera M. and Mitek M. 2007. Effect of osmotic dehydration on polyphenols content in dried pears (*Pyrus communis* and *Pyrus pyrifolia*). Food Science Technology Quality 63: 213.

L`homme C., Arbelot M., Puigserver A. and Biagini A. 2003. Kinetics of hydrolysis of fructooligosaccharides in mineral-buffered aqueous solutions: influence of pH and temperature. Journal of Agricultural and Food Chemistry 51: 224.

Linden G. and Lorient D. 1999. "New ingredients in food processing" 1st Ed. Woodhead Publishing Limited, Cambridge, UK.

Moraga M.J., Moraga G. and Martinez-Navarrete N. 2011. Effect of the re-use of the osmotic solution on the stability of osmodehydro-refrigerated grapefruit. LWT – Food Science and Technology 44: 35.

Moyano P.C. and Zúñiga R.N. 2003. Kinetic analysis of osmotic dehydration carried out witch reused sucrose syrup. Journal of Food Science 68: 2701.

Peiro R., Dias V.M.C., Camacho M.M. and Martinez-Navarrete N. 2006. Micronutrient flow to the osmotic solution during grapefruit osmotic dehydration. Journal of Food Engineering 74: 299.

Rastogi N.K., Raghavarao K.S.M.S., Niranjan K. and Knorr D. 2002. Recent developments in osmotic dehydration: methods to enhance mass transfer. Trends in Food Science and Technology 13: 48.

Ruiz R.Y., Caicedo L.A., Camacho G. and Clavijo L.M. 2005. Determining the effect of sucrose osmotic solution re-use on the pineapple's osmotic dehydration kinetic. 2nd Mercosur Congress on Chemical Engineering, 4th Mercosur Congress on Process Systems Engineering. Rio de Janeiro, Brasil.

Sangeetha P.T., Ramesh M.N. and Prapulla S.G. 2005. Recent trends in microbial production, analysis and application of fructooligosaccharides. Trends in Food Science and Technology 68: 57.

Sapata M.L., Ferreira A., Andrada L., Leitão A.E. and Candeias M. 2009. Osmotic dehydration of mandarins: Influence of reutilized osmotic agent on behaviour and product quality. Acta Scientarum Polonorum - Technologia Alimentaria 8: 23.

Singleton V.L. and Rossi J.A. 1965 Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144.

Tortoe C., Orchard J. and Beezer A. 2007. Comparative behavior of cellulosic and starchy plant materials during osmotic dehydration. Journal of the Science of Food and Agriculture 87: 1284.

Valdez-Fragoso A., Mujica-Paz H., Giroux F. and Welti-Chanes J. 2002. Reuse of sucrose syrup in pilot-scale osmotic dehydration of apple cubes. Journal of Food Process Engineering 25: 125.

Valdez-Fragoso A., Welti-Chanes J. and Giroux F. 1998. Properties of sucrose solution reused in osmotic dehydration of apples. Drying Technology 6: 1429.

Viberg U. and Sjöholm I. 1998. Sucrose inversion during osmotic pre-treatment of strawberries. Lebensmittel-Wissenschaft und-Technologie 31: 546.

Paper received October 1, 2010 Accepted January 29, 2011

PAPER

OVEN COOKING OF TURKEY MEAT AT DIFFERENT RELATIVE HUMIDITIES

B. MORA, D. BARBANTI*, G. BETTA and F. BOZZOLI
Department of Industrial Engineering, Viale G.P. Usberti 181/A, University of Parma, 43124 Parma, Italy
*Corresponding author: Tel. +39 0521 905706; Fax +39 521 905705,
email: davide.barbanti@unipr.it

ABSTRACT

Some selected physical characteristics of turkey meat (weight loss, texture, colour, volume shrinkage and cook value) were evaluated after oven cooking at 100° and 110°C. For each cooking temperature, dry air (only air convection, RH = 5%) and three predetermined relative humidity values (RH = 24, 43 and 62%) were obtained inside the oven cavity by means of a steam generator.

Significant cooking time decrement of about 70-90% (compared to air convection condition) was achieved by using steam, both at 100° and 110°C with the exception of the treatment at 100°C and RH= 24%. Moreover, the steam presence during cooking increased the cooking yield when steam treatments were shorter than the dry air convection treatment. About 10% cooking yield increase (as compared to air convection condition) was found at 100°C (at RH = 43 and 62%) and at 110°C (at RH = 62%). An additional cooking yield increase up to +15% was measured at RH = 24% and 43% at 110°C. Steam cooking led to a noticeable decreases of shrinkage, shear force and hardness of meat samples both at 100° and 110°C. The best cooking conditions, in terms of short cooking time, high cooking yield, high tenderness and low chewiness were obtained at 100°C with RH = 62% and 110°C with RH = 43%.

- Key words: cooking, steam, oven, meat, turkey, texture, colour, weight loss -

INTRODUCTION

Steam cooking is widely used both in industrial processing and domestic cooking for several foods (e.g. cooked ham, ready-to-eat meals, various vegetables and seafoods). Ovens operating by steam and air-convection (commonly known as "combi" ovens) are appliances which gained popularity since their introduction about 20 years ago (BARBANTI *et al.,* 2005). "Combi" ovens combine the accurate heat control of an air-convection oven with the efficiency of steam that can be used individually, sequentially or in combination to give the operator multiple cooking choices in a single appliance (VITTADINI *et al.,* 2005; CHIAVARO *et al.,* 2009). Two types of ovens are available on the market: the first type, commonly used in catering services, has the steam generator external to the oven cavity while in the second one, mainly for domestic use, the steam is produced inside the oven cavity. Whatever the type, ovens do not normally allow an accurate humidity control inside the cooking chamber: most of them only operates on the presence/absence of steam and only few of them have a steam modulation system but with a rough control of the steam quantity.

Concerning turkey meats, few data about the combined use of steam and air-convection during meat cooking are available. Moreover, it is quite difficult to compare results of different research papers on air-and-steam cooking mainly because of (i) different experimental conditions (e.g. oven temperatures or different cooking endpoint temperatures) and in particular (ii) unsatisfactory definitions of the thermodynamic conditions of steam (SKOG *et al.,* 2003; BARBANTI *et al.,* 2005; DANOWSKA-OZIEWICZ *et al.,* 2007; BIAŁOBRZEWSKI *et al.,* 2010).

At given temperature and atmospheric pressure, the relative humidity of an air-water mixture is the ratio of the partial pressure of water vapour in the mixture (P_{water}) to the saturated water vapour pressure ($P_{water-sat}$), expressed as percentage" (FELLOWS, 2000). If the system is isobarically heated (heating with no change in system pressure) at T > 100°C, the relative humidity of the system decreases because the $P_{water-sat}$ increases with increasing temperature. By considering the system at T < 100°C and at atmospheric pressure (P_{atm}), it is possible to achieve 100% of relative humidity at any temperature while for T > 100°C, the possibility of reaching the maximum relative humidity drastically falls, being $P_{water-sat} > P_{atm}$. As an example, pure water steam may reach 100% RH at 100°C, while it will only reach 20% RH at 150°C. Consequently, taking into account cooking trials with air-and-steam mixtures carried out at T > 100°C in an unpressurized system (oven cavity), it is ambiguous and not correct to use the phrase "at steam saturation condition" instead of "pure steam condition" or directly the RH value as previously defined.

Literature shows that the heat treatment conditions of food significantly influences cooking losses and content of basic chemical compounds (FILLION and HENRY, 1998; CLAUSEN and OVESEN, 2001; BADIANI *et al.,* 2002). On the other hand, steam-cooked foods are characterized by a valuable retention of substances with recognized nutritional value, i.e. vitamins and antioxidants (FERRACANE *et al.,* 2008) and attractive sensory properties (CHOUBERT and BACCAUNAUD, 2010). In particular for meat-based foods, steam cooking is reported to retain more proteins and less fat than traditionally oven-cooked samples. Additionally, operators gain other advantages, such as improved yields and lower energy consumption (DANOWSKA-OZIEWICZ *et al.,* 2007). DANOWSKA-OZIEWICZ *et al.* (2007) found that weight losses in chicken cooked in standard air-convection and combi ovens were comparable, while BARBANTI *et al.* (2005) showed that the weight loss of chicken breasts due to air-steam cooking was higher than that for the air method alone. VITTADINI *et al.* (2005) compared samples of pork meat cooked in a commercial oven using different cooking conditions: natural convection, forced convection or combined forced convection/steam (FC/S) heating. FC/S treatment showed shorter cooking times compared to natural and forced convection methods. FC/S samples also had significantly higher weight loss than samples cooked by the other methods and resulted in a paler colour on the meat surface. CHIAVARO *et al.* (2009) reported results for pork meat cooked in oven by forced convection and pure steam condition at four temperatures (100°, 110°, 120° and 140°C). Authors reported that the use of steam proved to be particularly attractive at lower temperatures (100° - 110°C) in comparison with air-convection cooking: the presence of steam gave similar or better cooked meat qualities (texture, colour, water holding capacity) in a shorter time.

MURPHY *et al.* (2001) analyzed the effects of different RH values (range, 40-95%) during oven cooking of chicken breast patties by means of the evaluation of heat transfer properties and product yield between 149° and 218°C. They reported increased heat flux and heat transfer coefficient (shorter cooking time) with increasing RH. An increased product yield was achieved by reducing oven temperature or final internal temperature of the product, and increasing RH.

Finally, in some papers steam cooking was exclusively carried out at pure steam condition and the RH value inside the oven cavity derived by the oven's temperature, according to the integration of Clausius-Clapeyron equation (VITTADINI *et al.,* 2005; CHIAVARO *et al.,* 2009).

At present, the behaviour of different relative humidities at T < 150°C is still quite unknown. Low cooking temperature could reduce energy consumption but the final internal temperature of about 74°C should be reached to ensure hy-

gienic safety of cooked meat (NACMCF, 2006). Treatments at low temperatures have also nutritional relevance because it has been shown that high temperature and high air velocity in the convection ovens may enhance food mutagenic activity. The presence of steam reduced the mutagenic activity, except when high temperature was used in combination with high air velocity (SKOG *et al.,* 2003).

The objective of this work was to study the effect of air-and-steam mixture on some selected physical characteristics of turkey meat samples. The cooking trials were carried out at three relative humidities (24, 43 and 62% RH) and two cooking temperatures (100° and 110°C) in an air-and-steam convection oven ("combi" oven). The reference standard cooking trials at 100° and 110°C were carried out without steam injection (RH = 5%).

The effects of the different cooking trials on turkey meat samples were evaluated in terms of heating profiles and analysing some selected physical characteristics such as cooking yield, shear force, hardness, cohesiveness, springiness, gumminess, chewiness, shrinkage and colour.

MATERIALS AND METHODS

Sample preparation

Turkey (*Meleagris gallopavo)* breast samples were purchased from a local supermarket and stored at 4°C. From halved samples 2 slices of 3 cm thickness from the same geometrical position were obtained. Each slice was divided into 4 samples of 3x3x6 cm with muscle fibres running parallel to the major dimension. Prior to cooking the meat samples were left at room temperature for 15 min.

Cooking equipment

An electric domestic convection oven (Whirlpool AKZ 190/IX, Whirlpool Europe, Varese, Italy) was used as cooking device. The oven was modified by connecting to its cavity an external steam generator (Tosca 100, Parma, Italy) in order to have a pre-determined quantity of steam injected into the cavity itself. A capacitive humidity-temperature sensor (Rotronic HygroClip HC2-IC302, Zurich, Switzerland) was fixed to the vertical right panel inside the oven cavity.

Type K, (Ni/Al–Ni/Cr) thermocouple wires were positioned inside the oven cavity in order to measure dry (T_{db}) and wet bulb (T_{wb}) temperatures as described by CHIAVARO *et al.* (2009).

Temperature and humidity data obtained from the capacitive sensor and from thermocouple wires were logged by a DAQ acquisition device (National Instruments, Austin, Texas*)* and recorded using LabVIEW 8.0 graphical interface software (National Instruments, Austin, Texas). The speed of forced air circulation inside the oven cavity (0.90±0.13 m s^{-1}) was measured with a hot-wire anemometer (Testo, Milan, Italy).

Cooking trials

The cooking treatments of the meat samples were performed at 100° and 110°C, each one at relative humidities of 5, 24, 43 and 62% under forced air circulation. Treatments at 5% of relative humidity were carried out without steam injection (convection cooking, coded as CC). The other conditions (RH = 24, 43 and 62%, ± 5%) were maintained by means of manual on-off control of the steam generator. They were coded as 24SCC, 43SCC and 62SCC respectively and all the steam convection cooking treatments were coded as SCC.

Cooking trials (n = 2 for each treatment) were carried out placing 4 meat samples on the tray in the middle position of the oven, hence 8 samples were analyzed for each cooking treatment. During cooking, the temperatures of one meat sample (always in a pre-fixed oven position) were measured at its surface and geometric centre using thermocouple wires. The cooking treatment ended when meat samples reached 74°C at their thermal centre, the recommended safety temperature for turkey meat (NACMCF, 2006). Cooked samples were cooled at room temperature for 30 min and then analysed.

Analyses

Cooking weight loss percentage (WL) was calculated as reported by VITTADINI *et al.* (2005).

Shear force test and texture profile analysis (TPA) of meat samples were carried out by means of Texture Analyser (TA-XT2, Stable Micro Systems, Goldalming, UK) equipped with a 25 kg load cell (equivalent to 245.2 N). For the shear force test, fresh and cooked meat samples (n = 8) obtained as previously described (3x3x6 cm) were cut perpendicularly to muscle fibers with a Warner-Bratzler blade (3 mm thickness) moving down at 2 mm s^{-1} and the shear force (N) was recorded.

Since the original dimensions of samples (3x3x6 cm) resulted influenced by the different cooking treatments, in order to reduce the variability of TPA measurements, the samples obtained from the shear force test (2 pieces from each original sample of 3x3x6 cm) were then manually cut into cubes of standardized dimensions (2x2x2 cm).

The TPA test was carried out perpendicularly to muscle fibres of fresh and cooked samples by means of a cylindrical probe (code P35, diameter = 35 mm) at 5 mm s^{-1}.

The contact area was 4 cm^2, the final strain was set at 50% and the time interval between the first and the second compression was 1 s.

8 TPA measurements were carried out for each treatment.

The TPA attributes (hardness, cohesiveness, springiness, gumminess and chewiness) were determined as reported by FRIEDMAN, WHITNEY, and SZCZESNIAK (1963) and BOURNE (1978). All experimental data were collected and analysed by Texture Expert software version 1.22.

Shrinkage of each meat sample (n=8) was calculated measuring length, width and thickness by means of a manual calliper before and after each cooking trial. Volume of raw (V_i) and cooked sample (V_f) was calculated and expressed as shrinkage ratio (V_f/V_i, dimensionless).

Colour of fresh and cooked samples was measured by means of a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) with the standard illuminant D65 at 10° position of the standard observer and illumination area of 8 mm diameter (CIE, 1978). For each sample and for each treatment, 4 readings at the surface and 4 at the central section were taken. The central section was obtained by halving the sample perpendicularly to fiber direction. Colour parameters L*(lightness), a*(redness) and b*(yellowness) were measured and colour difference ΔE, ΔL*, Δa* and Δb* between cooked and raw sample were calculated (SOSA-MORALES *et al.,* 2006) both at the surface and at the central section of each sample.

The degree of cooking at the thermal center and at the surface of each sample was expressed in terms of cook value C^z_{Tref}, taking into consideration only the heating phase, as reported by POON *et al.* (2001) and ZHANG *et al.* (2004). The cook value was calculated by the integration of the heat penetration curve obtaned from time–temperature data recorded at the thermal centre and at the surface of meat samples (VITTADINI *et al.,* 2005):

$$C^z_{T_{ref}} = \int_0^t 10^{(T-T_{ref})/z}\,dt,$$

where t is the time, T_{ref} the reference temperature set equal to 100°C; z the temperature increase that induces a 10-fold increase of the reaction rate of the chemical reaction taken as reference (thiamine degradation); z was set at 33°C (HOLDSWORTH, 1985).

Moisture content of turkey meat samples (raw and cooked ones) was determined by weight loss at 105°C (ISCO NSV 9035, ISCO, Milan, Italy) until constant weight and 4 measurements for each sample were carried out.

SPSS (Version 14.0, SPSS Inc., Chicago, Illinois, USA) statistical software was used to calculate (i) means and standard deviations (SD) of experimental data, (ii) one-way-analysis of variance (ANOVA) and (iii) least significant difference test (LSD at a 95% confidence level with $p < 0.05$) in order to identify differences among cooking conditions. Second degree regression equations between the surface temperature of the samples after 10 minutes and the RH of the air were obtained by means of Excel® spreadsheet.

RESULTS AND DISCUSSION

Thermal profiles

In order to have constant temperature and RH inside the oven cavity and also to reach 74°C at the thermal centre of each sample, a continuous check of these parameters for each cooking trial was carried out. As an example, in Fig. 1 (110°C, RH = 24%), the evolution of dry and wet bulb temperatures and RH inside the oven cavity and the temperatures of meat sample surface and centre are reported. Set oven temperature resulted very stable all along the cooking trial; the spikes of RH curve were caused by the on-off effect of the steam injector but this behaviour did not affect the attainment of constant RH values.

Surface and centre temperatures of meat samples increased with time during cooking but with different rates, as expected. Surface temperature of meat samples undergone little variations because of the effect of the latent heat of steam condensation at the sample surface.

In Figg. 2a and 2b, temperature profiles of sample surface and wet bulb at 100° and 110°C and different RH are reported. Each cooking treatment was interrupted when the respective meat sample reached 74°C at its thermal centre, hence different cooking lenghts were obtained. SCC and CC treatments resulted in different heating kinetics of the sample surfaces, both at 100° and 110°C. SCC treatments always showed an initial rapid increase of surface temperature; this behaviour was due to the latent heat of condensation transferred from the steam to the meat samples surface, about 2260 kJ kg^{-1}, (PERROT, 1998) and to the higher heat transfer coefficient of condensing water vapour ($h=10^4$-10^5 W m^{-2} K^{-1}) compared to that of forced convection air ($h \sim 10^2$ W m^{-2} K^{-1}) (DATTA, 2002). Moreover, at the beginning of the cooking process (after about 10 min) the sample's heating rate increased with increasing relative humidity of the air, as already reported by MURPHY *et al.,* (2001). The relationship between the heating rate of samples and the RH of the air in the oven was quantified taking into account the surface temperature of the samples after 10 minutes. Samples surface temperature resulted well correlated to the RH of the air by second degree regression equations:

$T = 100°C = -0.0055RH^2+1.06RH+44.237$; $R^2=0.99$

$T = 110°C = -0.0052RH^2+1.1317RH+46.985$; $R^2=0.99$

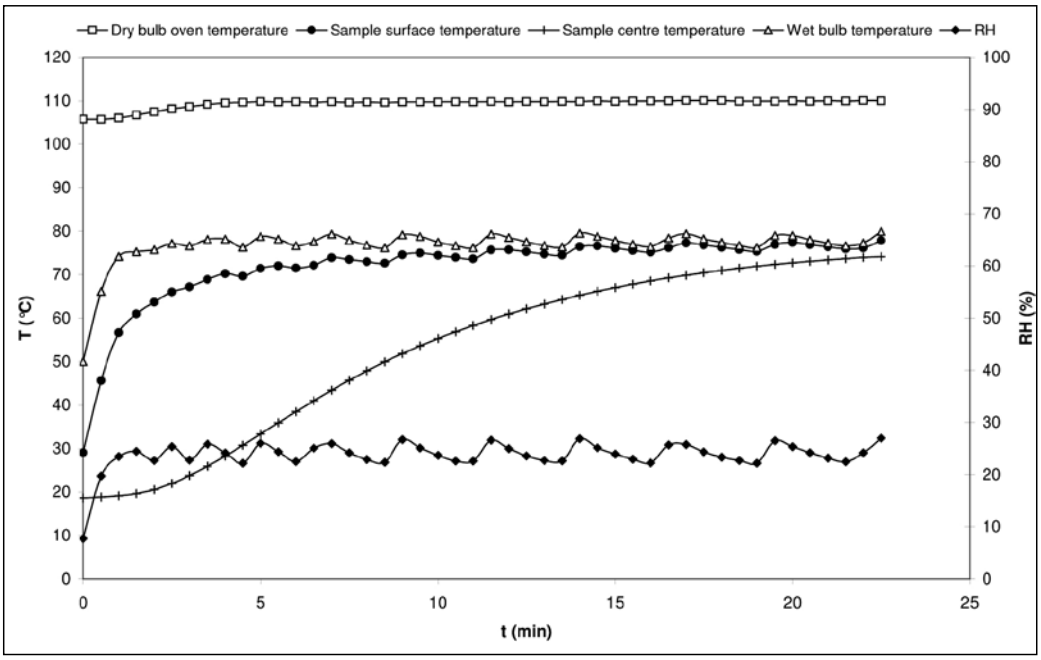


Fig. 1 - Example of the evolution during a cooking trial of dry bulb oven temperature, oven RH, temperatures of meat sample surface and centre. Referred condition: T°C = 110°C, RH = 24%.

After an initial phase characterized by an increase of the surface temperature, a constant temperature period was observed at 100°C, 24% and 43% RH and at 110°C, 24% RH. These conditions allowed sample surfaces to behave like a wet-bulb, in terms of mass transfer and temperature, according to the drying theory (FELLOWS, 2000). The temperature equilibrium is reached at the surface of the food if it is maintained perfectly wet by the migration of water from the interior of the food itself; when this condition occurs, the surface shows constant temperature, equal to wet bulb temperature and it is a function of cooking T and RH. At other SCC conditions (100°C, 62% RH, 110°C, 43 and 62% RH) meat sample reached 74°C at the thermal centre in about 10 min, hence the constant temperature period did not appear.

During CC treatment, the increase of surface temperature both at 100° and 110°C was due to the dehydration caused by the vapour partial pressure gradient between the food and the surrounding air, in agreement with the second phase of drying theory (FELLOWS, 2000). As a consequence, the surface temperature did not behave like a wet bulb and a slow and constant surface temperature increase during the entire cooking trial occurred.

Results of analyses on meat samples

In Table 1, a summary of final surface temperature, cooking time, cook values, moisture content and volume shrinkage has been reported. The final surface temperature of meat samples always turned out lower than 100°C because of water evaporation and consequent cooling effect at the meat surface. Nevertheless, the increasing of RH values (both at 100° and 110°C) reduced the vapour pressure gradient between the food and the surrounding air, thus resulting in a lower water evaporation and in an increase of meat surface temperature.

In SCC treatments the cooking time was in inverse proportion to the RH value, both at 100° and 110°C. At 100°C a significant cooking time reduction in comparison with CC condition was observed: -82% for 43SCC and -89% for 62SCC, with no significant differences between them. These values depended on the faster initial heating kinetic of SCC, as previously described.

An "apparent" abnormal value of cooking time was repeatedly observed at 24SCC and 100°C, significantly higher than CC at 100°C (+17.7%). Taking into account that during the 24SCC trials the wet bulb temperature (T_{wb}) was about 74.5°C, the extension of cooking time depended on the low temperature gradient between sample surface and centre until the end of cooking (T=74°C at sample's thermal centre). At 110°C, 24SCC, 43SCC and 62SCC showed a noticeable cooking time reduction in comparison to CC: -72, -86 and -87%, respectively.

Surface and centre cooking values (C^z_{Tref}) are the measure of the cumulative heat impact of the time/temperature history on a food quality attribute (POON *et al.*, 2001). The surface C^z_{Tref} value of SCC, except 24SCC at 100°C, resulted three to four times lower than CC at both tem-

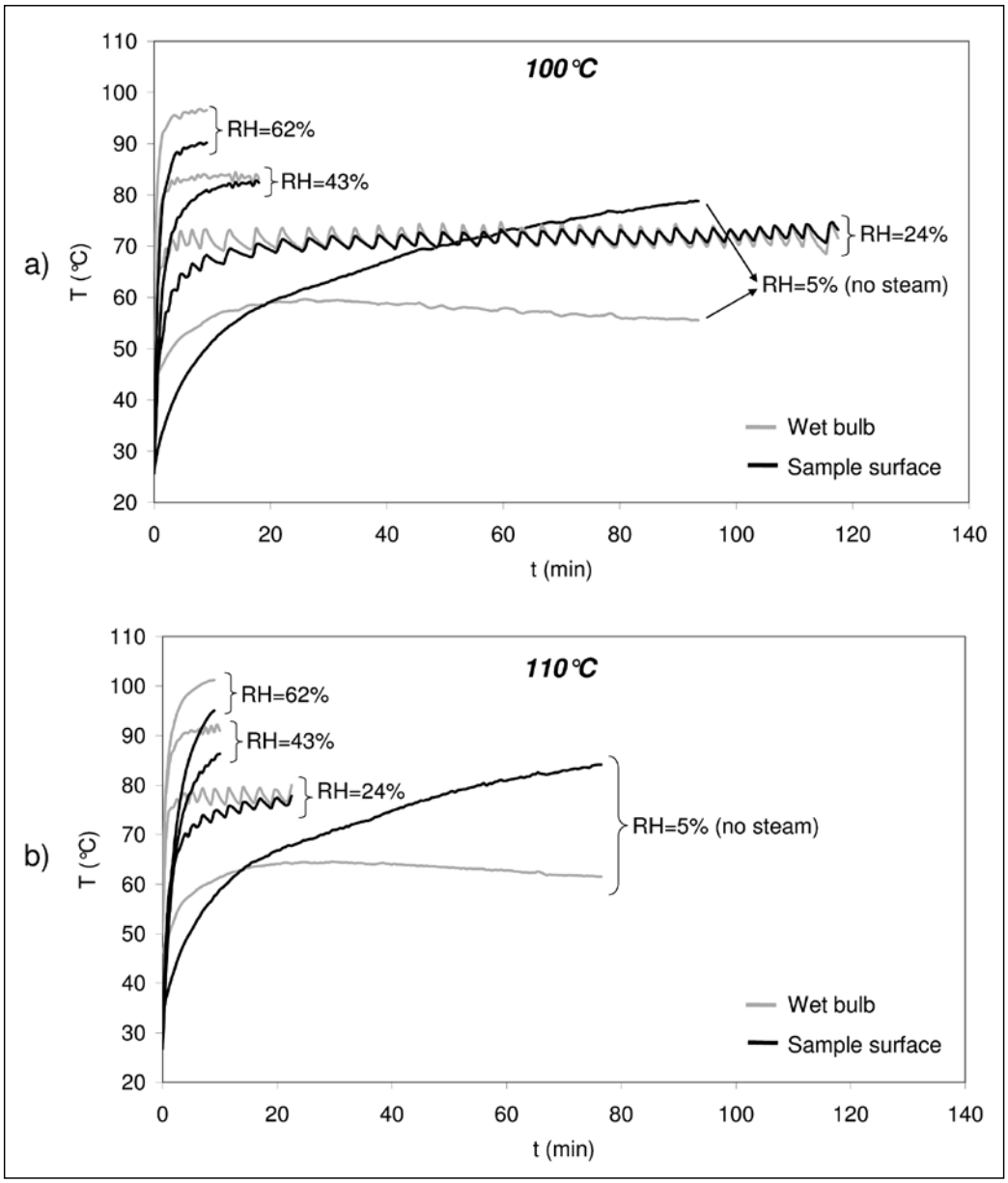


Fig. 2 - Temperature profiles of sample surface and wet bulb at 100° and 110°C (2a and 2b, respectively) at different RH. Different lenghts of cooking treatments were due to interruption when meat samples reached 74°C at their thermal centre.

peratures of 100° and 110°C, mainly due to their respective shorter cooking time.

By considering the limit threshold of thermal damage of 13.56 minutes at 100°C, defined as the time necessary to reduce thiamine content by 3% (POMPEI, 2009), the surface C^z_{Tref} of CC treatments and 24SCC at 100°C exceeded this value, while at the other SCC treatments a limited nutritional quality degradation of meat (C^z_{Tref} < 10min) can be hypothesized. The centre C^z_{Tref} value, both at 100° and 110°C was always lower than surface C^z_{Tref} value, due to the lower temperature reached in the centre of the sample (74°C).

The moisture content of heat treated samples with steam (at 100°C) decreased with the decreasing of RH. The lowest MC value was obtained at 24SCC because of the longest cooking time, while the highest MC value was measured at 62SCC (2.18 $kg_{water}/kg_{dry\ matter}$). At 110°C the higher MC decreasing was observed for the CC condition (1.56 $kg_{water}/kg_{dry\ matter}$), while for 24SCC, 43SCC and 62SCC the shorter cooking times reduced MC decrease (2.13, 2.1 and 2.06 $kg_{water}/kg_{dry\ matter}$, respectively).

The shrinkage value of SCC meat samples was always higher (hence their volume reduction was lower) than the corresponding CC samples both at 100° and 110°C. In details, at 100°C the V_f/V_i value of 43SCC samples was the highest (+20%) in comparison with CC sample. At 110°C the shrinkage value of all SCC samples result-

Table 1 - Meat cooking results at the different temperatures and hygrometric conditions: samples' final surface temperature (°C), cooking time (min), surface and center cook values (min), moisture content ($kg_{water}/kg_{dry\ matter}$) and volume shrinkage (dimensionless). Numbers in parenthesis are the standard deviation of the mean (+/-). Samples' final surface temperature, cooking time, surface and center cook values n=2, moisture content n=4, shrinkage n=8. Means with the same letter are not significantly different ($p<0.05$).

Treatment	Final surface temperature (°C)	Cooking time (min)	Cook values (min)		MC (kg water/kg dry matter)	Shrinkage (Vf/Vi)
			Surface	Center	raw meat: 2.7 (0.06) a	
100°C						
CC	80.1 (1.7) de	98.2 (5.5) b	12.4 (1.7) a	8.1 (0.1) b	1.52 (0.02) d	0.69 (0.08) c
24SCC	74.5 (2.2) e	115.7 (3.2) a	15.8 (0.7) a	14.2 (0.1) a	1.38 (0.07) e	0.73 (0.07) bc
43SCC	82.8 (0.4) cd	17.6 (0.6) de	3.9 (0.2) b	0.9 (0.1) e	2.08 (0.04) c	0.83 (0.09) a
62SCC	90.4 (0.3) ab	10.6 (1.8) e	3.4 (0.1) b	0.6 (0.1) e	2.18 (0.05) b	0.76 (0.08) abc
110°C						
CC	82.2 (2.8) cd	79.9 (4.7) c	12.1 (1.3) a	7.1 (0.3) c	1.56 (0.04) d	0.67 (0.05) c
24SCC	76.6 (1.2) e	22.3 (0.5) d	3.3 (0.3) b	1.5 (0.1) d	2.13 (0.08) bc	0.81 (0.06) ab
43SCC	87.4 (1.6) bc	11.3 (1.6) de	3.0 (1.3) b	0.6 (0.1) e	2.1 (0.06) c	0.80 (0.09) ab
62SCC	95.0 (0.2) a	10.1 (1.3) e	3.0 (0.9) b	0.4 (0.1) e	2.06 (0.03) c	0.85 (0.13) a

ed significantly higher than CC samples (+20-27%), and no significant differences were found among SCC samples.

Weight loss (WL) is the measure of the product cooking yield, the higher the weight loss the lower the cooking yield. The average WL values obtained at 100° and 110°C have been reported in Fig. 3. The presence of steam significantly reduced meat sample WL (with the exception of 24SCC at 100°C), in comparison with CC both at 100° and 110°C. At 100°C, 43SCC and 62SCC meat samples' WL resulted to be of about 20% (+10% of cooking yield compared to CC at the same temperature), whereas at 100°C, the WL of 24SCC was greater than CC mainly because of the long time needed for this cooking condition.

The lowest WL were observed at 110°C, 24SCC and 43SCC, where they were about 15% lower than CC. The presence of steam reduced the vapour pressure gradient between sample surface and surrounding air, hence steam cooked samples with a cooking length shorter than CC showed lower weight loss than those cooked without steam. When the cooking length of SCC samples was longer than CC (e.g., 24SCC at 100°C) an opposite result was obtained: higher WL in steam cooked samples was observed because of the crust formation in CC samples that reduced their water evaporation as previously described (VITTADINI *et al.*, 2005). Moreover, it is interesting to point out that at 110°C the sample's WL at RH = 62% (cor-

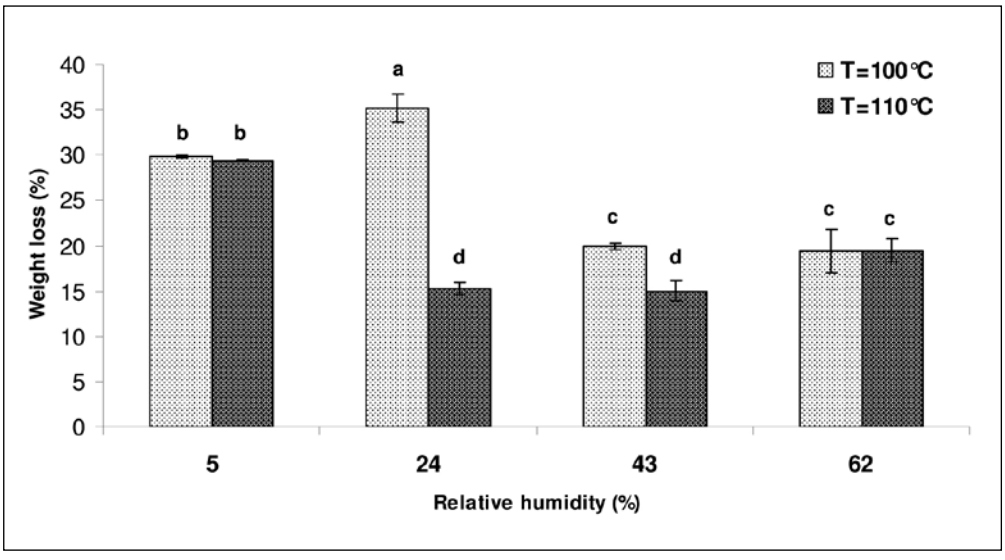


Fig. 3 - Average values of weight loss (%) of Turkey meat samples at 100° and 110°C, as a function of the different relative humidities.

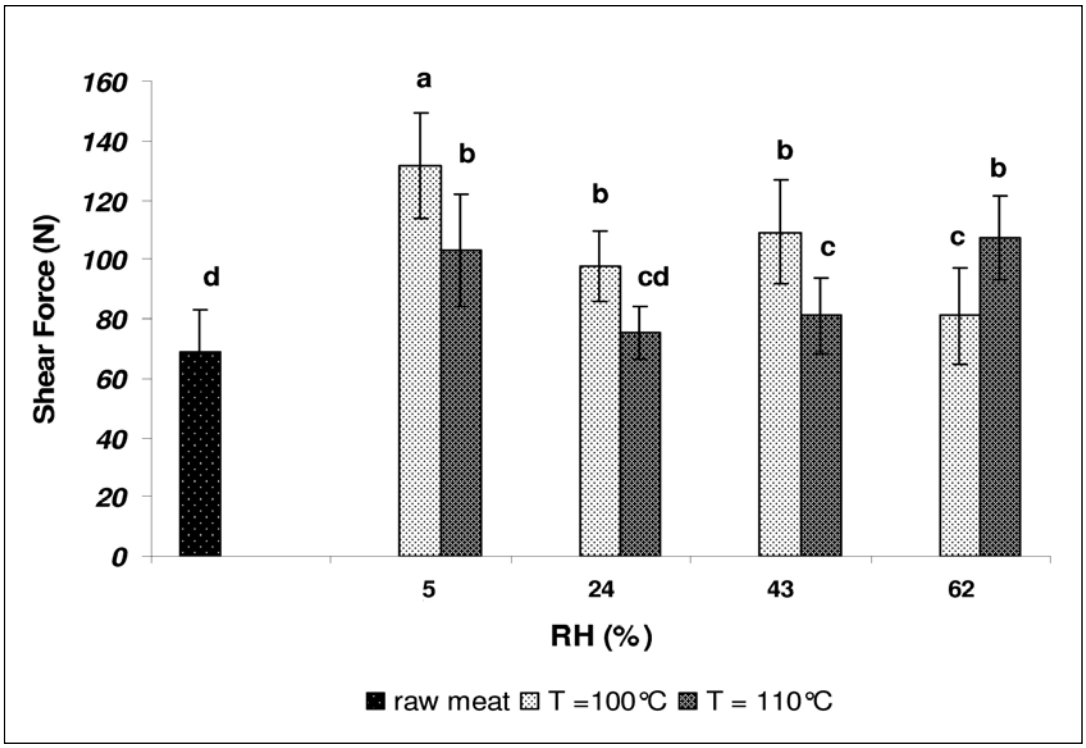


Fig. 4 - Average values of shear force (N) of raw and cooked Turkey meat samples at 100° and 110°C, as a function of the different relative humidities.

responding to the pure steam condition at this temperature) was higher than the WL at lower relative humidities probably because of the highest cooking rate of this particular thermohygrometric condition.

The shear force test is the most widespread method normally used as an indicator of meat tenderness; the shear force experimental values (N) of raw and cooked meat samples are shown in Fig. 4. Under our experimental conditions the injection of steam during cooking generally determined a shear force decrease of meat samples if compared to those cooked without steam. Only the sample 62SCC at 110°C (cooked at the pure steam saturation condition) showed the same shear force value of CC at the same temperature. Some significant differences of shear force were found among steamed samples, in particular, at 100°C, 62SCC resulted significantly more tender than 24SCC and 43SCC. A different behaviour was observed at 110°C, where the tenderest samples resulted 24SCC and 43SCC (with no significant difference between them). Considering only the SCC treatments at 110°C, the lowest RH values (lowest cooking rate) led to the lowest shear force values, whereas the injection of higher steam quantities (highest cooking rate) determined a tenderness reduction in agreement with KING *et al.* (2003).

Results of analyses of hardness, cohesiveness, springiness, gumminess and chewiness on raw and cooked samples are reported in Table 2.

At 100°C the hardest sample was 24SCC, not significantly different from CC; 62SCC resulted the tenderest sample, not significantly different from 43SCC. Samples cooked at 24SCC, 100°C resulted the highest in hardness if compared both to SCC and CC conditions because they underwent to an extended cooking time that caused strong samples dehydration. At 110°C the difference of hardness value among samples was lower than those at 100°C and only 43SCC resulted significantly tenderer than the CC one.

The cohesiveness values were very similar among cooked samples both at 100 and 110°C. At 100°C the only significant difference was found between 43SCC and 62SCC, with the former higher than the latter. As a general trend, the higher the RH the lower the cohesiveness (100°C 62SCC and 110°C 43SCC and 62SCC). No particular differences in springiness value were found among samples. At 100°C the less gummy sample was the 62SCC, significantly different from the others, while at 110°C the lower gumminess was observed at 43SCC with no statistical difference from 62SCC. The chewiness of cooked samples was lower at 43SCC and 62SCC in respect to all the other treatments at both temperatures. At 110°C the chewiness of 62SCC samples were not statistically different from CC and 24SCC.

Table 2 - TPA results of raw and cooked meat samples at the different temperatures and hygrometric conditions: hardness (N), cohesiveness, springiness, gumminess (N) and chewiness (N * mm) values are reported. Numbers in parenthesis are the standard deviation of the mean (+/-). n=8. Means with the same letter are not significantly different (p<0.05).

Treatment	Hardness (N)	Cohesiveness	Springiness	Gumminess (N)	Chewiness (N*mm)
raw meat	47.0 (20.5) f	0.40 (0.08) c	0.56 (0.12) a	17.77 (7.08) f	9.61 (3.83) f
100° C					
CC	103.3 (41.5) ab	0.45 (0.04) ab	0.57 (0.04) a	46.17 (15.70) ab	26.39 (8.64) ab
24SCC	113.0 (30.7) a	0.46 (0.05) ab	0.59 (0.04) a	51.43 (14.87) a	30.34 (9.99) a
43SCC	66.0 (21.6) cde	0.49 (0.06) a	0.53 (0.03) a	32.03 (10.47) cd	16.99 (5.99) de
62SCC	48.9 11.4) ef	0.43 (0.04) bc	0.57 (0.03) a	20.54 (3.40) ef	11.72 (2.30) ef
110°C					
CC	84.5 (29.1) bc	0.45 (0.03) ab	0.60 (0.05) a	38.22 (15.06) bc	22.96 (9.37) bc
24SCC	78.5 (21.5) cd	0.45 (0.08) ab	0.60 (0.05) a	36.14 (14.34) c	22.01 (9.95) bcd
43SCC	61.7 (13.5) def	0.43 (0.04) bc	0.58 (0.04) a	26.69 (7.25) de	15.45 (4.18) e
62SCC	65.3 (15.9) cde	0.43 (0.06) bc	0.61 (0.02) a	28.77 (10.60) cde	17.46 (6.27) cde

The differences of colour parameters between cooked and raw samples (Δ) are shown in Table 3. The surface colour parameters of the uncooked meat were characterized by lightness L* = 49.2±2.9, redness a* = -0.7±0.6, yellowness b* = 3.1±1.1. Cooking caused an increase of the sample's surface colour in terms of L*, a* and b*, consequently cooked meat sample's surfaces were brighter (higher L*), more red (higher a*) and yellow (higher b*) than the correspondent raw uncooked samples.

SCC samples compared to CC samples showed a paler colour: they resulted in lower a* and b* than CC sample and also by higher L*. Significant differences in terms of surface colour increase between SCC (with short cooking times) and CC were found. In SCC samples with short cooking times the presence of steam prevented surface dehydration thus reducing Maillard reactions while, as expected, under CC condition a uniform brown crust formation was clearly observed and instrumentally detected. No color differences were found between steam cooked samples at different RH with short cooking times. Meat samples cooked at 24SCC, 100°C (steam cooking with long cooking time) showed an increased a* values (browning degree) in-between CC and SCC samples: a non uniform crust formation, localized on edges and corners, the more dehydrated areas, was observed.

The results of the colour measures at the central sections of meat samples gave some contrasting results between the adopted temperatures. At 100°C the higher ΔE value was measured for CC sample, while at 110°C CC samples resulted in lowest ΔE, and the same behaviour was observed for ΔL* values. An high Δa* value was measured at 24SCC at 100°C no significantly different from CC at the same temperatures. Consequently, the presence of steam during the various cooking trials had limited or no influence on the chromatic properties of the inner part of the meat samples.

Table 3 - Meat cooking results at the different temperatures and hygrometric conditions: external surface and centrals section colour parameters. Differences (Δ) are between cooked and uncooked samples. Numbers in parenthesis are the standard deviation of the mean (+/-). n=32. Means with the same letter are not significantly different (p<0.05).

Treatment	Surface				Central section			
	ΔE	ΔL*	Δa*	Δb*	ΔE	ΔL*	Δa*	Δb*
100°C								
CC	24.4 (3.3) c	17.7 (4.3) c	6.6 (1.8) a	15.0 (2.3) a	36.3 (2.1) a	35.0 (2.2) a	1.6 (0.5) ab	9.3 (1.0) ab
24SCC	22.0 (5.0) c	17.7 (6.7) c	4.7 (1.8) b	11.2 (2.0) b	32.8 (2.1) de	31.9 (2.0) bc	1.9 (0.6) a	7.4 (1.1) d
43SCC	29.8 (2.6) b	27.7 (2.7) b	1.9 (0.6) c	10.4 (1.8) b	31.9 (1.8) e	30.9 (1.8) c	1.4 (0.5) b	7.7 (1.0) d
62SCC	33.3 (2.6) a	31.5 (2.8) a	1.3 (0.6) c	10.6 (1.4) b	33.5 (2.8) cd	32.0 (3.1) bc	1.4 (0.6) b	9.8 (1.6) a
110°C								
CC	24.4 (2.9) c	18.3 (3.5) c	6.6 (1.6) a	14.3 (2.5) a	34.1 (2.0) bc	32.8 (2.1) b	1.6 (0.5) b	9.1 (1.2) b
24SCC	32.1 (3.0) ab	30.2 (3.4) ab	1.9 (0.9) c	10.5 (1.3) b	35.2 (2.3) ab	34.1 (2.4) a	1.4 (0.6) b	8.4 (1.3) c
43SCC	31.4 (3.3) ab	29.6 (3.5) ab	1.9 (0.8) c	10.1 (1.6) b	35.7 (3.2) a	34.5 (3.2) a	0.9 (0.6) c	9.1 (1.4) b
62SCC	32.8 (3.9) a	30.8 (4.0) ab	1.7 (0.9) c	11.1 (1.7) b	35.7 (3.1) a	34.3 (3.1) a	1.4 (0.5) b	9.4 (1.4) ab

CONCLUSIONS

This study has been carried out by focusing the attention on the effects of different relative humidities on some quality characteristics of turkey meats submitted to oven cooking at 100° and 110°C.

Important information have been obtained from the analysis of the heating profiles of samples during cooking: at the beginning of the cooking process the presence of steam increased the heating rate of meat samples with increasing relative humidity of the air.

Different relative humidities led to different meat cooking performances. The presence of steam significantly reduced meat sample weight loss, increased their tenderness and reduced meat shrinkage when steam cooking times were shorter than dry air oven cooking. On the contrary, when long cooking times occurred (e.g. 100°C, RH = 24%) the steam presence led to a worsening of the overall quality of turkey meat.

The best cooking performances (maximum cooking yield, high tendernss and low chewiness) were found at RH = 62% at 100°C and at RH = 43% at 110°C. Relative humidity values lower than the pure steam condition, at 110°C, led to better quality characteristics of cooked meats with a potential saving of energy and water.

In conclusion, with this experimental approach to the study of oven cooking some improvements to the knowledge of quality changes of turkey meats as a function of different relative humidities have been obtained.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technicians of the Department of Industrial Engineering for their help to the set-up of the cooking device and Julian Penfold for the revision of the English manuscript.

REFERENCES

Badiani A., Stipa S., Bitossi F., Gatta P.P., Vignola G. and Chizzolini R. 2002. Lipid composition, retention and oxidation in fresh and completely trimmed beef muscles affected by common culinary practices. Meat Science, 60: 69.

Barbanti D. and Pasquini M. 2005. Influence of cooking conditions on cooking loss and tenderness of raw and marinated chicken breast meat. LWT-Food Science and Technology, 38: 895.

Białobrzewski I., Danowska-Oziewicz M., Karpinska-Tymoszczyk M., Nalepa B., Markowski M. and Myhan R. 2010. Turkey breast roasting – Process optimization. Journal of Food Engineering, 96: 394.

Bourne M.C. 1978. Texture profile analysis. Food Technology, 32(7): 62.

Cheng Q., Sun D.W. and Scannell A.G.M. 2005. Feasibility of water cooking for pork ham processing as compared with traditional dry and wet air cooking methods. Journal of Food Engineering, 67: 427.

Chiavaro E., Rinaldi M., Vittadini E. and Barbanti D. 2009. Cooking of pork *Longissimus dorsi* at different temperature and relative humidity values: effects on selected physico-chemical properties. Journal of Food Engineering, 93: 158.

Choubert G. and Baccaunaud M. 2010. Effect of moist or dry heat cooking procedures on carotenoid retention and colour of fillets of rainbow trout (*Oncorhynchus mykiss*) fed astaxanthin or canthaxanthin. Food Chemistry, 119(1): 265.

Clausen I. and Ovesen L. 2001. Proximate contents, losses or gains of fat, protein and water comparing raw, hospital- and household-cooked pork cuts. Journal of Food Composition and Analysis, 14: 491.

Danowska-Oziewicz M., Karpinska-Tymoszczyk M. and Borowski J. 2007. The effect of cooking in a steam-convection oven on the quality of selected dishes. Journal of Foodservice, 18(5): 187.

Datta A.K. (2002). Biological and bioenvironmental heat and mass transfer. Marcel Dekker, Inc., New York Basel., p. 111.

Fellows P. (2000). Food processing technology - Principle and practice. (2nd Ed.), Woodhead Publishing, Cambridge, England., p. 313 and p. XXV glossary.

Ferracane R., Pellegrini N., Visconti A., Graziani G., Chiavaro E., Miglio C. and Fogliano V. 2008. Effects of different cooking methods on antioxidant profile, antioxidant capacity, and physical characteristics of artichoke. Journal of Agricultural and Food Chemistry, 56: 8601.

Fillion L. and Henry C.J.K. 1998. Nutrient losses and gains during frying: a review. International Journal of Food Science and Nutrition, 49(1): 57.

Friedman H.H., Whitney J.E., and Szczesniak A.S. 1963. The texturometer: a new instrument for objective texture measurement. Journal of Food Science, 28: 390.

Holdsworth S.D. 1985. Optimization of thermal processing – a review. Journal of Food Engineering, 4: 89.

King D.A., Dikeman M.E., Wheeler T.L., Kastner C.L. and Koohmaraie M. 2003. Chilling and cooking rate effects on some myofibrillar determinants of tenderness of beef. Journal of Animal Science, 81: 1473.

Mansfield T. (1962). High temperature-short time sterilization. In: "Proceedings of the First International Congress on Food Science and Technology", London, UK.

Murphy R.Y., Johnson E.R., Duncan L.K., Clausen E.C., Davis M.D. and March J.A. 2001. Heat transfer properties, moisture loss, product yield, and soluble proteins in chicken breast patties during air convection cooking. Poultry Science, 80: 514.

National Advisory Committee on Microbiological Criteria for Foods (NACMCF) - United States Department of Agriculture Washington - FSIS Issues News Release on Single Minimum Internal Temperature for Cooked Poultry. 2006. Food Safety and Inspection Service, 7(14): 1.

Perrot P. 1998. A to Z of thermodynamics. Oxford University Press Inc, New York. Pp. 160-165.

Pompei C. 2009. Operazioni unitarie della tecnologia alimentare. Ed. Ambrosiana, Milan, Italy, p. 191.

Poon P.W.B., Durance T. and Kitts D.D. 2001. Composition and retention of lipid nutrients in cooked ground beef relative to heat-transfer rates. Food Chemistry, 74: 485.

Skog K., Eneroth A. and Svanberg M. 2003. Effects of different cooking methods on the formation of food mutagens in meat. International Journal of Food Science & Technology, 38(3): 313.

Sosa-Morales M.E., Orzuna-Espìritu R. and Vélez-Ruiz J.F. 2006. Mass, thermal and quality aspects of deep-fat frying of pork meat. Journal of Food Engineering, 77: 731.

Vittadini E., Rinaldi M., Chiavaro E., Barbanti D. and Massini R. 2005. The effect of different convection cooking methods on the instrumental quality and yield of pork *Longissimus dorsi*. Meat-Science, 69(4): 749.

Zhang L., Lyng J.G. and Brunton N.P. 2004. Effect of radio frequency cooking on the texture, colour and sensory properties of a large diameter comminuted meat product. Meat Science, 68: 257.

Paper received September 20, 2010 Accepted January 27, 2011

PAPER

BIOLOG METHODOLOGY FOR THE ANTIMICROBIAL ACTIVITY EVALUATION OF ESSENTIAL OILS' VOLATILE COMPOUNDS AGAINST FOODBORNE MICROFLORA OF FRESH-CUT SALAD

E. BERTOLONE[1], J.L. MINATI[1], B. ZANONI[2] and R. AMBROSOLI[1]*
[1]DIVAPRA, Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali
Settore Microbiologia agraria e Tecnologie alimentari, University of Turin,
Via Leonardo da Vinci 44, 10095 Grugliasco, TO, Italy
[2]DIBA, University of Florence, Italy
*Corresponding author: Tel. +39 11 6708554, Fax +39 11 6708549,
email: roberto.ambrosoli@unito.it

ABSTRACT

The antimicrobial properties of six volatile organic compounds (VOCs) of essential oils were tested on foodborne microflora of fresh-cut salad by plate-count method and by Biolog methodology, with lettuce-juice as nutritive medium. VOCs examined were eugenol, limonene, cinnamaldehyde, thymol, menthol and γ-terpinene, added in 2 concentrations in the wells of Biolog MT2 microplates for metabolic analisys, and in bacteriological tubes added with lettuce broth for growth observation. Both MT2 plates and broth tubes were inoculated with salad-extracted microflora. Chloramphenicol, pimaricin, sterile broth and inoculated broth with no compounds, were used as blanks.

The microbial metabolic activity was periodically measured by recording the absorbance variations in the wells at 590 nm during 144 hours of incubation. For growth measurements, the tubes were similarly incubated and periodically sampled for plate counting. The curves of absorbance temporal evolution, modelled with suitable mathematical equations, were used to express the effect of the VOCs under study on salad microbial population, and the results so obtained were compared with the ones obtained from growth measurements.

A good congruence was observed between the two methods for assessing the antimicrobial effect of the VOCs under study, while Biolog metabolic profiling provided finer pieces of information, being able to kinetically describe their antimicrobial behaviour (duration and evaluation of main and residual effect) during the chosen time of incubation. Such aptitude of Biolog methodology was verified in particular for the bacterial component of salad microflora, while a poor aptitude resulted for assessing the antimicrobial activity on the fungal component. A relevant inhibition against salad bacterial component was observed for eugenol (0.5 and 3 μL/mL), cinnamaldehyde (0.2 μL/mL) and thymol (0,5 mg/mL).

- Key words: antimicrobial activity, biolog methodology, essential oils, fresh-cut salad -

1. INTRODUCTION

In the last years the chemical composition of essential oils from different plants have been investigated by a variety of experimental researches and the antimicrobial properties of single volatile organic compounds (VOCs) or mixed fractions of them have been demonstrated (EDRIS, 2007), providing information on the activity spectrum of many essential oils (DELAQUIS *et al.*, 2002; SKOCIBUSIC *et al.*, 2006; SOYLU *et al.*, 2009). New plant sources of essential oils with high antimicrobial activity are also investigated for type and amount of active molecules present (SANDRI *et al.*, 2007) or their biochemical mode of action (ARFA *et al.*, 2006).

Other works are directed to understand the antimicrobial activity of single constituents of essential oils (DELAQUIS *et al.,* 2002), mainly against food-borne pathogens, such as *Salmonella typhimurium*, *Listeria monocytogenes, Escherichia coli O157:H7*, *Shigella dysenteriae*, *Bacillus cereus* and *Staphylococcus aureus* (BURT, 2004; TAO *et al.*, 2009). Thymol has been found active against *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (LAMBERT *et al.*, 2001; ULTEE *et al.*, 2002). Synergic antimicrobial effects have been demonstrated for combinations such as cinnamaldehyde/eugenol, thymol/eugenol and thymol/carvacrol (PEI *et al.*, 2009).

The pharmaceutical and therapeutic potential showed by essential oils and their single VOCs have been demonstrated in particular as chemopreventive and chemoterapeutic agents (myristicin, terpinen-4-ol, citral, limonene and monoterpenes in general), as anti-arteriosclerosis agents (γ-terpinene, thymol, garlic essential oil), as anti-thrombotic (allicin) and vasodilator (eugenol) compounds, and as antioxidant agents (thymol, carvacrol, α- and γ-terpinene) (EDRIS, 2007). Because of these properties, the addition in foods of specific molecules for the control of spoiling food-borne microorganisms can lead also to obtain functional foods for consumer health.

The traditional methods used for the evaluation of microbial sensitivity to chemical compounds are the so-called MIC (Minimum Inhibiting Concentration) test in liquid medium, and the antibiogram tests (BARRY, 1976; AUSTRIAN, 1980). The first represents the measure of microbial sensitivity/resistance to a certain antibiotic, allowing to establish the minimum inhibiting concentration to block microbial growth. The second is based on the diameter measure of the inhibition zones in agar medium. Both provide a single-reading measure, based on an evaluation of microbial growth. For both of them problems for volatility and poor solubility of oils or their constituents are reported, in view of their dilution and diffusion in microbiological media. Moreover, they are methods strictly linked to specific procedures, quite functional when the aim is measuring the sensitivity/resistance of a single microbial species (test-microorganism) but of limited efficacy when mixed populations, affected by numerous and interconnected factors, have to be investigated (BURT, 2004).

This is the case when fractions or single compounds constituents of essential oils are studied for their addition to fresh cut vegetables and fruits in order to investigate the possibility to increase the product shelf-life (JACXSENS *et al.*, 2003), also maintaining the good sensorial quality of the product (AYALA-ZAVALA *et al.*, 2009; VALERO *et al.*, 2006). For example, the possibility to create film-forming solutions containing antimicrobial substances into edible films for the storage of fruits and vegetables (DU *et al.,* 2009) is very interesting. Little is known about the effect of single constituents or mixed fractions of oils in experimental conditions similar to those of food matrices, where a microbial community in complex growth conditions is present. If the direct VOCs application in food matrices is the final aim of a study, the search for molecules with particular antimicrobial properties has to be combined with an effective method to ascertain their effect in real situations.

Aim of this work was to test an innovative analysis of antimicrobial activity, based on an application of Biolog methodology of metabolic fingerprinting. Traditional applications of Biolog methodology are for microbial identification (KLINGER *et al.*, 1992; PRAPHAILONG *et al.*, 1997) and microbiological characterization of soils (GOBERNA *et al.*, 2005) in presence of a wide array of carbon sources contained in the microwells of Elisa type plates. Other possible applications are also suggested, for monitoring microbial presence in biotechnological processes (DENITTIS *et al.*, 2010; WILLIAMS *et al.*, 2001). The potentialities built in Biolog methodology indicate the possibility to use it to assess the effect on microbial metabolism by environmental and/or chemical factors, in particular substances with supposed antimicrobial activity, obtaining finer pieces of information than the ones provided by current single-reading growth-based methods. In the case of VOCs, Biolog methodology seems also suitable to overcome volatility and poor solubility problems reported above. To test such possibilities in the present research, traditional Biolog methodology has been modified, inoculating the "total" microbiota extracted from a commercial ready-to-use salad in Biolog MT2 microplates in presence of different VOCs (eugenol, limonene, cinnamaldehyde, thymol, menthol and γ-terpinene) and a Redox indicator, whose changes are periodically monitored during a suitable incubation time to provide a measure of microbial activity evolution. A similar approach has been employed in previous

works where Biolog methodology was used to asses the toxicity of industrial waste streams on activated sludge microrganisms (FREITAS DOS SANTOS *et al.*, 2002).

2. MATERIALS AND METHODS

2.1 Experimental design

The antimicrobial action of eugenol, limonene, cinnamaldehyde, thymol, menthol and γ-terpinene against the microbial community of commercial ready-to-eat lettuce was studied in nutritional conditions as far as possible similar to the real ones.

Microbial metabolism in presence of VOCs was ascertained in Biolog MT2 microplates (Biolog Inc., CA, USA), whose wells contain a tetrazolium violet Redox indicator and no carbon source. Lettuce-juice (see 2.3) was added in the wells as growth medium, along with measured amounts of the VOCs (see 2.2) and inoculated with the microbial population previously extracted from commercial ready-to-use lettuce (see 2.4). During incubation, the Redox indicator undergoes a progressive colour change in consequence of the reduction caused by microbial metabolism, with an absorbance increase at λ=590 nm that can be used as a measure of the intensity of microbial activity (PRESTON-MAFHAM *et al.*, 2002). Successive absorbance readings during incubation were used to produce metabolic evolution curves that were modelled with suitable mathematical models (LINDSTROM *et al.*, 1998; ZWIETERING *et al.*, 1990) to express the kinetics of the metabolic activity and obtain a finer description of possible antimicrobial behaviour of the compounds tested.

Comparisons were made with the results obtained by analysing the quantitative evolution of microbial populations by the traditional plate count methodology.

2.2 Chemicals

Six single VOCs (eugenol, limonene, cinnamaldehyde, thymol, menthol and γ-terpinene), each of them in two concentrations, have been tested. They were dissolved in ethanol 99.8% and sterilized by 0.22 μm membrane filter (Schleicher & Schuell, FP30/0.2, Germany). Ethanol solutions were diluted in sterile water to avoid any interference on microbial growth and metabolism due to the solvent. Currently used VOCs' concentrations (VALERO *et al.,* 2006; SKOCIBUSIC *et al.*, 2006), were chosen: 0.5 and 3 μL/mL for eugenol and limonene, 0.1 and 0.2 μL/mL for cinnamaldheyde, 0.2 and 0.5mg/mL for thymol and menthol, 3 and 5 μL/ml for γ-terpinene. As reference antimicrobial molecules for bacteria and fungi inhibition, solutions of chloramphenicol (0.95 g/L) and 0.25% pimaricin (20 μL/mL), respectively, were prepared in inoculated broth. Inoculated and sterile broth, without any compound addition, were used as blanks. All chemicals were purchased from Sigma Aldrich (Germany).

2.3 Preparation of lettuce-juice broth

To simulate the growth condition in the food matrix, a nutritive broth from commercial lettuce was prepared for microbial count and metabolism analyses. The lettuce was washed with water, 40 g of the leaves were mixed in 200 mL of sterile quarter strength Ringer solution and centrifuged in a kitchen centrifuge for 60 s; the juice was paper filtered, then centrifuged (ALC, Multispeed Centrifuge, PK131) at 8,000 rpm for 5 min to separate chlorophyll and bulky material; the supernatant was filtered through 0.22 μm pore size filter (Schleicher & Schuell, FP30/0.2, Germany) and then autoclaved at 85°C for 30 min, three times at 24 hours intervals (JACXSENS *et al.*, 2003). Final pH was 5.7. Sterility and ability of juice to sustain microbial growth was confirmed in preliminary tests. Absence of absorbance at λ=590 nm to avoid any interferences with Biolog signal was verified by spectrophotometric reading (Shimadzu UV-1800). Absence of any of the VOCs used for the experiment was checked by Solid Phase Microextraction - Mass Spectrometry (SPME-MS) analysis.

2.4 Extraction of lettuce microflora and preparation of microbial growth and metabolism analyses

The antimicrobial effect of the six VOCs was tested against the total microflora extracted from commercial ready-to-eat lettuce. The leaves of the product were sterilely removed from the package and homogenized in sterile quarter strength Ringer solution in ratio 1:10 (w:v) by Stomacher Lab-Blender 400 (PBI International, Italy) for 3 min at 230 rpm. The resulting suspension was successively used to inoculate lettuce-juice nutrient broth for growth and metabolism analyses (1:10 v:v). Initial microbial presence in the nutrient broth was assessed with 3M Petrifilm™ "Aerobic Count Plate" (ACP) and 3M Petrifilm™ "Yeasts & Mould Count Plates" (YMCP), which gave respectively $1.7x\ 10^6$ and $3.4x10^2$ cfu/mL.

Immediately after inoculation, the lettuce-juice broth was added with the compounds to be tested. A total of 12 broth-compound combinations were so prepared, for the 6 VOCs in 2 concentrations each. Two combinations were also prepared with control antimicrobial compounds (pimaricin, chloramphenicol) and 2 controls with inoculated and sterile broth without any addition.

Microbial growth was performed in bacterio-

logical tubes containing the above reported combinations/controls and assessed after one and three days of incubation at 24°C in the dark by plate-count on 3M Petrifilm™ "Aerobic Count Plate" and "Yeasts & Moulds Count Plate". All the analyses were made in triplicate.

Microbial metabolic activity was assessed in Biolog MT2 microplates (Biolog, Inc., Hayward, CA, USA), which were inoculated by transferring 120 µL of every combination in 8 of their 96 wells and incubated for one week at 24°C in the dark. Metabolic activity was measured through absorbance (OD) readings at λ=590 nm with E-Max Reader (Biolog, Inc., Hayward, CA, USA) approximately every 10 h. All the analyses were made in triplicate.

Optical densities measured at 24 and 72 h of incubation in Biolog MT2 microplates were correlated to the results of ACP and YMCP counts for the screening of antimicrobial compounds. Absorbance readings obtained during approximately one week of incubation were used to produce a kinetic analysis of microbial metabolic profiles in presence of the examined compounds for every combination prepared.

All activities for the preparation of microplates and bacteriological tubes were performed under a laminar-flow hood to minimize risk of contamination.

2.5 Data analysis

2.5.1 Measure of microbial growth and metabolism inhibition

In order to obtain a numerical index describing the inhibiting activity of the VOCs, so providing a quantitative expression of VOCs' effect, for a better discrimination within the various cases under study, their effect on microbial growth and metabolism was expressed as *percentage of inhibition* on the basis of the increment of the experimental data at the beginning of incubation and after 24 and 72 h of it, by comparison with the blank containing only the inoculated broth. For growth analyses, the increment was calculated from the cfu/ml value before compounds addition, while for metabolic analyses it was calculated from the OD value measured for each combination immediately after the microplates preparation.

2.5.2 Cluster analysis

In order to identify differences and/or similarities in the inhibiting action of the VOCs tested and verify the possible congruence of the response obtained with the two methodologies, absorbance values (OD) and microbial counts (cfu/mL) at 24 and 72 hours of incubation were submitted to cluster analysis by SPSS 17 software. The clustering distance was measured as Euclidean distance and group average method was applied as linkage strategy. Resulting clusters were represented in dendograms.

2.5.3 Microplates readings and kinetic analysis

MT2 microplates were read at λ=590 nm (wavelength of maximum absorbance of the reduced tetrazolium dye) at approximately every 10 h for one week following inoculation. Data were then elaborated using the mean of the 8 OD replications for each combination and subtracting to each averaged OD value at λ=590 nm the averaged OD value measured at λ=750 nm (which accounts for turbidity). To further minimize the interference due to cell precipitation in the wells, the microplates were shaken for 10 s before reading.

A representation of the metabolic activity evolution for microbial population in presence of every VOC studied was so obtained. Experimental data were treated with Tablecurve®2D software 5.01 (SYSTAT Software Inc., USA) to carry out the non-linear regression based on Levenberg-Marquardt (LM) algorithm. Because of sigmoid shape of metabolic curves, two logistic functions were chosen for the fit, Lindstrom's equation ($y=K/[1+exp(-r*(t-s))]$) and Gompertz's modified equation ($y=A*exp[-exp(\mu*e*1/A*(\lambda-t)+1)]$), where A and k represents the maximum OD value, μ and r the colour development ($OD*h^{-1}$) in the exponential phase, λ the lag phase duration (h) and s the time (h) to reach the midpoint of the exponential phase of the curve.

Discrimination of the tested molecules based on the kinetic parameters was performed considering for each constant the values of 95% confidence limits, calculated by Tablecurve®2D software. Molecules with constant values found to lie within the same confidence limits were considered as being characterized by antimicrobial activity not significantly different.

3. RESULTS AND DISCUSSION

3.1 Percentage of inhibition of tested compounds (plate count and Biolog data)

Results, expressed as percentage of inhibition for mesophilic microorganisms by the presence of VOCs, are reported in Figg. 1, 2, and 3. Percentage of inhibition was calculated in comparison with the results obtained in blank conditions (inoculated broth without any compound).

In general, the range of growth inhibition evaluated with plate-count method (Figg. 1, 2) was wide, from 100% to 0 at both 24 and 72 hours. Biolog's metabolic measurements (Fig. 3) showed a shorter range of inhibition percentage, from a maximum of approximately 90% for chloramphenicol to a minimum of about 22% for menthol at 0.5 mg/mL. While plate-count method allowed discriminating between VOCs activity on bacteria and fungi, this was not possible with Biolog methodology, whose data are the result of the combined effect exerted by the two com-

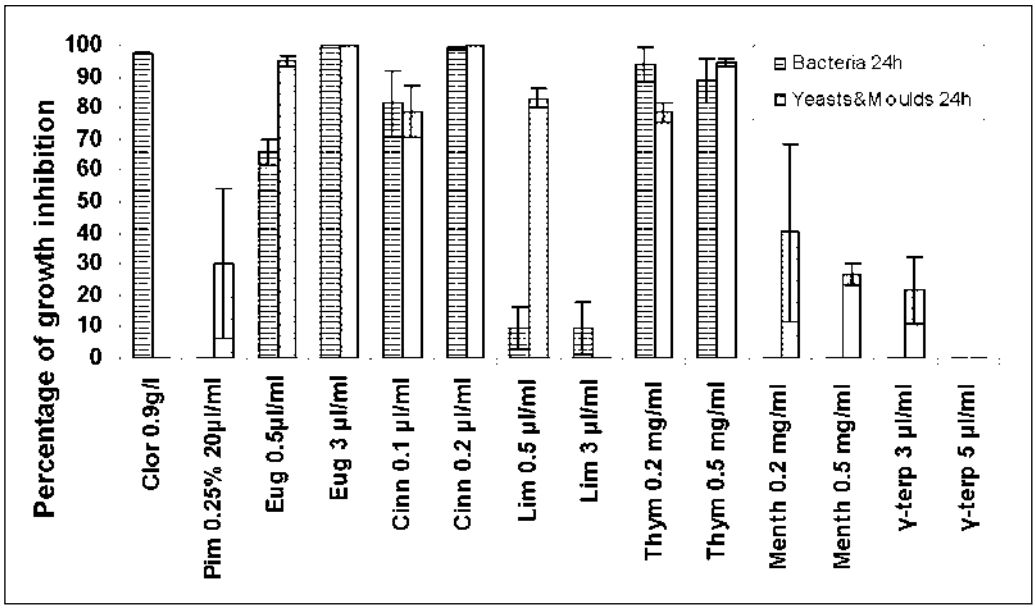


Fig. 1 - Inhibition percentage of microbial growth evaluated by plate count method at 24h incubation, in comparison with inoculated broth without antimicrobial compounds. Clor: Cloramphenicol; Pim: Pimaricin; Eug: Eugenol; Cinn: Cinnamaldheyde; Thym: Thymol; Lim: Limonene; Menth: Menthol; γ-terp: γ-terpinene.

ponents of the population inoculated. Nevertheless, as bacterial metabolism is generally faster than fungal metabolism, the bacterial component is likely to have been predominant in the plate wells, so that Biolog data can be hypothesized to account mainly for bacterial activity.

With the plate-count methodology, the two concentrations tested for eugenol, cynnamaldehyde and thymol showed the best antimicrobial activity at 24 and 72 h. Eugenol and cynnamaldehyde resulted active on both bacterial and eucariotic microflora, while thymol presented such behaviour only at 24 h, losing its activity against yeasts and moulds after 72 h of incubation.

For menthol and γ-terpinene, an increment of their antibacterial activity was recorded from 24 to 72 h, while their fungicidal activity decreased during the same period.

Antimicrobial activities measured with Biolog methodology presented some differences from the ones recorded for bacteria with plate-count method, in the sense that microorganisms in the inoculated population were affected (although in different measure) by the presence of *all* the molecules added. Molecules showing

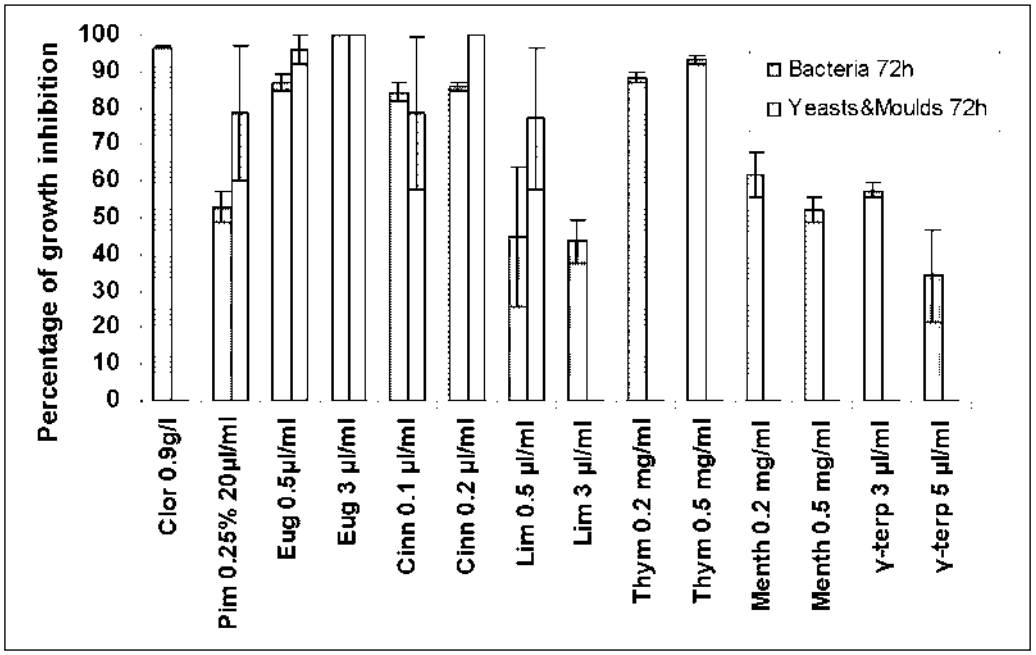


Fig. 2 - Inhibition percentage of microbial growth evaluated by plate count method at 72h incubation, in comparison with inoculated broth without antimicrobial compounds. Clor: Cloramphenicol; Pim: Pimaricin; Eug: Eugenol; Cinn: Cinnamaldheyde; Thym: Thymol; Lim: Limonene; Menth: Menthol; γ-terp: γ-terpinene.

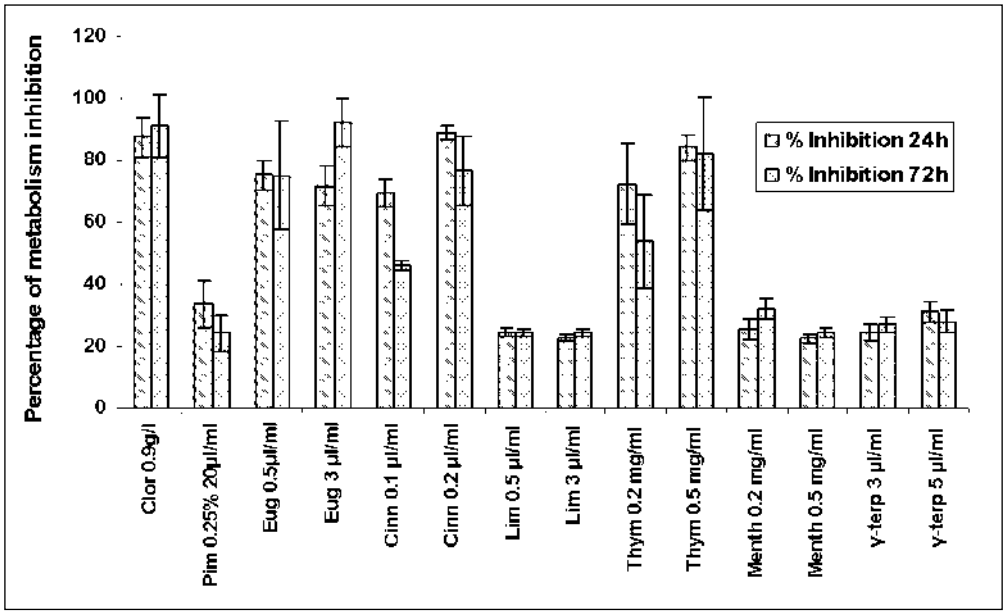


Fig. 3 - Inhibition percentage of microbial metabolism evaluated by Biolog methodology at 24h and 72h incubation, in comparison with inoculated broth without antimicrobial compounds. Clor: Cloramphenicol; Pim: Pimaricin; Eug: Eugenol; Cinn: Cinnamaldheyde; Thym: Thymol; Lim: Limonene; Menth: Menthol; γ-terp: γ-terpinene.

no or very low antibacterial activity with plate-count method (limonene, menthol, γ-terpinene) resulted in this case able to exert a not negligible effect, with an inhibition percentage of approximately 20-25%.

Anyway, in accordance with the results of plate-count method, the best inhibiting action was found for eugenol, cynnamaldehyde and thymol, although with some differences from plate-count results, probably due to the different sensitivity of plate-count and Biolog methodologies, because of the diverse microbial signal taken into consideration (microbial growth as expressed by colony formation in the former case, metabolic activity as expressed by OD variations in the latter).

For example, microbial growth in sample added with eugenol at 3 µL/mL resulted to be nil (100% inhibition) in agar medium both at 24 and 72 h. Anyway a metabolic activity significantly different from the uninoculated broth, was detected (corresponding to an inhibition of 70 and 90%, respectively at 24 and 72 h). These results indicated a better ability by Biolog methodology than traditional plate-count method to detect also low microbial presence. Moreover, Biolog's response was able to better describe variations of the inhibiting effect showed by low VOCs concentrations in comparison with the information obtained by plate-count. In the case of cynnamaldehyde at 0.1 µL/mL and thymol at 0.2 mg/mL, a decrease of their inhibiting power between 24 and 72 h (from 70 to 46% and from 72 to 53%, respectively) was detected by Biolog and not by plate-count. Contrarily, cinnamaldheyde and thymol at their greater concentrations, showed the best inhibiting action on microbial metabolism (superior to 85%, equal to reference compound, cloramphenicol) independently from incubation time.

As already noted, limonene, menthol and γ-terpinene showed the lowest antimicrobial activity. No particular behaviour was detected by Biolog methodology for limonene at 0.5 µL/mL, that by plate-count method was indicated as comparable to pimaricin, cynnamaldehyde and eugenol for its activity against yeasts and moulds. This again suggests that the fungal component was not relevant in the wells of Biolog plates in these experimental conditions.

3.2 Cluster analysis of plate-count and Biolog data

Data obtained after 24 and 72 hours of incubation by Biolog and plate-count methodologies were submitted to cluster analysis to compare the results obtained by these techniques. The results are summarized in Fig. 4.

High congruence between Biolog results and bacterial plate-count results was observed for data at 24 h. In both cases, two big groups can be seen, each of them linked at very low Euclidean distance. The first contains all the experiments where a weak antimicrobial activity was detected (limonene, menthol, γ-terpinene, at the different concentrations tested). Results for pimaricin and the blank without any compound were also included in this group, as it could be expected. The second group links the results obtained for the VOCs with highest antimicrobial activity (eugenol, cynnamaldehyde and thymol, at all the concentrations tested) and chloramphenicol (reference molecule for antibacterial activity). Some minor differences can be observed between Biolog and plate-count data as for relative posi-

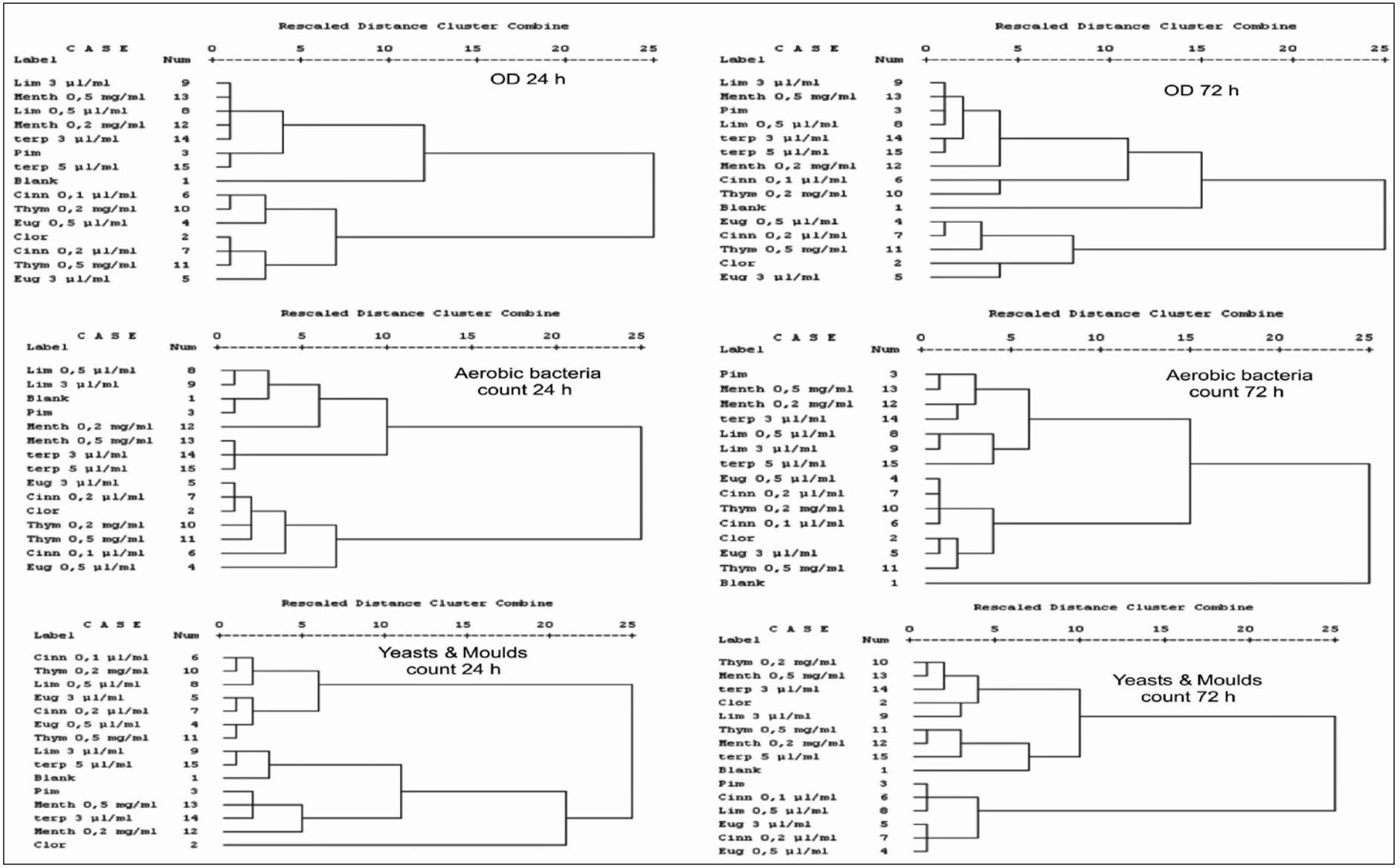


Fig. 4 - Cluster analysis of volatile compounds' antimicrobial activity evaluated as cfu/mL with plate count method (total aerobic bacteria and yeasts and moulds) and metabolic activity (OD) at 24h and 72h of incubation. Distance between samples was measured as Euclidean distance. Group average method was applied as linkage stategy.
Clor: Cloramphenicol; Pim: Pimaricin; Eug: Eugenol; Cinn: Cinnamaldheyde; Thym: Thymol; Lim: Limonene; Menth: Menthol; γ-terp: γ-terpinene.

tion of the compounds in the sub-groups, due to the different sensitivity of the two methodologies in evaluating the amount of antimicrobial activity. Anyway, as all the sub-groups are linked at an Euclidean distance even lower than the one of the main groups, such differences do not invalidate the general congruence of these results. Such finding seems to again confirm the hypothesis that the Biolog methodology applied in the present study accounts particularly for the bacterial component of the populations tested.

The above reported congruence is not entirely maintained for the results at 72 h. While plate-count results are grouped practically in the same way as the ones at 24 h, at even lower Euclidean distances (with the only exception of blank, that is positioned outside all groupings), for Biolog results a main group can be observed, where the weak activity compounds are included together with two of the ones previously positioned in the active group, cynnamaldehyde and thymol at their minimal concentration. The other high activity compounds are strictly linked in a separate group (blank is positioned outside all groupings, here also).

The reason for this result probably lies in the already noted higher sensitivity of Biolog methodology, that detected a decrease of antibacterial activity at 72 h for the lowest concentrations of cynnamaldehyde and thymol, while plate-count method was not able to do so.

No congruence with Biolog data was found for the ones obtained with plate-count for yeasts and moulds, due to the unsuitability of Biolog methodology to account for the activity of such microorganisms in our experimental conditions. Cluster analysis of data at 24 h showed two main groupings of the VOCs tested. The first (with higher antifungal activity) is constituted by cynnamaldehyde, eugenol, thymol at their low and high concentrations, and by limonene at 0.5 µL/mL, while in the second all the other cases are linked along with pimaricin, chloramphenicol and blank. Two main clusters can be seen also for the results at 72 h, but with a quite different distribution of cases in each of them, according with the time-dependent activity variation which the tested compounds at their different concentrations underwent during incubation.

In particular, an interesting piece of information was obtained about the action of pimaricin, which required more than 24 hours to exert a microbial inhibition.

33. Kinetic analysis of metabolic profiles

In general, both Lindstrom's and modified Gompertz's functions showed good fitting properties for the experimental metabolic profiles obtained with Biolog methodology. In fact, R^2 values (Table 1) for Lindstrom's model ranged between 0.972 and 0.997 and for modified Gompertz's between 0.891 and 0.999. Only the OD curve corresponding to cloramphenicol did not significantly ($P>0.05$) fit to Lindstrom's function and those corresponding to eugenol 3 µL/mL did not significantly ($P>0.05$) fit both to Lindstrom's and modified Gompertz's models.

In Tables 2 and 3, the values of the constants characteristic of the two equations are reported, along with their confidence limits, while in Table 4 the Pearson correlation between such parameters and the inhibition percentage evaluated with Biolog methodology can be seen. From Pearson correlation analysis, both Lindstrom's and modified Gompertz's parameters result suitable to describe the inhibition measured through OD data. As percentages of inhibition increase, asymptotes (k, A) and maximum rates of OD development decrease, while lag phase (λ) and time to reach inflection point (s) increase. Nevertheless, Pearson coefficients resulted higher for the parameters corresponding to Lindstrom's model than for the ones corresponding to modified Gompertz's model.

Modified Gompertz's function, unlike Lindstrom's, is affected by the duration of lag phase (λ) and for such reason is more suitable to describe the behaviour of molecules with high inhibiting power, accounting for the delay in metabolic activity that they initially produce. Lindstrom's model, on the other hand, can be more suitable for molecules with low or medium inhibition power, moderately affecting the initial metabolic activity of the population under study.

Thus, the choice of the most suitable mathematical model, in order to obtain the best description of the bacterial metabolic activity in presence of the different molecules, should be

Table 1 - Fitting properties (R^2) of model equations applied to experimental metabolic curves for different volatile compounds at different concentrations.
[a] Fitting not statistically significant at 95% level.

Samples	R^2	
	Lindstrom model	Modified Gompertz model
Blank (inoculated broth)	0.992	0.994
Cloramphenicol 0,95 g/L	/[a]	0.891
Pimaricin 0,25% 20 µL/mL	0.995	0.990
Eugenol 0,5 µL/mL	0.997	0.990
Eugenol 3 µL/mL	/[a]	/[a]
Cinnamaldehyde 0,1 µL/mL	0.972	0.988
Cinnamaldehyde 0,2 µL/mL	0.994	0.999
Limonene 0,5 µL/mL	0.990	0.992
Limonene 3 µL/mL	0.982	0.987
Thymol 0,2 m g/mL	0.996	0.993
Thymol 0,5 m g/mL	0.989	0.999
Menthol 0,2 m g/mL	0.982	0.984
Menthol 0,5 m g/mL	0.985	0.990
γ-terpinene 3 µL/mL	0.985	0.990
γ-terpinene 5 µL/mL	0.990	0.993

Table 2 - Kinetic parameters (K , R and S) and 95% confidence limits (L.C.L., lower confidence limit; U.C.L., upper confidence limit) for microbial metabolic curves fitted to Lindstrom equation.
[a] Fitting not statistically significant at 95% level.

Samples	K 95% C.L.			R 95% C.L.			S 95% C.L.		
	K (OD)	L.C.L.	U.C.L.	r (OD/h)	L.C.L.	U.C.L.	S (h)	L.C.L.	U.C.L.
Blank (inoculated broth)	1,6029	1,5488	1,6570	0,0787	0,0682	0,0891	34,32	32,28	36,35
Cloramphenicol 0,95g/l [a]	/	/	/	/	/	/	/	/	/
Pimaricin 0,25% 20µl/ml	1,3906	1,3481	1,4332	0,0624	0,0562	0,0685	43,48	41,54	45,41
Eugenol 0,5 µl/ml	0,7446	0,7214	0,7677	0,0351	0,0327	0,0375	70,13	67,35	72,91
Eugenol 3 µl/ml [a]	/	/	/	/	/	/	/	/	/
Cinnamaldheyde 0,1 µl/ml	1,3999	1,2726	1,5273	0,0422	0,0324	0,0521	61,19	54,09	68,29
Cinnamaldheyde 0,2 µl/ml	0,9098	0,8416	0,9779	0,0522	0,0397	0,0648	80,98	74,65	87,32
Limonene 0,5 µl/ml	1,3324	1,2798	1,3850	0,0667	0,0575	0,0758	38,73	36,25	41,21
Limonene 3 µl/ml	1,4364	1,3541	1,5187	0,0572	0,0468	0,0676	41,46	37,64	45,27
Thymol 0,2 mg/ml	0,9944	0,9652	1,0236	0,0526	0,0481	0,0570	54,08	52,09	56,07
Thymol 0,5 mg/ml	0,4753	0,4483	0,5022	0,0383	0,0331	0,0435	66,20	61,44	70,95
Menthol 0,2 mg/ml	1,3668	1,2851	1,4485	0,0511	0,0420	0,0601	44,86	40,69	49,03
Menthol 0,5 mg/ml	1,4077	1,3358	1,4797	0,0613	0,0510	0,0716	40,55	37,24	43,85
γ-terpinene 3µl/ml	1,3452	1,2782	1,4122	0,0617	0,0515	0,0719	40,25	37,04	43,46
γ-terpinene 5µl/ml	1,2733	1,2220	1,3245	0,0690	0,0593	0,0787	39,06	36,57	41,54

made on the basis of both the R^2 values of OD curves, which are represented in Table 1.

Should OD curves with the same behaviour at the beginning of the kinetic be compared, the Lindstrom's model with the parameter "s" as a descriptor would allow a more useful characterization. This is the case, for instance, of pimaricin (0.25%, 20 µL) and cinnamaldehyde at 0.1 µL/mL (Fig. 7), which not significantly differed for Gompertz's "λ" parameter and were similar for asymptotic values, but showed significant difference for Lindstrom's "s" parameter (Tables 2 and 3). Lindstrom's model is also more useful for the discrimination between substances with weak inhibiting action (such as pimaricin, menthol and γ-terpinene) and the blank: in fact, Lindstrom's model provides two kinetic constants (k, s) usable for the purpose of differentiation from the blank, while only one (A) is provided by modified Gompertz's model (Tables 2 and 3).

Modified Gompertz's model results more suitable to fit the OD curves corresponding to molecules with a percentage of inhibition at 24 h higher than 80% (corresponding to OD values between 0.055 and 0.077), as in the case of cloramphenicol at 0.95 gL, thymol at 0.5 mg/mL

Table 3 - Kinetic parameters (A, µ and λ) and 95% confidence limits (L.C.L., lower confidence limit; U.C.L.. upper confidence limit) for microbial metabolic curves fitted to modified Gompertz equation.
[a] Fitting not statistically significant at 95% level.

Samples	A 95% C.L.			µ 95% C.L.			λ 95% C.L.		
	A (lnOD)	L.C.L.	U.C.L.	µ (lnOD/h)	L.C.L.	U.C.L.	λ (h)	L.C.L.	U.C.L.
Blank (inoculated broth)	3,2920	3,2190	3,3650	0,1333	0,1099	0,1566	6,810	3,889	9,730
Cloramphenicol 0,95g/l	0,9334	0,8307	1,0361	0,1058	0	0,3138	24,146	19,084	31,208
Pimaricin 0,25% 20µl/ml	3,1564	3,0526	3,2601	0,0920	0,0754	0,1086	5,393	1,843	8,943
Eugenol 0,5 µl/ml	2,3917	2,2655	2,5179	0,0309	0,0271	0,0347	5,293	0,821	9,766
Eugenol 3 µl/ml [a]	/	/	/	/	/	/	/	/	/
Cinnamaldheyde 0,1 µl/ml	2,9692	2,8316	3,1067	0,0650	0,0536	0,0764	9,333	5,298	13,369
Cinnamaldheyde 0,2 µl/ml	2,7766	2,7407	2,8124	0,0546	0,0524	0,0568	35,991	35,060	36,922
Limonene 0,5 µl/ml	3,1308	3,0492	3,2125	0,1058	0,0881	0,1234	5,856	2,818	8,894
Limonene 3 µl/ml	3,0497	2,9481	3,1513	0,1061	0,0818	0,1305	6,538	2,465	10,610
Thymol 0,2 mg/ml	2,7812	2,6885	2,8738	0,0558	0,0493	0,0622	8,172	5,268	11,076
Thymol 0,5 mg/ml	1,9594	1,9196	1,9992	0,0309	0,0293	0,0325	22,211	20,648	23,775
Menthol 0,2 mg/ml	2,9925	2,8756	3,1094	0,0927	0,0719	0,1135	5,153	0,866	9,439
Menthol 0,5 mg/ml	3,1	3,0072	3,1928	0,1040	0,0837	0,1242	6,152	2,622	9,683
γ-terpinene 3µl/ml	3,0074	2,9128	3,1020	0,1013	0,0799	0,1227	6,599	2,807	10,391
γ-terpinene 5µl/ml	2,9869	2,9060	3,0679	0,0976	0,0800	0,1152	6,996	3,749	10,243

Table 4 - Pearson's correlation coefficients between the metabolic inhibition percentage at 24 h and 72 h and the kinetic parameters of Lindstrom and modified Gompertz models.

Pearson correlation	% metabolic inhibition 24 h	% metabolic inhibition 72 h
K	-0.840	-0.931
R	-0.813	-0.853
S	0.941	0.937
A	-0.713	-0.836
μ	-0.790	-0.726
λ	0.707	0.724

and cinnamaldehyde at 0.2 μL/mL (Fig. 2). For them, the duration of lag phase (λ, Tab. 3) allows an immediate perception of their antimicrobial activity, in comparison with that of the other compounds.

The kinetic characterization of OD curves allows to obtain a finer description and discrimination of possible antimicrobial behaviour of the compounds tested. In Fig. 5 the OD curves (Gompertz's model) corresponding to cinnamaldehyde at 0.2 μL/mL, eugenol at 0.5 μL/mL and thymol at 0.5 mg/mL (all high inhibiting activity compounds) are represented in comparison with the OD curve of the blank (inoculated broth with no VOCs). While all the curves show inhibiting action on microbial metabolism, specific trends can be seen for each of them.

In the case of cinnamaldehyde (0.2 μL/mL), the long lag phase followed by a rise of the exponential phase indicates that the initial relevant antimicrobial activity was subsequently lost, with a restart of microbial vitality. The data obtained for eugenol (0.5 μL/mL) suggest a progressive increase of the antimicrobial action of this molecule during incubation, as accounted by a short lag phase and a low asymptotic value. For thymol (0.5 mg/mL) a long lag phase and a low asymptote value was seen, suggesting that this compound maintains a high antimicrobial action during the entire incubation. Such observations agree with the ones drawn from the consideration of Table 3, and can be used to assess how different molecules or concentrations could be mixed to obtain the desired antimicrobial action in a specific range of time.

Figure 6 reports the different effects on microbial metabolism when two compounds (cinnamaldehyde and thymol) were used at different concentrations. The use of thymol at 0.5 mg/mL decreased the level of microbial metabolism with greater effectiveness than at the lower concentration (0.2 mg/mL). In the case of cinnamaldehyde, on the other hand, the greater initial antimicrobial activity of the higher concentration

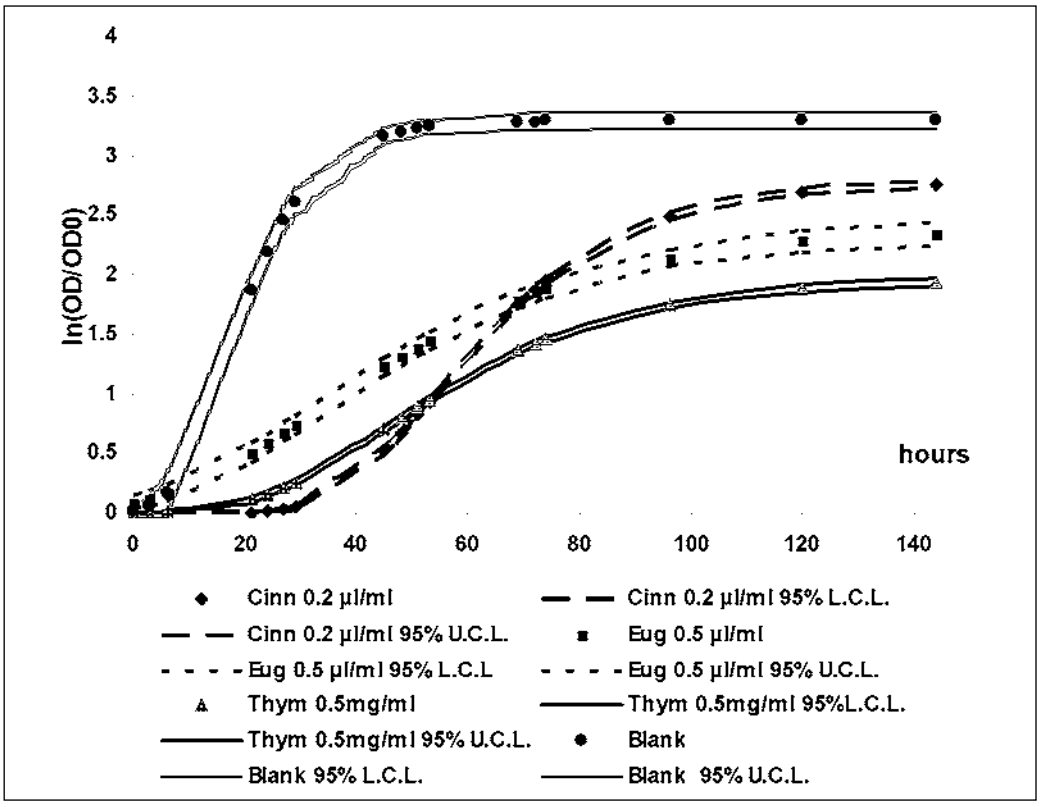


Fig. 5 - OD curves fitted to Gompertz modified model in presence of cinnamaldheyde at 0.2 μL/mL, eugenol at 0.5 μL/mL and thymol at 0.5 mg/mL in comparison with blank without any compound.
Cinn: Cinnamaldheyde; Eug: Eugenol; Thym: Thymol; L.C.L.: Lower Confidence Limit; U.C.L.: Upper Confidence Limit.

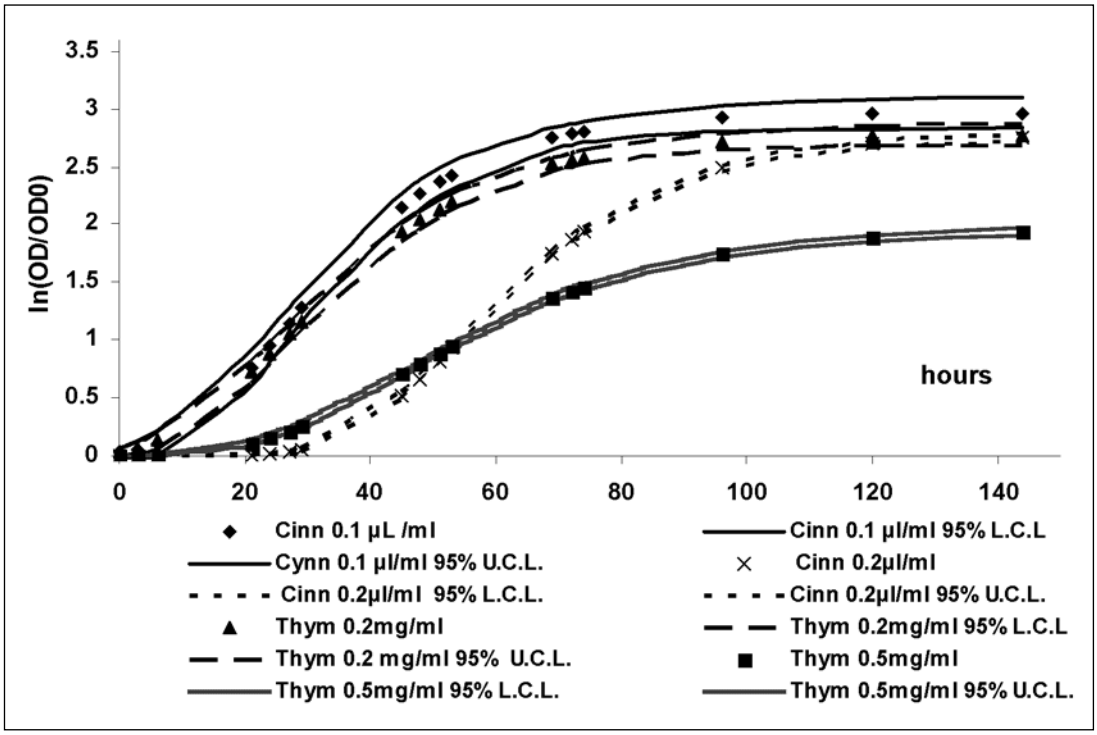


Fig. 6 - OD curves fitted to Gompertz modified model in presence of cynnamaldheyde at 0.1 and 0.2 µL/mL and thymol at 0.2 and 0.5 mg/mL.
Cinn: Cinnamaldheyde; Thym: Thymol; L.C.L.: Lower Confidence Limit; U.C.L.: Upper Confidence Limit.

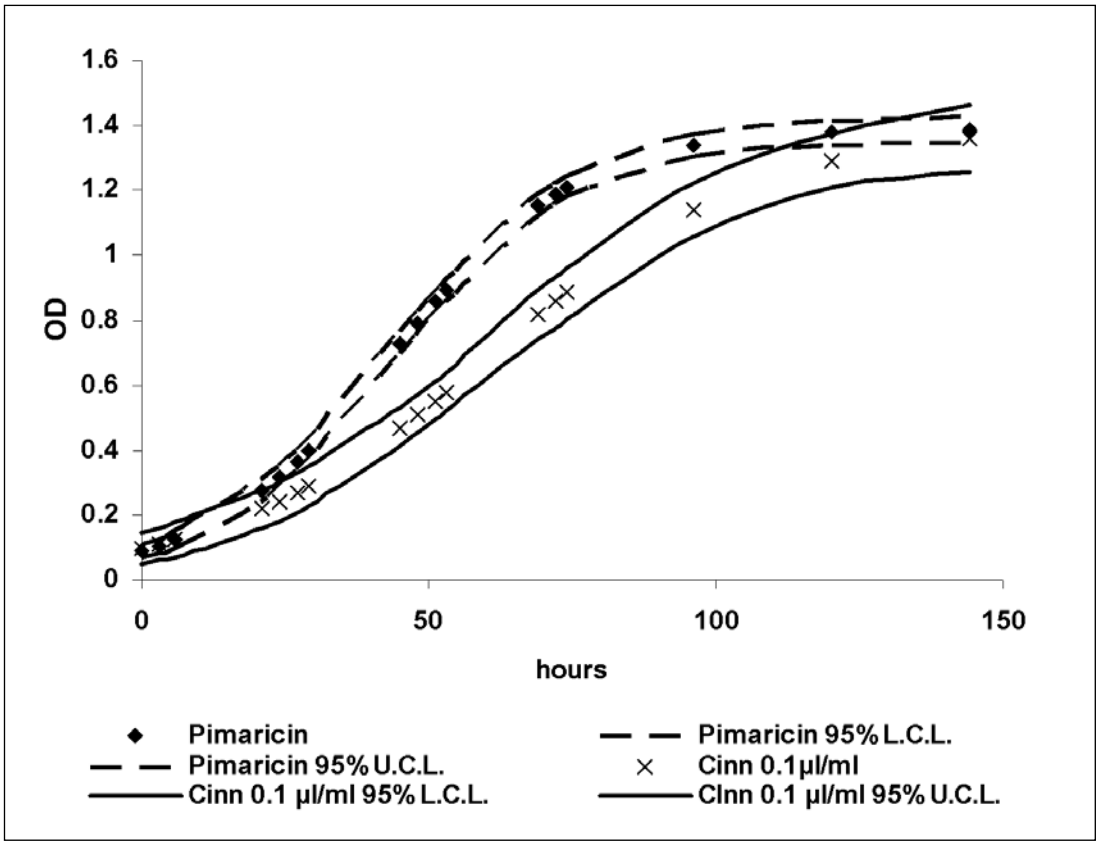


Fig. 7 - OD curves fitted to Lindstrom model in presence of cynnamaldheyde at 0.1 and 0.2 µL/mL and pimaricin 0.25% at 20 µL/mL.
Cinn: Cinnamaldheyde; L.C.L.: Lower Confidence Limit; U.C.L.: Upper Confidence Limit.

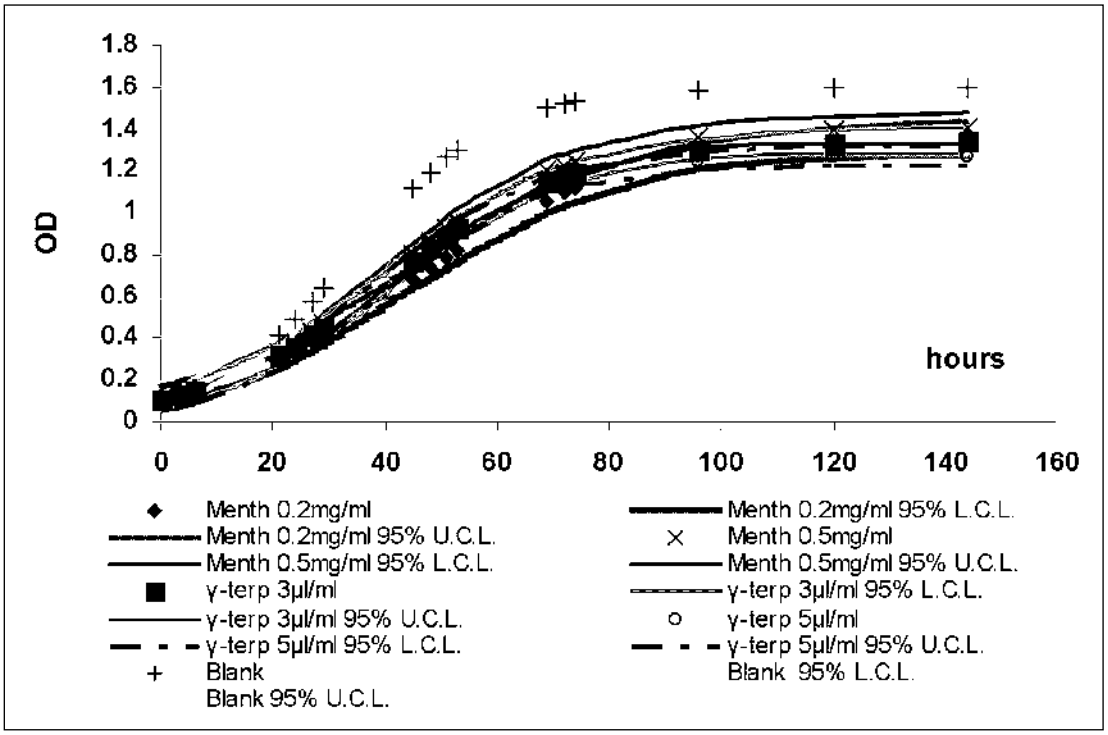


Fig. 8 - OD curves fitted to Lindstrom model in presence of menthol at 0.2 and 0.5 mg/mL, γ-terpinene at 3 and 5 µL/mL in comparison to the blank without any compound.
Menth: menthol; γ-terp: γ-terpinene; L.C.L.: Lower Confidence Limit; U.C.L.: Upper Confidence Limit.

(0.2 µL/mL) was lost during the first hours of incubation, as already noted, so that the final asymptotic value reached was not very different from the lower one (0.1 µL/mL), although a longer time was needed.

Comparing the antimicrobial action of pimaricin and cinnamaldehyde (0.1 µL/mL) (Fig. 7) a time interval was identified (approximately 40-100 hours of incubation) in which the inhibiting activity of the molecules significantly differed, pimaricin being less effective than cinnamaldehyde. No real differences in the concentration effect were observed for the γ-terpinene and menthol (Fig. 8).

In general, the consideration of the curves' trends allowed to obtain information about the temporal effect of the compounds tested. In particular, more molecules could be added together in order to exploit different properties to control food hygiene and quality during the shelf-life.

CONCLUSIONS

The antimicrobial behaviour of six *VOCs* costituents of essential oils (eugenol, cinnamaldehyde, thymol, menthol, limonene, γ-terpinene) against fresh-cut lettuce microflora has been investigated through plate-count and an application of Biolog methodology. Among the molecules examined, eugenol, cinnamaldheyde and thymol showed the best performances, so confirming previous observations about microbial sensitivity to certain *VOCs* (VALERO and GINER, 2006; LANCIOTTI *et al.*, 2004).

Plate-count and Biolog data resulted congruent for the detection of the inhibiting action of natural compounds on the bacterial component of the lettuce microflora, confirming the suitability of the methodology under study for the screening of the antibacterial action of volatile organic compounds. No volatility and/or poor solubility problems resulted able to affect the results. More important, the possibility to fit the experimental OD curves to suitable mathematical models (with their characteristic parameters) allows a better discrimination within the different modes of action of the examined compounds, providing (unlike traditional methods for antimicrobial activity evaluation) multiple information about duration of the main effect of a certain substance (lag-phase), residual effect (growth velocity) and final antimicrobial activity (asymptote). In presence of the kinetic characterization of a previously ascertained "ideal" antimicrobial behaviour, the experimental OD values of a certain compound could be compared with it (taking into account the parameters of the curves) for the assessment of the possible differences between the two cases and the eval-

uation of compound's suitability in view of the desired result.

An asset of this methodology is also the possibility to simultaneously study the different activity of multiple molecules, using Biolog MT2 microplates with a microbial growth medium that better reproduce the nutritional situation of a certain food matrix, provided that it does not interfere with the OD values assessment. From this point of view, this application of Biolog methodology can be useful also for monitoring microbial activity related in food spoilage and the effect of microbial strains artificially added to the matrix.

The application of the traditional tests for the evaluation of microrganisms sensitivity to antimicrobial compounds (MIC, antibiogram) together with Biolog analysis can lead to a specific selection of the molecules showing the best antimicrobial behaviour not only on cell multiplication but also on cell metabolic activity, which is a main factor of food spoilage.

REFERENCES

Arfa A.B., Combes S., Preziosi-Belloy L., Gontard N. and Chalier P. 2006. Antimicrobial activity of carvacrol related to its chemical structure. Letters in Applied Microbiology, 43: 149.

Austrian R. 1980. Chemioterapia delle malattie batteriche: prove di sensibilità ai farmaci antibatterici. In: Trattato di Microbiologia, 25: 689.

Ayala-Zavala J.F., Gonzales-Aguilar G.A. and del Toro-Sanchez L. 2009. Enhancing safety and aroma appealing of fresh-cut fruits and vegetables using the antimicrobial and aromatic power of essential oils. Journal of Food Science 74, 7: 84.

Barry A.L. 1976. The Antimicrobics Susceptibility Test: Principles and Practices. Lea and Febiger, Philadelphia, USA.

Burt S. 2004. Essential oils: properties and potential applications in foods - a review. International Journal of Food Microbiology, 94: 223.

Delaquis P.J., Stanich K., Girard B. and Mazza G. 2002. Antimicrobial activity of individual and mixed fraction of dill, cilantro, coriander and eucalyptus essential oils. International Journal of Food Microbiology, 74: 101.

DeNittis M., Querol A., Zanoni B., Minati J.L. and Ambrosoli R. 2010. Possible use of Biolog methodology for monitoring yeast presence in alcoholic fermentation for winemaking. Journal of Applied Microbiology, 108, 4: 1199.

Du W-X., Olsen C.W., Avena-Bustillos R.J., McHugh T.H., Levin C.E. and Friedman M. 2009. Effect of allspice, cinnamon, clove bud, essential oil in edible apple films on physical properties and antimicrobial activities. Journal of Food Science, 74, 7: 372.

Edris A.E. 2007. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytotherapy research, 21: 308.

Freitas dos Santos L., Defrenne L. and Krebs-Brown A. 2002. Comparison of three microbial assay procedures for measuring toxicity of chemical compounds: ToxAlert®10, CellSense and Biolog MT2 microplates. Analytica Chimica Acta, 456: 41.

Goberna M., Insam H., Pascual J.A. and Sanchez J. 2005. Storage effects on the community level physiological profiles of Mediterranean forest soils. Soil Biology&Biochemistry, 37: 173.

Jacxsens L., Devlieghere F., Ragaert P., Vanneste E. and Debevere J. 2003. Relation between microbiological quality, metabolite production and sensory quality of equilibrium modified atmosphere packaged fresh-cut produce. International Journal of Food Microbiology 83: 263.

Klingler J.M., Stowe R.P., Obenhuber D.C., Groves T.O., Mishra S.K. and Pierson D.L. 1992. Evaluation of the Biolog automated microbial identification system. Applied and Environmental Microbiology, 58, 6: 2089.

Lambert R.J.W., Skandamis P.N., Coote P. and Nychas G.-J.E. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology, 91: 453.

Lanciotti R., Gianotti A., Patrignani F., Belletti N., Guerzoni M.E. and Gardini F. 2004. Use of natural aroma compounds to improve shelf-life and safety of minimally processed fruits. Trends in Food Science & Technology, 15: 201.

Lindstrom J.E., Barry R.P. and Braddock J.F. 1998. Microbial community analysis: a kinetic approach to constructing potential C source utilization patterns. Soil Biology & Biochemistry, 30: 231.

Pei R.-S., Zhou F., Ji B.-P. and Xu J. 2009. Evaluation of combined antibacterial effects of eugenol, cinnamaldehyde, thymol and carvacrol against *E.coli* with an improved method. Journal of Food Science, 74: 379.

Praphailong W., Van Gestel M., Fleet G.H. and Heard G.M. 1997. Evaluation of the Biolog system for the identification of food and beverage yeasts. Letters in Applied Microbiology, 24: 455.

Preston-Mafham J., Boddy L. and Randerson P.F. 2002. Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles - a critique. FEMS, Microbiology Ecology, 42:1.

Sandri I.G., Zacaria J., Fracaro F., Delamare A.P.L. and Echeverrigaray S. 2007. Antimicrobial activity of the essential oils of Brazilian species of the genus *Cunila* against foodborne pathogens and spoiling bacteria. Food Chemistry, 103: 823.

Skocibusic M., Bezic N. and Dunkic V. 2006. Phytochemical composition and antimicrobial activities of the essential oils from *Satureja subspicata* Vis. growing in Croatia. Food Chemistry, 96, 20.

Soylu S., Soylu E.M. and Evrendilek G.A. 2009. Chemical composition and antibacterial activity of essential oils of bitter fennel (*Foeniculum Vulgare* Mill.Var. *Vulgare*) and dill (*Anethum Graveolens* L.) against the growth foodborne and seed borne pathogenic bacteria. Italian Journal of Food Science, 3, 21:347.

Tao N., Liu Y. and Zhang M. 2009. Chemical composition and antimicrobial activities of essential oil from the peel of bingtang sweet orange (*Citrus sinensis* Osbeck). International Journal of Food Science & Technology, 44: 1281.

Ultee A., Bennink M.H.J. and Moezelaar R. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-born pathogen *Bacillus cereus*. Applied and Environmental Microbiology 68: 1561.

Valero M. and Giner M.J. 2006. Effects of antimicrobial components of essential oils on growth of Bacillus cereus INRA L2104 and sensory quality of carrot broth. International Journal of Food Microbiology, 106: 90.

Williams A.G., Noble J. and Banks J.M. 2001. Catabolism of amino acids by lactic acid bacteria isolated from Cheddar cheese. International Dairy Journal 11:203.

Zwietering M.H., Jongenburger I., Rombouts F.M. and Van'T Riet K. 1990. Modeling of the bacterial growth curve. Applied and Environmental Microbiology, 56: 1875.

Paper received September 6, 2010 Accepted March 25, 2011

PAPER

STABILITY OF INVERTASE IMMOBILIZED ON THREE DIFFERENT SUPPORTS

TAISEER M. ABOU BAKER*, SAMIR M. EL-IRAQI, LAILA A. SHEKIB and HANAA A. ABDELLAH
Food Science and Technology Department, Faculty of Agriculture,
Alexandria University 21545, El-Shatby, Alexandria, Egypt
*Corresponding author: Fax +203 5922780,
email: taiseerbakr@yahoo.com

ABSTRACT

β-D-fructofuranosidase, (EC 3.2.1.26) was immobilized on DEAE-cellulose, polyacrylamide and hen-egg white. Storage at 4°C for 5 months indicated that the polyacrylamide and DEAE-cellulose preparations were more stable. The effect of lyophilization on immobilized invertase activity was also studied. It was found that lyophilization of the enzyme attached to DEAE-cellulose caused a decrease in its activity as compared to the other two enzymes. All lyophilized preparations could be stored for 4-5 months with a slight decrease in their activities. Batch processes were used to trace the activity of immobilized invertase. The results showed that the enzyme preparations could be re-used for eight cycles with slight effect on their activities. The inversion degree of sucrose at different concentrations and flow rates using the polyacrylamide-enzyme gel ranged between 52 to 99%. A polyacrylamide-invertase gel was operated continuously for 5 days with a very low decrease in its retained activity (3%). After storage for one month the polyacrylamide-enzyme matrix conserves 83% of its initial velocity.

- Key words: DEAE-cellulose, hen-egg white, polyacrylamide, invertase, invert syrup -

1. INTRODUCTION

Invertase β-D-fructofuranosidase (EC 3.2.1.26) is mainly used to hydrolyze sucrose in the production of invert sugar. Invert sugar is sweeter and easier to incorporate in industrial preparations and has a lower crystallinity at higher concentrations than is granular sucrose (CHOU and JASOVSKY, 1993; SANJAY and SUGUNAN, 2005). The invert syrups were used extensively in the production of soft drinks (BASSETTI *et al.*, 2000), non-crystallizing creams, jams and glazed fruits (EMREGUL *et al.*, 2006). Due to its hygroscopic nature, invert syrup is utilized in the manufacture of soft-centered candies and fondants (MILOVANOVIĆ *et al.*, 2007, KOTWAL and SHANKAR, 2009). Enzymatic hydrolysis of sucrose is preferable to acid hydrolysis as it does not result in the formation of undesirable flavouring agents as well as coloured impurities (MONSAN and COMBES, 1984; ARRUDA and VITOLE, 1999; KOTWAL and SHANKAR, 2009). Although invertase is a relatively inexpensive enzyme, its use in the free form necessitates a batch operation which reduces process efficiencies. So, the application of immobilized invertase may offer technical and economical advantages, such as the reduction of enzyme usage because once the enzyme is immobilized, it can be used for a much longer period than in its soluble form. In addition, the immobilized enzyme can lead to preferred continuous processes that may use either fixed or fluidized bed reactors, in which it is possible to use a higher enzyme dosage per volume of reactor than in the soluble enzyme process, and this contributes to high reaction rates and consequently, small reactor sizes (KENNEDY and WHITE, 1985; ZANIN and DE MORAES, 1994; AKGOL *et al.*, 2001; KOTWAL and SHANKAR, 2009). These technical advantages allow a reduction in the operational and capital process costs (WISEMAN, 1985; BERGAMASCO *et al.*, 2000; EMREGUL *et al.*, 2006).

The present work aimed to study the stability and storage life of invertase immobilized on three different novel inexpensive carriers, and simple techniques. These carriers were diethylaminoethyl-cellulose (DEAE-cellulose),polyacrylamide and hen-egg which is a novel nontoxic support. The ability to re-use of these preparations in batch processes was investigated. Also, a continuous column reactor for production of invert syrups at different sucrose concentrations was studied.

2. MATERIALS AND METHODS

2.1 Enzyme

Invertase, β-D-fructofuranosidase, (EC 3.2.1.26) was obtained from Sigma Chemical Co (United States of America).

2.2 Chemical reagents

3,5 dinitrosalicylic acid, dimethylaminopropionitrile (DMAPN) and glutaraldehyde were obtained from BDH Ltd. (England), DEAE-cellulose from Whatman Chemicals (England) and acrylamide (crystallized pure), N,N-methylenebisacrylamide and ammonium persulphate were purchased from Serva Feinbiochemica GmbH & Co. Sucrose was obtained from Koch Light Co (England). Hen eggs were purchased from the local market. All other chemicals were analytical grade.

2.3 Immobilization of invertase

2.3.1 DEAE-cellulose

Invertase was immobilized on DEAE-cellulose following the method of HUITRON and LIMON-LASON (1978) as described by ABDELLAH *et al.* (1992). A suspension of DEAE-cellulose (0.01 g/mL distilled water) was prepared. Eight mL of Invertase solution (10 mg/mL distilled water) were added to100 mL of the DEAE-cellulose suspension and stirred gently for 30 min at 4°C. The enzyme support complex obtained was filtered and washed three times with distilled water (100 mL) through filter paper (Watman No.1). Finally the pellets obtained were suspended in 50 mL distilled water and kept at 4°C.

2.3.2 Polyacrylamide

Polyacrylamide gel was prepared by dissolving 23.67g acrylamide and 1.24 g N,N-methylenebisacrylamide in 100 mL phosphate buffer pH 7. Five mL of the enzyme solution (10 mg/mL of phosphate buffer) were added to an equal volume of polyacrylamide gel and the suspension was shaken mechanically for 10 min at room temperature. The suspension was cooled in an ice bath for 5 min, then one drop of DMAPN was added. After 2 min, 0.5 mL of 5% ammonium persulphate was added to the mixture and the cooling was continued for 20 min until gel was formed. The gel obtained was shattered by passing it through a syringe needle and washing it with phosphate buffer (100 mL), then it was filtered (Whatman No.1) and kept at 4°C (KOBAYASHI *et al.*, 1975; ABDELLAH *et al.*, 1992).

2.3.3 Hen-egg white

Invertase was attached to hen-egg white according to the method of D'SOUZA and NADKARNI (1981) as described by ABDELLAH *et al.* (1992). The hen-egg white was carefully separated from the yolk. Thirty-five mL of the egg-white were mixed thoroughly with 3.5 mL of invertase solution (10 mg/mL distilled water) and then 3.5 mL of glutaraldehyde (25%) were added. The mixture was stirred well (3 min) with a magnetic stirrer and allowed to stand for 2.5 h at room temperature. The hard gel obtained was then

shattered by passing through a syringe needle and washed with distilled water (100 mL), and then filtered. The free aldehyde groups present in the gel were blocked by suspending the gel overnight at 4°C in 100 mL phosphate buffer (pH 7) containing 0.1 M lysine. The immobilized preparation was washed with distilled water, filtered and stored at 4°C.

2.4 Assay of invertase activity

Invertase activity was estimated as the amount of reducing sugars (sucrose inverted) by the 3,5-dinitrosalicylic acid method described by Anonymous (1978). Samples of the different immobilized enzyme preparations (100 mg) were stirred gently at 25°C for 30 min with 20 mL sucrose solution (0.25 M in acetate buffer pH 4.5). The immobilized enzyme was removed by filtration and the reducing power of the filtrate was estimated. For the lyophilized preparations, 100 mg were swollen by suspending in the acetate buffer for 12 h at 4°C. The sucrose solution was then added and the enzyme activity was measured as before. For batch or continuous inversion processes, the invertase activity was measured directly as the reducing power of an aliquot of the reaction mixture filtrates or of the column effluents using the same method. In each case 0.5 mL of the test solution was added to a test tube containing 2 mL of dinitrosalicylic acid reagent maintained for 5 min on a boiling water bath, cooled and diluted to 30 mL with distilled water. The absorbance was measured at 540 nm using a Specol Spectrophotometer with quartz cuvette 1 cm (Carl Zeiss, Jena, DDR). A standard curve was constructed under the same conditions using pure glucose. The enzyme activity was expressed in terms of units. One unit of invertase was defined, according to HRDIL and SVEC (1981), as µmol sucrose inverted/min/mg of invertase.

2.5 Lyophilization of immobilized invertase preparations

Forty grams of each immobilized invertase preparation were lyophilized at –80°C for 48 h, using Edwards High Vacuum Freeze Drier. Model No. EFO3. USA.

2.6 Reusability of immobilized invertase in a batch process

Samples of invertase immobilized on DEAE-cellulose, polyacrylamide and hen-egg white were stirred gently for 30 min with 20 mL sucrose solution (0.25 M) in acetate buffer at pH 4.5. Then, the immobilized enzyme matrix was recovered by filtration. The enzyme-complex (support or gel) was washed repeatedly with acetate buffer pH 4.5 and re-suspended in a fresh batch of sucrose solution. This process was repeated eight times for each immobilized enzyme preparation and the amount of reducing sugars in the respective filtrates was estimated.

2.7 Continuous inversion at different sucrose concentrations

The experiments were carried out using 20g gel containing 60 mg enzyme. The enzyme-gel complex was packed in a 32×1.5 cm glass column (Pharmacia Fine Chemical, Sweden). The column was surrounded by a jacket allowing circulation of water. The height of the gel was 10 cm. Sucrose solution of various concentrations (20-50%) was passed through the column at 50°C applying different flow rates (40-120 mL/h) and the reducing sugars were estimated in the effluents.

3. RESULTS AND DISCUSSION

3.1 Storage stability of immobilized invertase

Fig. 1 shows that the activity of the three immobilized enzyme preparations decreased gradually with the prolongation of the storage period. The enzyme immobilized on DEAE-cellulose retained about 74% of its initial activity after storage for one month. However, it kept about 23.0% of its activity even after six months of storage. DANISMAN *et al.* (2004) studied the stor-

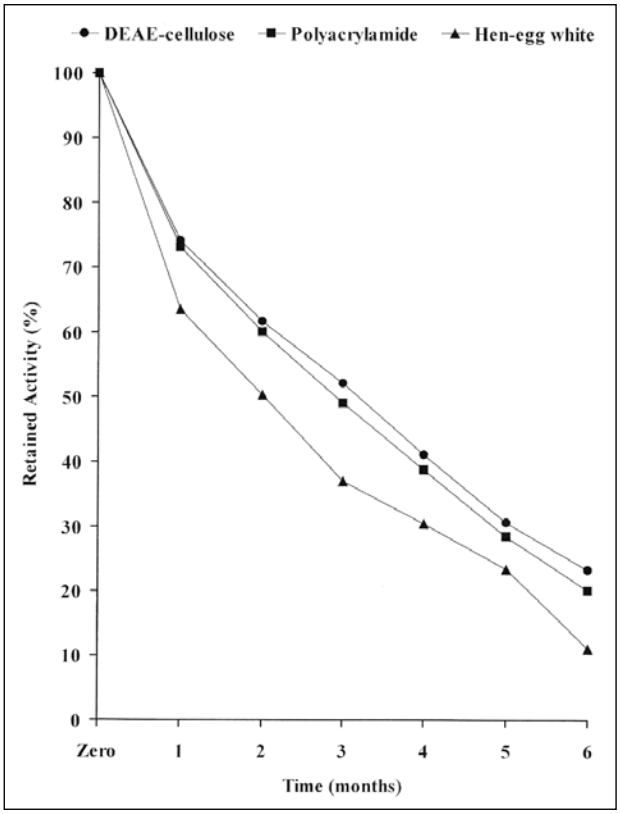


Fig. 1 - Storage stability of immobilized invertase at 4°C.

age stability of invertase immobilized on micro porous poly (2-hydroxyethyl methacrylate-glycidyl methacrylate). They found that the enzyme lost about 22% of its activity during the storage period (35 days). GOMEZ *et al.* (2006) reported that invertase immobilized on DEAE-cellulose retained 95% of its activity after 50 days of storage at 37°C. Invertase bound to polyacrylamide lost about 27% of its activity after one month of storage (Fig. 1). Then the reduction in activity was gradually on further storage until it reached 20% of the initial activity after six months of storage. Invertase fixed on hen-egg white retained about 63% of its original activity after storage for one month, then it fell to only 11% of the initial value after six months of storage (Fig. 1). The reduction in activity may be attributed to the drop out of some of the enzyme from the gel since the remaining storage solution was enzymatically active. IMAI *et al.* (1986) showed that the activity of immobilized invertase bound to the poly (ethylene-vinyl alcohol) membrane fell rapidly in an early stage of storage in pure water at 5°C, then it remained at 60% of its initial activity even after 160 days of storage. AKGOL *et al.* (2001) reported that invertase bound covalently onto magnetic polyvinyl alcohol microspheres retained 62% of its initial activity after 28 days of storage in acetate buffer (pH 5.5) at 4°C. Entrapped invertase in polyvinyl alcohol hydrogel capsules retained 78% of its initial activity after 8 months of storage in acetate buffer (pH 4.5) at 4°C (REBROS *et al.*, 2007). VU and LE (2008) reported that the activity of invertase immobilized in alginate gel was found to be quite stable after 40 days at 2°-4°C in 0.1M acetate buffer (pH 4.5).

It can be concluded that invertase immobilized on DEAE-cellulose and that entrapped in polyacrylamide were more stable during storage at 4°C than the enzyme fixed on hen-egg white.

3.2 Effect of lyophilization on immobilized invertase

Samples of enzyme immobilized on DEAE-cellulose, polyacrylamide and hen egg white were lyophilized and stored at 4°C for 7 months. The data shown in Table 1 indicates that lyophilization of the enzyme attached to DEAE-cellulose caused a decrease in its activity from 110.5 to 44.5 units. This decline in activity may be attributed to the hydrophobic microenvironment of this support which cause the loss of activity upon lyophilization of the enzyme which was stable in the aqueous suspension (KENNEDY and WHITE, 1985; GÓMEZ *et al.*, 2006). Lyophilization had negligible effect on the activity of the enzyme fixed on polyacrylamide and hen egg white.

3.3 Stability of immobilized invertase preserved by lyophilization

In general during the storage period the activity of all lyophilized preparations decreased gradually. After seven months of storage, activity retention was 45.4, 47.5 and 38.1% for the enzyme immobilized on DEAE-cellulose, polyacrylamide and hen-egg white, respectively.

From these results it could be concluded that the enzyme fixed on polyacrylamide and DEAE-cellulose and stored in lyophilized form was more stable during storage than that bound to hen-egg white, in spite of the deep loss during lyophilization of the enzyme fixed on DEAE-cellulose. Also, it was observed that the enzyme preparations stored in lyophilized form could retain around 40% of their initial activities (zero activities) after storage for seven months, in comparison with those stored in an aqueous suspension at 4°C, they retained only from 10 to 20% of their initial activities after storage for six months (Fig. 1).

Table 1 - Storage stability of immobilized invertase preserved by lyophilization.

	Immobilized invertase preparations		
	DEAE-cellulose	**Poly- acrylamide**	**Hen-egg white**
Control* (units)	110.5	111.5	107.6
Zero** (units)	44.5	108.6	102.0
Storage time (months)	**Retained activity (%)**		
1	92.6	90.1	86.1
2	83.3	83.1	77.7
3	76.6	74.3	73.3
4	68.0	67.7	68.0
5	60.4	59.4	59.5
6	51.7	54.4	50.4
7	45.4	47.5	38.1

Control* and Zero**: activity of invertase before and after lyophilization, respectively.

3.4 Reusability of immobilized invertase in a batch process

One of the major objectives of immobilization of an enzyme is to allow its recovery for re-use. Accordingly, the following experiments were carried out to study the inversion of sucrose by the immobilized invertase preparations in a batch process. Fig. 2 shows that, after 8 repeated cycles, the enzyme attached on DEAE-cellulose and polyacrylamide lost only 6.4 and 5.1% of its initial activity, respectively.

SHIOMI *et al.* (1988) reported that the activity of invertase immobilized on poly (ethylene-co-vinyl alcohol) fell to approximately 60% of the initial activity in the early stage for both repeated and continuous usage and after that kept almost that value. NAKANE *et al.* (2001) found that the activity of invertase immobilized on composite gel fibre of cellulose acetate and zirconium alkoxides dropped to 90% after 10 repeated runs. BAHAR and TUNCEL (2002) reported that the activity of invertase immobilized onto crosslinked poly (p-chloromethylstyrene) decreased by 20% after 25 batch experiments. Invertase immobilized on microporous montmorillonite could be reused for 15 continuous cycles without loss in its activity, whereas after 21 continuous cycles, the covalently bound invertase retained 80% of its initial activity. The loss in activity was attributed to inactivation of the enzyme due to continuous use (SANJAY and SUGUNAN, 2005). GÓMEZ *et al.* (2006) reported that invertase immobilized on carboxymethylcellulose retained 98% of the original catalytic activity after 10 cycles of reuse. EMREGUL *et al.* (2006) showed that when polyacrylamide gelatine was used for invertase immobilization, it could be used 20 times over a period of 2 months without considerable loss of activity.

Also, Fig. 2 shows that the stability of the enzyme fixed on hen-egg white was less than that of the enzyme immobilized on DEAE-cellulose or polycrylamide. It lost about 9.1% of its original activity after eight times of repeated usage. D'SOUZA and NADKARNI (1981) reported that invertase fixed on hen-egg white was stable during repeated use for eight times.

3.5 Continuous inversion at different sucrose concentrations

Continuous column reactors are more efficient than batch reactors and are also simple and easy to operate and automate. Large-scale and continuous operations minimize labor costs and facilitate control and products of more reliable quality than the traditional batch process (KENNEDY and WHITE, 1985; AKGOL *et al.*, 2001).

Invertase immobilized on polyacrylamide was selected to study the continuous inversion of sucrose since it was the more effective one as compared with the other supports tested. The data in Fig. 3 show that in a continuous column operation, an important relationship has been observed between the initial sucrose concentrations and the other operating parameters. For each of the initial sucrose concentrations used, the percentage of inversion decreased within a narrow range (28 to 35%) by increasing the flow rate from 40 to 120 mL/h. The amount of sucrose hydrolysed also decreased, but differed greatly by increasing the flow rate. The amount of sucrose inverted was 581 and 405 at flow rates 40 and 120 mL/h, respectively when the initial sucrose concentration was 20%. These two values rose to 1260 and 764, respectively for the treatment started by 50% sucrose solution.

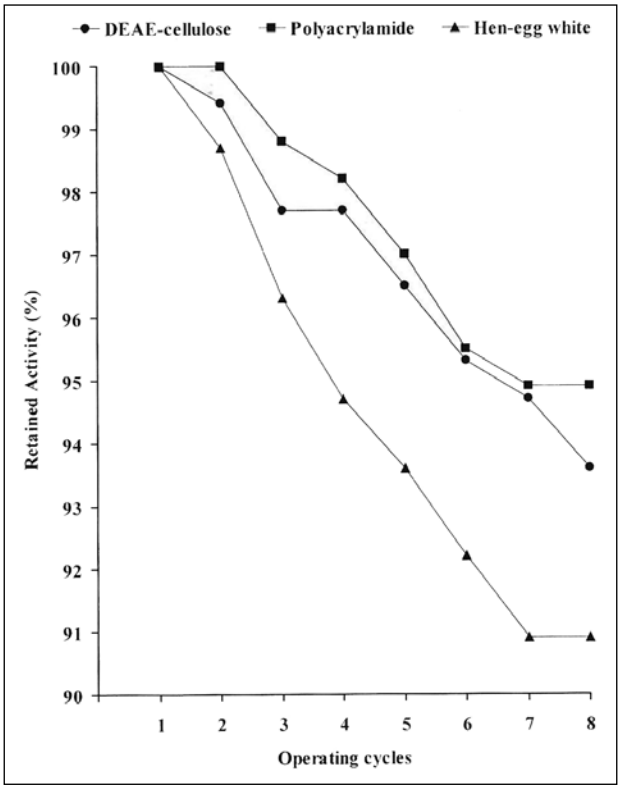


Fig. 2 - Reusability of immobilized invertase in batch process for 8 cycles.

These results indicated that increasing the operating flow rate will lower the contact or reaction time between sucrose and invertase and hence will decrease both the percentage of the inversion and the amount of glucose formed.

These results are in agreement with those reported by GOLDSTEIN *et al.* (1977) who found that inversion levels in excess of 90% could be achieved when sucrose was fed to the column containing invertase immobilized on collagen membranes at low concentrations (<0.3 M) of sucrose solutions. Encapsulated invertase in polyelectrolyte complexes showed a high operational stability if low substrate concentrations were applied (MANSFELD *et al.*, 1992). KOTWAL and SHANKAR (1997) reported that invertase immobilized on indion 48-R showed inhibition at high substrate concentrations.

The effect of the initial sucrose concentrations within all the treatments tested in the present study was different from that of each treatment. The initial sucrose concentrations had different effects on the degree of inversion and the amount of sucrose inverted. The percentage of inversion decreased while the amount of sucrose inverted increased by increasing the initial sucrose concentration. The degree of inversion was 99% and dropped to 86% when the initial sucrose concentration increased from 20 to 50% at flow rate of 40 mL/h. Whereas, the amount of sucrose inverted was 581 mmol (at 20% concentration) and rose to 1,260 mmol (at 50% concentration) for the same flow rate.

The amount of sucrose inverted is more important than the percentage of hydrolysis. So the treatment, which has higher inverted sucrose, is commercially viable due to the decrease in the processing cost (AGGARWAL *et al.*, 2001).

However, operation at low solids is not commercially preferred due to the increase in the evaporation cost, the need for larger hydrolysis tanks and the risk of microbial contamination (GRABER and COMBES, 1990; HEBEDA, 1992).

AMAYA-DELGADO *et al.* (2006) used a tubular fixed bed reactor for continuous sucrose hydrolysis by invertase immobilized on nylon-6-microbeads. They demonstrated very good productivity, up to 2.0 M sugar concentration, with inversion factors of 0.95 and 0.97 depending on sucrose concentration in the feeding.

3.6 Continuous operational stability of invertase entrapped in polyacrylamide

The most effective continuous inversion treatment (polyacrylamide- invertase gel, 50% initial sucrose concentration and a flow rate of 40 mL/L) was used to estimate stability using the continuously operated column. Data in Fig. 4 show the retained activity tested daily through 5 days of continuous operation. Also, the activity was measured on the 15th and 30th days of storage at 4°C. It was found that the immobilized enzyme preparation was nearly completely stable through five days of continuous operation. It lost only 3% of its initial activity during this period. After 15 and 30 days of storage, the retained activity was 91 and 83%, respectively. These results indicate that the hydrolysis of sucrose using continuous column reactors could be operated for long periods.

GINALSKA *et al.* (1999) used a column with immobilized invertase on controlled pore glass and was able to degrade sucrose to glucose and fructose in an amount of 60 g/h and the enzyme in the column remained fully active for 120 days. AKGOL *et al.* (2001) investigated the operational stability of immobilized invertase onto magnetic polyvinyalcohol in a packed bed reactor. Their results showed that during the initial 12 h, the enzyme system preserved all of its initial activity. After 40 h, the immobilized enzyme lost about 17% of its initial activity. Invertase immobilized on a poly (maleic anhydride-hexen-1) membrane

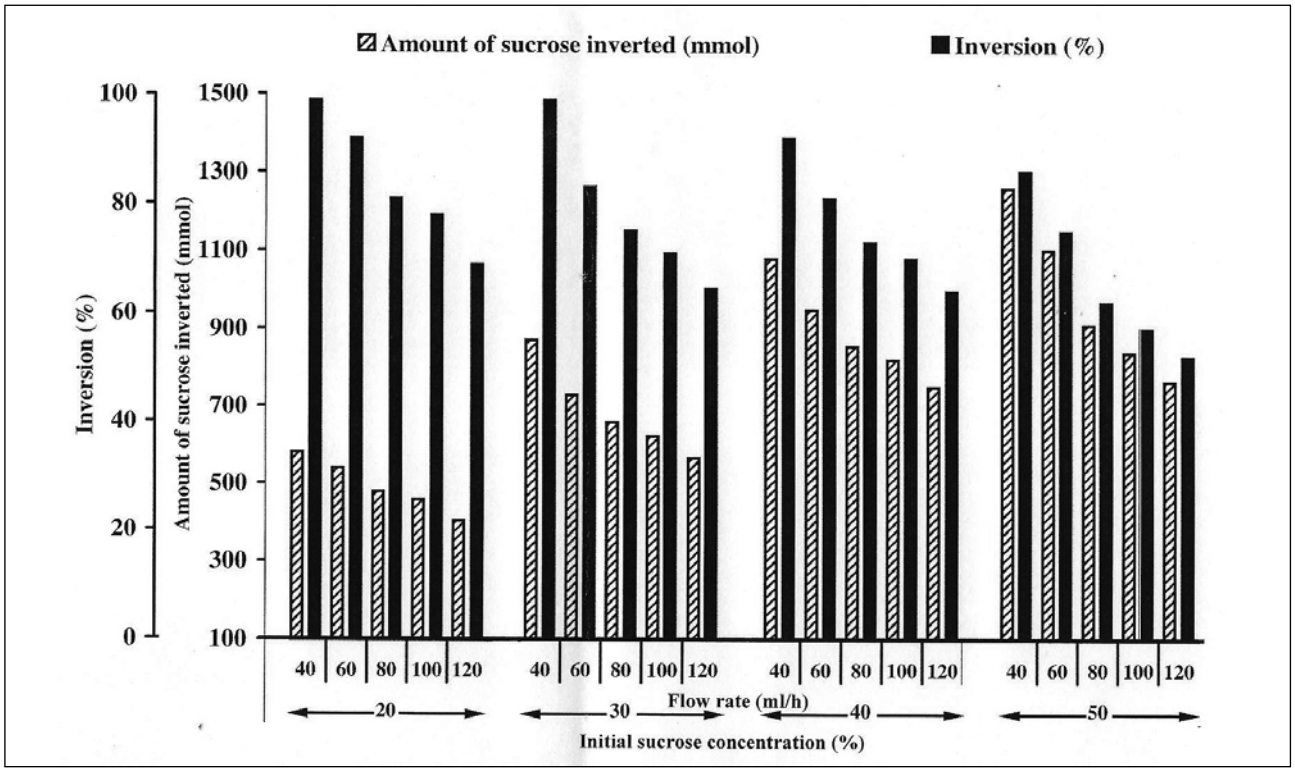


Fig. 3 - Continuous inversion at different sucrose concentrations (20 to 50%) and flow rates (40-120 mL/h).

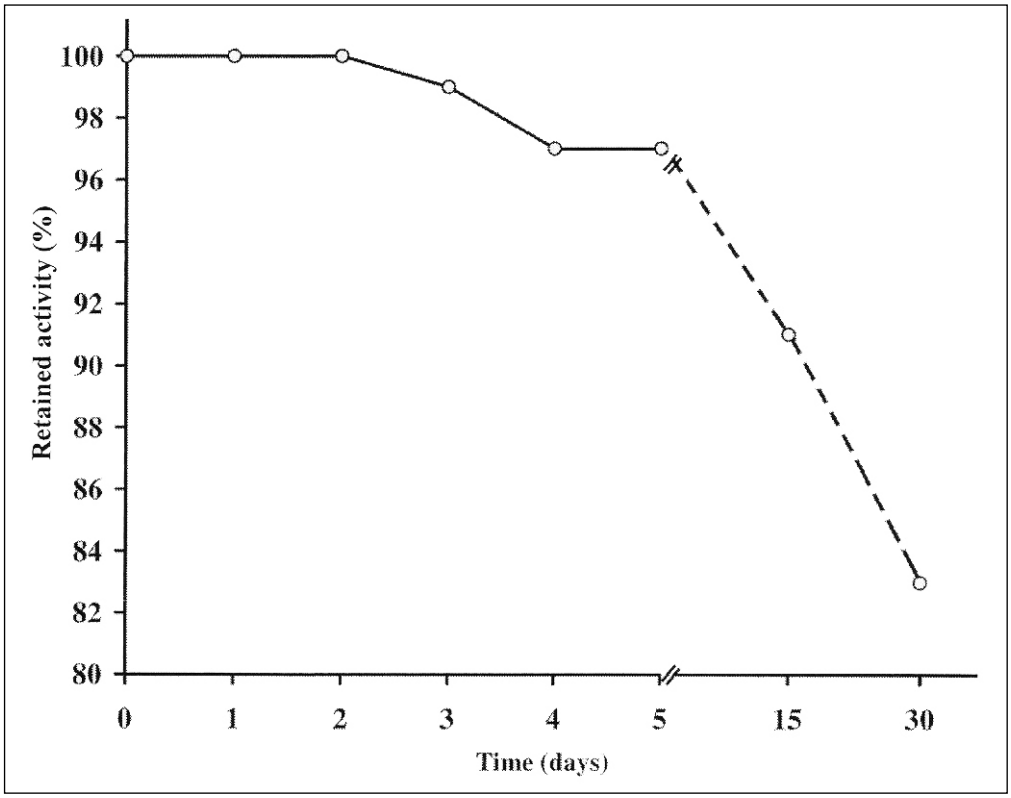


Fig. 4 - Continuous operational stability of invertase entrapped in polyacrylamide (50% initial sucrose concentration and flow rate 40 mL/l).

was stable and could be used many times over a period of 2 months without considerable activity loss (HIDAYET *et al.*, 2006).

SANJAY and SUGUNAN (2006) reported that invertase immobilized on montmorillonite by covalent binding retained complete activity for up to 64 h of continuous reaction in a packed bed reactor and after 96 h, 75% activity was retained.

4. CONCLUSION

Immobilization of invertase to the three different supports used showed good stability for 5 months at 4°C. The immobilized preparations could be re-used for eight cycles with slight decrease in their initial activity. Also, they could be stored in lyophilized form for 4-5 months. The polyacrylamide enzyme gel showed satisfactory activity in high sucrose concentrations and a good operation stability.

REFERENCES

Abdellah H.A., Abou Baker T.M., Shekib L.A. and El-Iraqi S.M. 1992. Characteristics of invertase immobilized on three different types of supports. Food Chem., 43 (5): 369-375.

Aggarwal N.K., Yadav S.K., Dhamija S.S. and Yadav B.S. 2001. Optimization of enzymatic hydrolysis of pearlmillet for glucose production. Starch, 53 (7): 330-335.

Akgol S., Kacar Y., Denizli A. and Arica M.Y. 2001. Hydrolysis of sucrose by invertase immobilized onto novel magnetic polyvinylalcohol microspheres. Food Chem., 74 (3): 281-288.

Amaya-Delgado L., Hidalgo-Lava M.E. and Montes-Harcasitas M.C. 2006. Hydrolysis of sucrose by invertase immobilized on nylon-6 microbeads. Food Chem., 99 (2): 299-304.

Anonymous. 1978. Enzymes. BDH biochemicals, Ltd., England, pp. 6.

Arruda L.M.O. and Vitole M. 1999. Characterization of invertase entrapped into calcium alginate beads. Appl. Biochem. Biotechnol., 81 (1): 23-33.

Bahar T. and Tuncel A. 2002. Immobilization of invertase onto crosslinked poly (p-chloromethylstyrene) beads. J. Appl. Polym. Sci., 83 (6): 1268-1279.

Bassetti F.J., Bergamasco R., Moraes F.F. and Zanin G.M. 2000. Thermal stability and deactivation energy of free and immobilized invertase. Braz. J. Chem. Eng., 17 (4-7): 841-848.

Bergamasco R., Bassetti F.J., de Moraes F.F. and Zanin G.M. 2000. Characterization of free and immobilized invertase regarding activity and energy of activation. Braz. J. Chem. Eng., 17 (4-7): 873-880.

Chou C.C. and Jasovsky G.A. 1993. Advantages of Ecosorb TM precoats in liquid sugar production. Int. Sugar J., 95 (1138): 425-430.

D'souza S.F. and Nadkarni G.B. 1981. Hen-egg white: A novel support for the immobilization of enzymes. Biotechnol. Bioeng., 23 (2), 431-436.

Danisman T., Tan S., Kacar Y. and Ergene A. 2004. Covalent immobilization of invertase on microporous pHEMA-GMA membrane. Food Chem., 85 (3): 461-466.

Emregul E., Sungur S. and Akbulut U. 2006. Polyacrylamide gelatine carrier system used for invertase immobilization. Food Chem., 97 (4): 591-597.

Ginalska G., Belcarz A., Lobarzewski J. and Wolski T. 1999.

The application of immobilized invertase for continuous production of inverted sucrose. Biotechnologia, 1: 226-237.

Goldstein H., Barry R.W., Rizzuto A.B., Venkatasubramanian K. and Vieth W.R. 1977. Continuous enzymatic production of invert sugar. J. Ferment. Technol., 55 (4): 516-524.

Gómez L., Ramirez H. and Villalonga R. 2006. Immobilization of Chitosan-invertase neoglycoconjugate on carboxymethyl cellulose modified chitin. Prep. Biochem. Biotechnol., 36 (3): 259-271.

Graber M. and Combes D. 1990. Action pattern of alpha-amylase from Aspergillus oryzae in concentrated media. Biotechnol. Bioeng., 36 (1), 12-18.

Hardil J. and Svec F. 1981. Invertion of sucrose with β-D-fructofuranosidase (invertase) immobilized on bead DEAHP-cellulose: Batch process. Enzyme Microb. Technol., 3(4), 331-335.

Hebeda R.E. 1992. Syrups. In: "Encyclopedia of Food Science and Technology". Hui Y.H. (Ed.). Vol. 4 John Wiley and Sons, Inc., New York.

Hidayet M., Emel E., Zakir R.M.O. and Gunay K. 2006. Preparation and properties of invertase immobilized on a poly (maleic anhydride-hexen-1) membrane. J. Biomat. Sci., 17 (7): 831-835.

Huitron C. and Limon-Lason J. 1978. Immobilization of glucose isomerase to ion-exchange materials. Biotechnol. Bioeng., 20 (9), 1377-1391.

Imai K., Shiomi T., Uchida K. and Miya M. 1986. Immobilization of enzyme into poly (ethylene-vinyl alcohol) membrane. Biotechnol. Bioeng., 28 (2): 198-203.

Kennedy J.F. and White C.A. 1985. Principles of immobilization of enzymes. In: A Handbook of Enzymes Biotechnilogy. Wiseman (Ed.), Ellis Horwood Ltd. U.K., pp. 147-380.

Kobayashi T., Ohmiya K. and Shimizu S. 1975. Immobilization of β-galactosidase by polyacrylamide gel. In: Immobilized Enzyme Technology Research and Applications, Weetall H.H. and Suzki, S. (Eds) Plenum Press, New York and London, pp. 169-183.

Kotwal S.M. and Shankar V. 1997. Preparation and properties of Sclerotium rolfsii invertase immobilized on indion 48-R. Appl. Biochem. Biotechnol., 62 (2-3): 151-158.

Kotwal S.M. and Shankar V. 2009. Immobilized invertase: Research review paper. Biotechnol. Adv., 27 (4): 311-322.

Mansfeld J., Schellenberger A. and Rombach J. 1992. Application of polystyrene-bound invertase to continuous hydrolysis on pilot scale. Biotechnol. Bioeng., 40 (9): 997-1003.

Milovanović A., Božić N. and Vujčić Z. 2007. Cell wall invertase immobilization within calcium alginate beads. Food Chem., 104 (1): 81-86.

Monsan P. and Combes D. 1984. Application of immobilized invertase to continuous hydrolysis of concentrated sucrose solutions. Biotechnol. Bioeng., 26 (4): 347–351.

Nakane K., Ogihara T., Ogata N. and Kurokawa Y. 2001. Entrap-immobilization of invertase on composite gel fiber of cellulose acetate and zirconium alkoxide by sol-gel process. J. Appl. Polym. Sci., 81 (9): 2084-2088.

Rebros M., Rosenberg M., Mlichova Z. and Kristofikova L. 2007. Hydrolysis of sucrose by invertase entrapped in polyvinyl alcohol hydrogel capsules. Food Chem., 102 (3), 784-787.

Sanjay G. and Sugunan S. 2005. Invertase immobilized on montmorillonite: reusability enhancement and reduction in leaching. Catal. Commun., 6 (1), 81-86.

Sanjay G. and Sugunan S. 2006. Fixed bed reactor performance of invertase immobilized on montmorillonite. Catal. Commun., 7 (12): 1005-1011.

Shiomi T., Tohyama M., Satoh M., Miya M. and Imai K. 1988. Properties of invertase immobilized on the poly (ethylene-co-vinyl alcohol) hollow fiber membrane. Biotechnol. Bioeng., 32 (5): 664-668.

Vu T.K.H. and Le V.V.M. 2008. Biochemical studies on the immobilization of the enzyme invertase (EC .3.2.1.26) in alginate gel and its kinetics. ASEAN Food J., 15 (1), 73-78.

Wiseman A. 1985. Principles of industrial enzymology: Basis of utilization of soluble and immobilized enzymes in industrial process. In: Handbook of Enzymes Biotechnology, Cheetham, P.J. (Ed.), p. 74.

Zanin G.M. and de Moraes F.F. 1994. Performance of fixed and fluidized reactors with immobilized enzyme. Appl. Biochem. Biotechnol., 45/46, 627-640.

Paper received September 6, 2010 Accepted January 7, 2011

PAPER

DIET FAT LEVEL AND SEASON EFFECT ON HEAVY PIG LIPID PROFILE

L. ROTOLO[1], K.J. GUO[2] and I. ZOCCARATO[1]*
[1]Dipartimento di Scienze Zootecniche, Turin University,
Via Leonardo da Vinci 44, 10095 Grugliasco, Italy
[2]College of Animal Science and Technology, Beijing University of Agriculture,
Beinong Road 7, Huilongguan, Changping District, Beijing 102206, China
Authors contribuited equally to the paper
*Corresponding author: Tel. +39 0116708568, Fax +39 0116708563,
email: ivo.zoccarato@unito.it

ABSTRACT

144 pigs were fed 2 diets: LL and HL (41 *vs* 51 fat/kg DM) in summer or winter. Diet and season affected fatty acids profile. Diet LL increased significantly $C_{14:0}$ $C_{16:0}$ $C_{16:1}$ and SFA, while HL improved significantly $C_{14:1}$ $C_{17:0}$ $C_{17:1}$ $C_{18:2}$ and PUFA. $C_{16:0}$ and SFA levels resulted significantly higher in summer than in winter, whereas $C_{18:2}$ $C_{18:3}$ $C_{20:1}$ $C_{22:0}$ PUFA and IV showed opposite trend. $C_{14:0}$ $C_{16:0}$ $C_{18:3}$ and $C_{22:0}$ showed significant interaction. For $C_{18:3}$, during winter low temperature reduced diets difference, while for $C_{14:0}$ $C_{16:0}$ and $C_{22:0}$ summer reduced diets difference for depletion of fat tissue turnover.

- Key words: dietary fat, fatty acid profile, pig, season -

INTRODUCTION

Fatty acid profile is an important parameter of meat quality and it is a fundamental characteristic that must be considered in order to preserve cured bacon and hams against oxidation and "soft fat" defects (WOOD *et al.*, 2008).

Many biotic and abiotic factors can affect lipid profile. Amongst biotic factors, the increasing "health" consciousness of consumers, has led to rear lean genetic pool in Italian heavy pig farming, resulting in high UFA profile and consequently causing several problems to meat processing technologists (LO FIEGO *et al.*, 1990). In this process, linoleic fatty acid ($C_{18:2}$) is the main agent in guaranteeing good fat characteristics with its high iodine value (IV) contribution. In order to obtain adequate final products, a $C_{18:2}$ threshold value must be lower than 15% in covering fat of raw ham and iodine value no higher than 70 (LO FIEGO *et al.*, 2005). In order to obtain a firm fat, $C_{18:2}$ content in pig diet must be no more than 2% on dry matter basis (DELLA CASA *et al.*, 2010). Diet optimisation has a pivotal role in order to meet the animal growth and improve carcass quality traits. Pigs have great potential in regulating fatty acid proportions which cannot be synthesised by the animal tissue (WISEMAN and AGUNBIADE, 1998) and fatty acid profile of their adipose depots can be modulated (NGUYEN *et al.*, 2003; HUGO and ROODT, 2007). For this reason, changes in dietary fat can greatly modify the fatty acid profile of fat deposited (NURNBERG *et al.*, 1998) and consequently rebound on both nutritional and technological properties of final products. Indeed, it is important to remember that by mirroring the fatty acid composition of the diet, in a short time period within 2 to 6 weeks, the pig is able to modify the backfat fatty acid profile (WISEMAN and AGUNBIADE, 1998; GATLIN *et al.*, 2002).

WISEMAN and AGUNBIADE (1998) reported that the dietary fat sources could influence the fatty acid profiles of finishing pigs resulting in lipid oxidation and rancidity phenomena by UFA fat in carcass and consequently a poor quality of meat.

The use of high PUFA diet can play a pro-oxidant effect of lipid subfraction phospholipids with consequent development of warmed-over flavour in meat (WARNANTS *et al.*, 1996).

Usually, pigs are fed diets rich in corn and the risk of fat high in UFA is not infrequent because of the modern corn hybrids are characterized by high variability in UFA contents (DELLA CASA *et al.*, 2010). This situation has led to negatives consequences for pig producers, because they may produce pigs inappropriate for cured meat production. In addition, another well-known abiotic factor is the rearing temperature which has a primary importance for productive performance. It is well known that exposure to different environmental conditions (temperature, relative humidity, photoperiod) could affect swine metabolism influencing many physiological responses and carcass quality traits, including fatty acids profile (RINALDO and LE DIVIDICH, 1991; WHITE *et al.*, 2008). Conversely, few studies are available regarding the possible effects of rearing season, on fatty acid profile in pigs (RODRIGUEZ-SANCHEZ *et al.*, 2009).

The goal of this research was to study the effect of season (winter and summer) in heavy pigs fed increasing fat level diets, aiming to evaluate variation of fatty acids profile in subcutaneous thigh fat.

MATERIAL AND METHODS

Animals and diets

A 60 d trial consisting in a 2 x 2 complete factorial design, rearing season and diet was performed. Pigs have been reared in winter (from January to March) and in summer (from July to August). Trials were repeated twice, during the same period, in 2008 and 2009. Each year, 72 castrated males (210±3 days of age) and 120 (±3.45) kg belonging to a GOLAND C21 commercial type have been randomly allotted to each one of 4 diet-season combinations (n=18). Pigs have been fed in a restricted way with two commercial diets based on cereals, cereal by-products and soybean-meal, with increasing levels of fat (g fat/kg DM) as follows: Low Level=LL (41); High Level=HL (51).

Proximate composition and fatty acids profile of diets are reported in Table 1. Diet was distributed twice a day and the average quantity of diet delivered during the feeding period was 2.6 kg/die for each animal. Feed from each batch were sampled 30 days and 7 days prior to slaughtering, vacuum-packaged and stored at -20°C until subsequent chemical analysis were performed. Pigs were reared indoor and not in climatized barn in common husbandry conditions of Piedmont, in the same farm located in Caraglio, (north-western Italy; 44.42°N latitude and 7.43°W longitude, 575 m asl). The daily temperature series have been taken from Regional Service for Weather Forecast (ARPA http://www.arpa.piemonte.it/).

Fat sampling

Following the regulations of the Italian Parma ham consortia, at the age of 270 days, all pigs were slaughtered after fasting of 12 h in a commercial abattoir, according to industry-accepted procedure. Slaughtering average weight was 163.1 (±4.75). Fat-O-Meater (FOM) measurements were recorded to calculate carcass lean percentage. After a chilling period of 24 h at 0° to 2°C, fat thickness on right thighs (*caput ossis femoris*) was measured. A sample of 50 g

Table 1 - Chemical composition (g/kg DM) and main fatty acids composition of diets (% on total FAME).

	[1]Low Level (LL)	SD	[2]High Level (HL)	SD
Dry Matter	890	4.4	890	4.3
Crude Protein	155	5.4	155	5.4
Ether extract	41.2	1.0	51.4	0.5
Crude Fiber	48.6	1.4	47.2	1.3
Ash	59.5	1.0	59.3	0.5
[3]Digestible energy (MJ/kg DM)	14.15	-	14.34	-
C 14:0	0.14	0.02	0.17	0.05
C 15:0	0.50	0.04	0.98	0.14
C 16:0	23.11	1.51	19.22	0.03
C 18:0	10.27	1.10	7.42	0.23
SFA	34.01	2.59	27.78	0.07
C 14:1	0.48	0.02	0.36	0.01
C 15:1	0.08	0.01	0.12	0.02
C 16:1	0.67	0.16	1.05	0.01
C 18:1	28.06	0.37	28.48	0.13
MUFA	29.29	0.25	30.00	0.13
C 18:2n6	37.55	0.45	39.66	0.75
C 18:3n3	1.57	0.09	2.12	0.04
PUFA	39.11	0.54	41.78	0.71

[1]LL= 41 g EE/kg DM diet;
[2]HL= 51 g EE/kg DM diet.
[3]Digestible energy was calculated in according to English *et al.* (1988).

of subcutaneous fat, including both outer and inner layer, was collected from each pig. Samples were stored at -20°C for subsequent chemical analysis.

Analytical methods and iodine value calculation

Diet fat content was determined in accordance with AOAC rules (2000), while lipids were extracted according to FOLCH *et al.* (1957) for determining fatty acid composition. For lipid diets and subcutaneous fat samples, fatty acid methyl esters (FAME) were prepared following AOAC rules (2000) and analysed by gas chromatography. A SHIMADZU – GC 17A equipped with a HP88 capillary column (100 m × 0.25 mm ID, 0.2 μm film thickness; Varian) was used. The column temperature was held at 45°C for 5 min and then rose to 20°C min^{-1} to a final temperature of 190°C, where it remained for 50 min. Temperature of the injector and flame-ionization detector were maintained at 250° and 280°C, respectively. The injection volume was 0.1 μL, nitrogen column flow was 1.4 mL/min with nitrogen average velocity at 22 cm/sec. The individual fatty acids were identified by comparing their retention times with those of standard fatty acids (FAME Mix 30 mg/mL methylene chloride, Restek Corporation – 110 Benner Circle, Bellefonte PA 16823). Results were expressed as percentage of total methyl esters (FAME). The percentages of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and unsaturated (UFA = MUFA + PUFA) fatty acids were calculated. The iodine value was calculated from the fatty acid composition (g/100 g of total FA) according to AOCS (1998) as follows:

$$\text{Iodine value} = (C16{:}1 \times 0.950) + (C18{:}1 \times 0.860) + (C18{:}2n\text{-}6 \times 1.732) + (C18{:}3n\text{-}3 \times 2.616) + (C20{:}1 \times 0.785) + (C22{:}1 \times 0.723)$$

Statistical analysis

The effects of fixed factors (rearing season, diet and year) and respective interactions on subcutaneous fatty acid profile were evaluated by a three-way analysis of variance using the GLM procedure of SPSS v.17.0 for Windows (SPSS Inc. Chicago Illinois, 2008). Since the year of pig-raising factor was not significant, data were analyzed by two-way ANOVA considering season and diet. Significance was accepted at $P<0.05$.

RESULTS AND CONCLUSIONS

Collected temperature data showed that on 2008 the average temperatures were 5.7°C (-3.5° to 27.6°C) in winter (Fig. 1), and 22.0°C (10.6° to 34.3°C) in summer (Fig. 2). In 2009 the average temperatures were 5°C (-4.3° to 22.7°C) in winter (Fig. 3), and 22.2°C (12.5° to 32.8°C) in summer (Fig. 4). In winter in both years, occurrences of hot days with temperature that strongly deviated from the average values have been observed. The reason of this phenomenon is due to specific meteorological conditions. Indeed, this geographical area is blown by a northern wind, typical of the winter period that sweeps down from alpine elevations to the plains increasing the atmospheric pressure and consequently heating the air. Irrespectively to the year and season, pigs have been reared out of the comfort zone for many days during the finishing period, as indicate by WHITTERMORE (1993) and CLOSE (1983). This occurrence is typical in fattening piggery of continental climate area and can affect the composition of carcass and meat. Considering both years, irrespectively of the dietary treatment, thigh fat thickness (mm) ranged from 20.9 (±0.8) to 21.3 (±1.2) and the percentage of carcass lean estimated by FOM at slaughter ranged between 52.00 (±1.1) and 51.41 (±0.8) for LL and HL, respectively. The fatty acid profiles of fat are shown in Table 2. Effect of season is observed for $C_{16:0}$ ($P<0.01$), $C_{22:0}$ ($P<0.01$) and SFA ($P<0.05$). $C_{16:0}$ and SFA resulted higher in summer than in winter whereas $C_{22:0}$ showed opposite trend. Diet significantly affected the

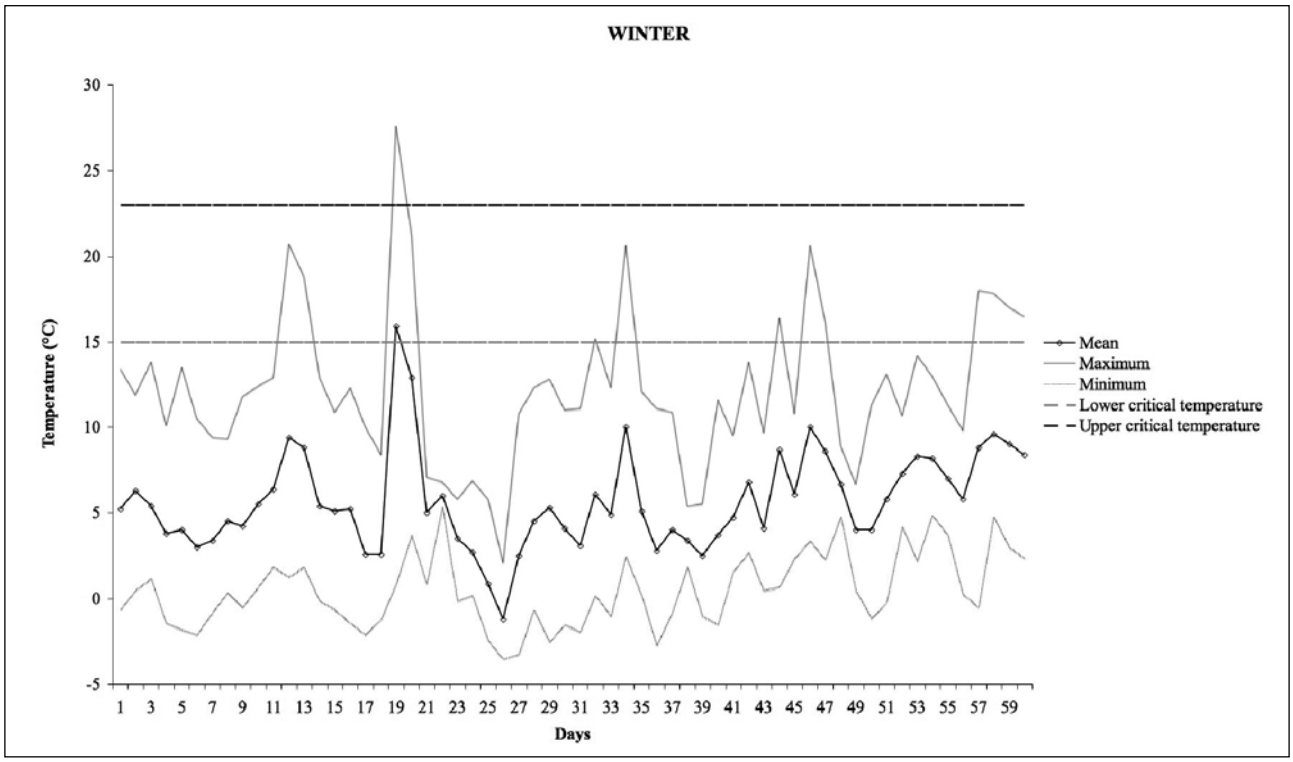


Fig. 1 - 2008 Winter - Daily temperature during feeding period.

level of $C_{14:0}$, $C_{16:0}$, $C_{17:0}$ and SFA (P<0.01) with $C_{14:0}$, $C_{16:0}$ and SFA higher in LL than HL and $C_{17:0}$ lower in LL than HL. Amongst MUFA only eicosenoic acid ($C_{20:1}$) was significantly affected by season (P<0.05) increasing during the winter.

Amongst MUFA, myristoleic acid ($C_{14:1}$) (P<0.01), palmitoleic ($C_{16:1}$) (P<0.05) and heptadecenoic acid ($C_{17:1}$) (P<0.01) were affected by diet. In particular $C_{14:1}$ and $C_{17:1}$ are increased in HL, while $C_{16:1}$ increased in LL. Season significantly affect-

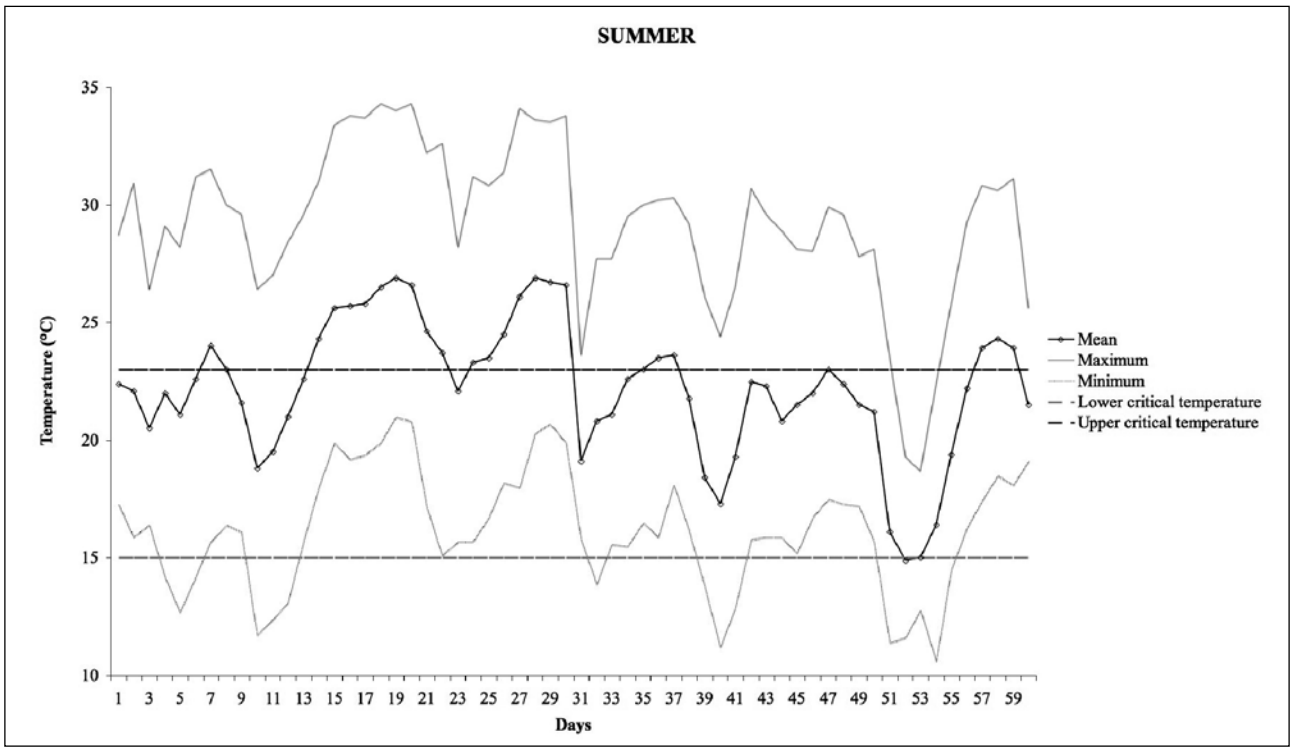


Fig. 2 - 2008 Summer - Daily temperature during feeding period.

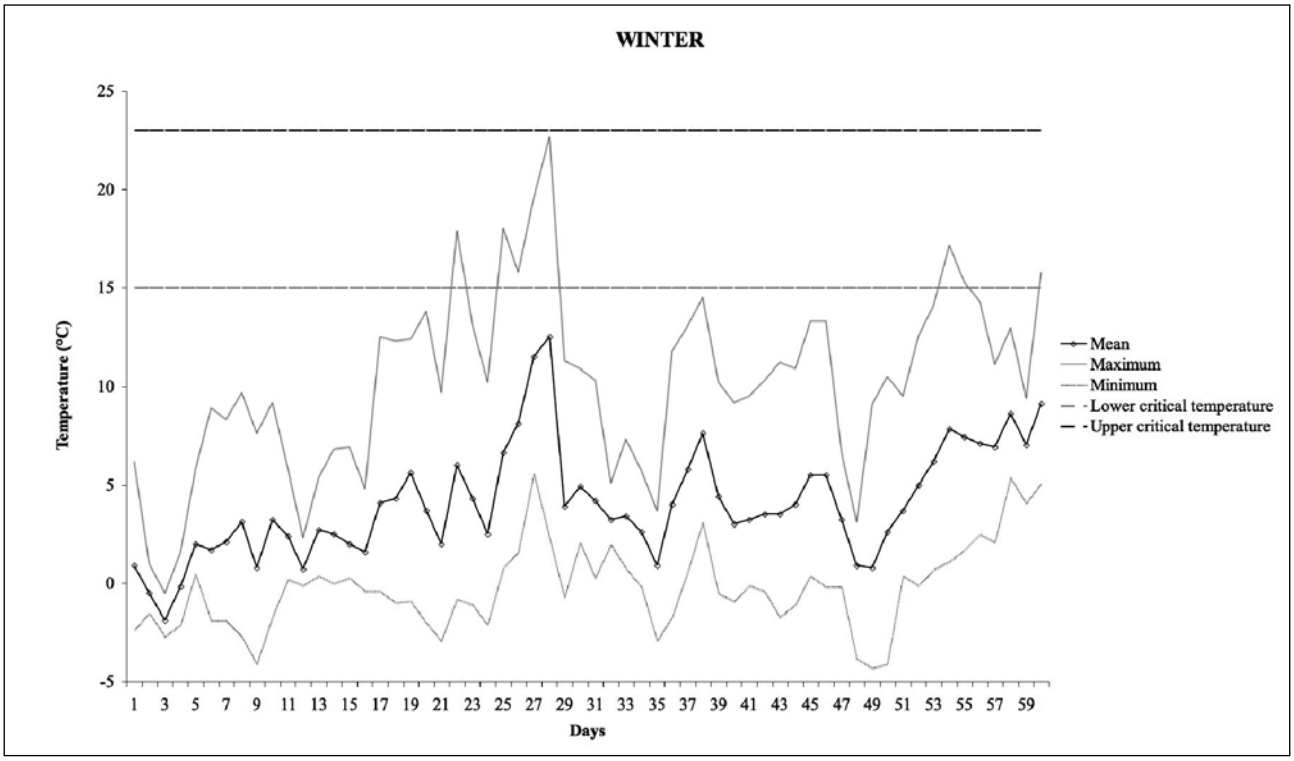


Fig. 3 - 2009 Winter - Daily temperature during feeding period.

ed linoleic ($C_{18:2}$), linolenic ($C_{18:3}$), PUFA and IV ($P<0.01$), that resulted all increased in winter.

Moreover linoleic, linolenic and IV were significantly affected by diet ($P<0.01$). Diet high in ether extract induced an increasing in $C_{18:2}$ and consequently in PUFA. Also IV was increased by HL diet. Significant interaction was observed for $C_{14:0}$, $C_{16:0}$, $C_{18:3}$ ($P<0.05$) and $C_{22:0}$ ($P<0.01$). The results showed that diet is more effective than season to induce fatty acid profile modifications,

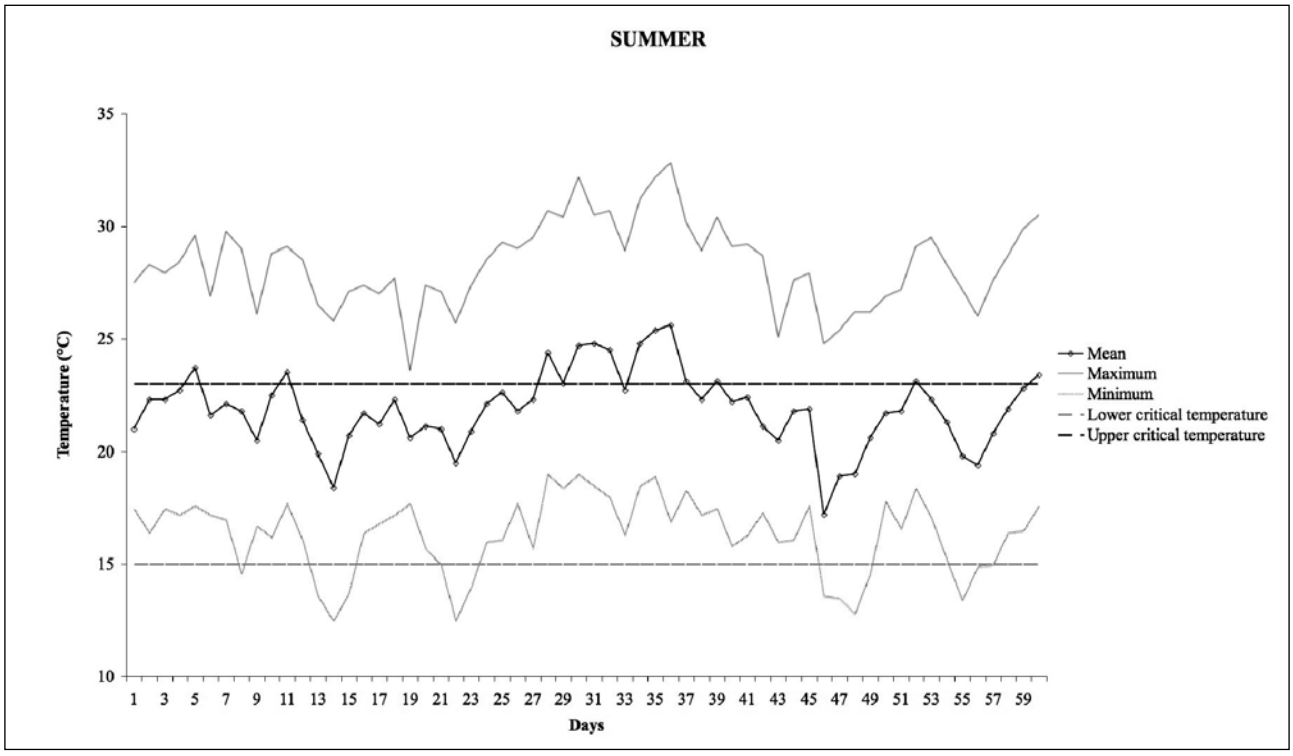


Fig. 4 - 2009 Summer - Daily temperature during feeding period.

Table 2 - Effect of season and dietary fat level on heavy pigs fatty acids profile (N=144) (% on total FAME).

	Winter		Summer		SEM	Significance		
	LL	HL	LL	HL		Season	Diet	S × D
C12:0	0.07	0.08	0.07	0.08	0.00	NS	NS	NS
C14:0	1.31	1.25	1.33	1.36	0.01	NS	**	*
C16:0	24.30	23.22	24.43	24.00	0.09	**	**	*
C17:0	0.28	0.34	0.28	0.31	0.01	NS	**	NS
C18:0	12.44	12.27	12.87	12.58	0.10	NS	NS	NS
C22:0	0.56	0.64	0.55	0.51	0.01	**	NS	**
SFA	39.01	37.80	39.54	38.79	0.16	*	**	NS
C14:1	0.04	0.05	0.04	0.05	0.00	NS	**	NS
C16:1	2.15	1.97	2.11	2.05	0.03	NS	*	NS
C17:1	0.25	0.34	0.26	0.32	0.01	NS	**	NS
C18:1	44.62	45.06	44.97	44.90	0.15	NS	NS	NS
C20:1	0.95	1.01	0.82	0.92	0.02	*	NS	NS
MUFA	48.00	48.43	48.20	48.24	0.16	NS	NS	NS
C18:2	12.42	13.11	11.80	12.35	0.10	**	**	NS
C18:3	0.56	0.67	0.46	0.63	0.01	**	**	*
PUFA	12.98	13.77	12.26	12.98	0.11	**	**	NS
[1]IV	64.14	65.86	62.96	64.32	0.19	**	**	NS

NS: not significant; *P<0.05; **P<0.01.

[1]Iodine value = (C16:1×0.950) + (C18:1×0.860) + (C18:2n-6×1.732) + (C18:3n-3×2.616) + (C20:1×0.785) + (C22:1×0.723).

in particular for saturated and monounsaturated fatty acids types. Conversely, PUFA fatty acids are affected both by season and diet in independent way. In a few cases, mainly on saturated lipids, a significant interaction has been observed, whereas no interaction is evident about MUFA. $C_{18:3}$ showed interaction season-diet and during the cold season, differences between diets seems to be reduced by low temperature.

In the case of interaction observed for $C_{14:0}$, $C_{16:0}$ and $C_{22:0}$, season seems to play an opposite effect with a reduction of difference due to diet in hot season when the rate of turnover of fat tissue is reduced (LEBRET and MOUROT, 1998).

Fatty acid profile results in accordance with data reviewed by HUGO and ROODT (2007) and WOOD *et al.* (2008). Both SFA and MUFA profiles reflected the typical traits of heavy pigs commonly reared for Italian cured ham related industry and confirmed the data of LO FIEGO *et al.* (2005; 2010).

In literature, to our knowledge, there are controversial references about seasonal effect on adipose tissue composition of pig, and most studies useful to explain the effect, are related to differences due to environmental temperature. In earlier studies, MAC GRATH *et al.* (1968) and STAHLY and CROMWELL (1979) observed that environmental temperature was a discriminating factor on physical characteristics of fat as melting point and modify the lipid profile response in unsaturated fatty acids proportion. Moreover, it is well known that body fat distribution is affected by environmental temperature and its effect is independent of feeding conditions. In practice, the percentage of abdominal fat and perirenal depots increase at the expense of backfat when temperature increases and the mechanism is inverted when temperature drops (NOBLET *et al.*, 2001).

In lamb, the effect of season on fatty acids profile of fat is well known as reported by ENSER and WOOD (1993) and more recently in suckling lamb by MAZZONE *et al.* (2010). The effect is due to the variation of the quality and quantity of sheep pasture that modify the proportion in volatile fatty acids in rumen. Conversely in pig, this explanation cannot be accepted because of pig is a monogastric commonly indoor reared in not climatized barn and fed in a constant way all around the year. Nevertheless in practice, despite diets are quantitatively and qualitatively controlled, cases of high fat unsaturation level in backfat (iodine value >70) are often reported. LEFAUCHER *et al.* (1991) observed that a difference of 16°C in pig housing temperature, struck again on unsaturated backfat proportion. RINALDO and MOUROT (2001) reported that Large White pigs grown in a tropical climate compared to a group reared in thermally neutral zone (20°C) showed an increased level of $C_{18:2}$ and $C_{18:3}$ in backfat. Recently, WHITE *et al.* (2008) reported that a temperature of 32.2° *vs* 23.9°C had an independent effect on increasing iodine value of back and belly fat with a significant change in $C_{18:2}$ proportion. RODRIGUEZ-SANCHEZ *et al.* (2009) observed a significant effect of seasonality on SFA, MUFA and UFA while linoleic and linolenic acids were not significantly affected. Our results agree partially with these authors. In fact a significant effect of rearing season on SFA and PUFA was observed but no significant differences were re-

ported for MUFA. DAUNCEY and INGRAM (1988) suppose that increasing unsaturation in winter could be explained considering the stimulation of respiratory enzyme activities in skeletal muscles. LE DIVIDICH *et al.* (1987) and KATSUMATA *et al.* (1995) stated that, compared to thermoneutral and cold temperatures, high temperatures could reduce the backfat unsaturation. Furthermore, it must be remembered that during cold stress, thermoregulatory mechanisms are activated and the feeding level affects the requirements for metabolizable energy to maintain homeothermy. In particular, CLOSE (1983) stated that for pigs of 120 kg (BW) fed restricted from 2.5 to 3.0 kg/die, the lower critical temperature ranged from 15° to 11°C. Nevertheless, effect of seasonality on fatty acid profile are less important than the effect of diet as indicated by results of this study.

However the lack of significant interaction between season and diet, observed on two consecutive years, for the main fatty acid ($C_{18:2}$), suggest that season of rearing can affect the unsaturation level of fat.

As reported in Table 2, regarding the diet, the results confirmed the data reported in literature. Diet significantly affects the main fatty acids, in particular PUFA, SFA and iodine value, while MUFA are unaffected. From this point of view, the quantity of fat and the linoleic acid level of diet exerted a primary role to induce changes in subcutaneous fat lipid profile. Results of this study ranged in acceptable values of linoleic acid below 15% and iodine value below 70. Nevertheless, a significant increase in linoleic and iodine value from LL to HL diet is observed. The low level of linoleic acid in subcutaneous fat can be due to fat thickness, which was more than 20 mm in thighs examined. This inverse relationship is well known (WOOD *et al.*, 2008). Moreover it must be remembered that these pigs were slaughtered at 270 d of age and it is established the relationship between age of slaughtering and fatty acid profile of adipose tissue (KOUBA *et al.*, 2003; LO FIEGO *et al.*, 2010).

Approximately the quantity of linoleic in the diet of trial ranged between 16 to 20 g/kg on dry matter basis. The effect of intake of diet characterized by this quantity of $C_{18:2}$ is in agreement with BOCHICCHIO *et al.* (2005) who obtained similar results with diets containing approximately 23 g and 21 g of $C_{18:2}$ resulted in about 12 to 13% $C_{18:2}$ in subcutaneous fat of thigh. Our results are in agreement with the observations of CORINO *et al.* (2008), who showed that a 5% extruded linseed diet corresponded in backfat to 10.45% of $C_{18:2}$ on total FAME, for 160 kg BW slaughtered pigs. FONTANILLAS *et al.* (1997) observed values ranging from 10 to 12% of $C_{18:2}$ in backfat of pig fed using diet containing 15 g linoleic acid/kg. Linoleic acid linear proportion between dietary intake and tissue content is well described (WOOD *et al.*, 2008). The heavy pigs show different fat characteristics, in comparison to light pigs, probably due to genetic selection and age of slaughtering, in order to obtain a specific product for cured meat industry.

In conclusion it is possible to affirm that seasons have a role in modifying lipid profile in heavy pig. Probably, the effect is due to the temperature that in the season is the main factor of variability affecting the rearing environment. High unsaturation degree in animals reared in winter was observed. The season exerted an effect irrespectively of diet, and in some cases interacts with diet as for linolenic acid. From this point of view, the results confirm that when the feed level is maintained up to 20 g $C_{18:2}$ /kg of DM, the differences in fatty acids proportion, do not compromise the suitability of thighs for ham production and the parameters for satisfactory quality of fat are obtained: $C_{18:0}$>12% and $C_{18:2}$<15% (LO FIEGO *et al.*, 2005). In practice, Italian typical ham industry must consider the eventuality that season changes the fatty acid profile of thighs, and then the attitude of meat to transformation. This aspect should be remembered also by feedstuff manufacturers and providers in order to modulate the feed formulation in different seasons, and to reduce the risk of increasing unsaturation of fat during the cold season.

ACKNOWLEDGMENTS

Research has been supported by Regione Piemonte and Regional Swine Producer Association (A.P.S. Piemonte). Authors are grateful to Dr. Carola Lussiana and Mrs. Vanda Malfatto, for fatty acids and proximate analysis. The valuable cooperation of Dr. Valentine Brossette is also acknowledged.

REFERENCES

AOAC 2000. "Official methods of analysis" 17th Ed. Association of Official Analytical Chemists, Gaithersburg, MD.

AOCS 1998. "Official Methods and Recommended Practices of the AOCS" 5th Ed.; American Oil Chemists' Society. Champaign, IL.

Bochicchio D., Faeti V., Marchetto G., Poletti E., Maranesi M., Mordenti A.L. and Della Casa G. 2005. Effect of feeding partially hydrogenated lard on trans-fatty acid content of muscle and backfat of heavy pigs. Meat Sci. 71: 651-656.

Close W.H. 1983. Interaction of environment on pig production. Guelph Pork symposium, 12-21. Quoted in Perez J.M., Mornet P. and Rérat A. (eds.) "Le porc et son élevage, bases scientifiques et techniques", p.356,. Maloine, Paris.

Corino C., Musella M. and Mourot J. 2008. Influence of extruded linseed on growth, carcass composition, and meat quality of slaughtered pigs at one hundred ten and one hundred sixty kilograms of liveweight. J. Anim. Sci. 86: 1850-1860.

Dauncey M.J. and Ingram D.L. 1988. Influence of environmental temperature and energy intake on skeletal muscle respiratory enzymes and morphology. Eur. J. Appl. Physiol. Occup. Physiol. 58: 239-244.

Della Casa G., Bochicchio D., Faeti V., Marchetto G., Poletti E., Rossi A., Panciroli A., Mordenti A.L. and Brogna N. 2010. Performance and fat quality of heavy pigs fed maize differing in linoleic acid content. Meat Sci. 84: 152-158.

English P.R., Fowler V.R., Baxter S. and Smith B. 1988. "The growing and finishing pig: improving efficiency" Farming Press Books, Ipswich.

Enser M. and Wood J.D. 1993. Effect of time of year on fatty acid composition and melting point of UK lamb. Proceedings of the *39th International Congress of Meat Science and Technology*, Calgary, Canada, 74.

Folch J., Lees M. and Stanley G.H.S. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226: 497-509.

Fontanillas R., Barroeta A., Baucells M.D. and Codony R. 1997. Effect of feeding highly *cis*-monounsaturated, *trans*, or *n*-3 fats on lipid composition of muscle and adipose tissue of pigs. J. Agric. Food Chem. 45: 3070-3075.

Gatlin L.A., See M.T., Hansen J.A., Sutton D. and Odle J. 2002. The effects of dietary fat sources, levels, and feeding intervals on pork fatty acid composition. J. Anim. Sci. 80: 1606-1615.

Hugo A. and Roodt E. 2007. Significance of porcine fat quality in meat technology: A review. Food Rev. Int. 23: 175-198.

Katsumata M., Hirose H., Kaji Y. and Saitoh M. 1995. Influence of a high ambient temperature and dietary fat supplementation on fatty acid composition of depot fats in finishing pigs. J. Anim. Sci. Technol. 66: 225-232.

Kouba M., Enser M., Whittington F.M., Nute G.R. and Wood J.D. 2003. Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. J. Anim. Sci. 81: 1967-1979.

Le Dividich J., Noblet J. and Bikawa T. 1987. Effect of environmental temperature and dietary energy concentration on the performance and carcass characteristics of growing-finishing pigs fed to equal rate of gain. Livest. Prod. Sci. 17:235-246.

Lebret B. and Mourot J. 1998. Caracteristique et qualité des tissus adipeux chez le porc. Facteurs de variation non génétiques. INRA Prod. Anim. 11: 131-143.

Lefaucheur L., Le Dividich J., Mourot J., Monin G., Ecolan P. and Krauss D. 1991. Influence of environmental-temperature on growth, muscle and adipose-tissue metabolism, and meat quality in swine. J. Anim. Sci. 69: 2844-2854.

Lo Fiego D.P., Macchioni P., Minelli G. and Santoro P. 2010. Lipid composition of covering and intramuscular fat in pigs at different slaughter age. Ital. J. Anim. Sci. 9: 200-205.

Lo Fiego D.P., Nanni Costa L. and Santoro P. 1990. Caratteristiche del tessuto adiposo nel suino pesante italiano. Riv. Ital. Suinic. 31: 41-47.

Lo Fiego D.P., Santoro P., Macchioni P. and De Leonibus E. 2005. Influence of genetic type, live weight at slaughter and carcass fatness on fatty acid composition of subcutaneous adipose tissue of raw ham in the heavy pig. Meat Sci. 69: 107-114.

MacGrath W.S. Jr., Noot G.W., Gilbreath R.L. and Fisher H. 1968. Influence of environmental temperature and dietary fat on backfat composition of swine. J. Nutr. 96: 461-466.

Mazzone G., Giammarco M., Vignola G., Sardi L. and Lambertini L. 2010. Effects of the rearing season on carcass and meat quality of suckling Apennine light lambs. Meat Sci. 86: 474-478.

Nguyen L.Q., Nuijens M., Everts H., Salden N. and Beynen A.C. 2003. Mathematical relationships between the intake of n-6 and n-3 polyunsaturated fatty acids and their contents in adipose tissue of growing pigs. Meat Sci. 65: 1399-1406.

Noblet J., Le Dividich J. and Van Milgen J. 2001. Thermal environment and swine nutrition. In: "Swine Nutrition" A.J. Lewis and L.L. Southern (Eds.), pp. 519-544. CRC Press Boca Raton, FL.

Nurnberg K., Wegner J. and Ender K. 1998. Factors influencing fat composition in muscle and adipose tissue of farm animals. Livest. Prod. Sci. 56: 145-156.

Rinaldo D. and Le Dividich J. 1991. Assesment of optimal temperature for performance and chemical body-composition of growing pigs. Livest. Prod. Sci. 29: 61-75.

Rinaldo D. and Mourot J. 2001. Effects of tropical climate and season on growth, chemical composition of muscle and adipose tissue and meat quality in pigs. Anim. Res. 50: 507-521.

Rodriguez-Sanchez J.A., Ripoll G., Calvo S., Arino L. and Latorre M.A. 2009. The effect of seasonality of the growing-finishing period on carcass, meat and fat characteristics of heavy barrows and gilts. Meat Sci. 83: 571-576.

Stahly T.S. and Cromwell G.L. 1979. Effect of Environmental temperature and dietary Fat supplementation on the performance and carcass characteristics of growing and finishing swine. J. Anim. Sci. 49: 1478-1488.

Warnants N., Van Oeckel M.J. and Boucque C.V. 1996. Incorporation of dietary polyunsaturated fatty acids in pork tissues and its implications for the quality of the end products. Meat Sci. 44: 125-144.

White H.M., Richert B.T., Schinckel A.P., Burgess J.R., Donkin S.S. and Latour M.A. 2008. Effects of temperature stress on growth performance and bacon quality in grow-finish pigs housed at two densities. J. Anim. Sci. 86: 1789-1798.

Whittermore C. 1993. "The science and practice of pig production" Longman Group UK Limited. Harlow.

Wiseman J. and Agunbiade J.A. 1998. The influence of changes in dietary fat and oils on fatty acid profiles of carcass fat in finishing pigs. Livest. Prod. Sci. 54: 217-227.

Wood J.D., Enser M., Fisher A.V., Nute G.R., Sheard P.R., Richardson R.I., Hughes S.I. and Whittington F.M. 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat Sci. 78: 343-358.

Paper received September 3, 2010 Accepted January 10, 2011

PAPER

THE EFFECT OF WATER-SOLUBLE ROSEMARY EXTRACT, SODIUM ERYTHORBATE, THEIR MIXTURE AND PACKAGING METHOD ON THE QUALITY OF TURKEY MEATBALLS

MIROSŁAWA KARPIŃSKA-TYMOSZCZYK
Department of Human Nutrition, University of Warmia and Mazury,
10-726 Olsztyn, Plac Cieszyński 1, Poland
Corresponding author: Tel./Fax +48 89 523 49 91
email: mikar@uwm.edu.pl

ABSTRACT

The effect of antioxidants (water-soluble rosemary extract, sodium erythorbate and their mixture), packaging method (VP and MAP-20% CO_2 / 80% N_2) and storage time on the quality of turkey meatballs were studied. The applied antioxidants delayed lipid oxidation and improved the sensory quality of turkey meatballs. The rate of lipid oxidation was lower in vacuum packaged samples than in MA-stored samples. Water-soluble rosemary extract was less effective than sodium erythorbate in delaying lipid oxidation, regardless of the packaging method, and it protected samples against redness fading during modified atmosphere storage. Sodium erythorbate slowed down the hydrolytic process in turkey meatballs more effectively during vacuum storage than during MA storage.

- Key words: antioxidants, lipid oxidation, microbial quality, physicochemical quality, sensory properties, turkey meatballs -

INTRODUCTION

Over the past decade, poultry-based products have become increasingly popular worldwide due to their high nutritional quality. Such products are available either fresh or precooked, and after subsequent packaging they are usually stored under refrigeration. Since poultry is perishable food, the main concern of the meat-processing industry is to extend the shelf-life of poultry products. The susceptibility of poultry meat to microbial spoilage presents a potential health hazard, since it may harbor pathogenic microorganisms. Spoilage is commonly detected by a sensory and/or microbiological analysis (PATSIAS *et al.*, 2006).

Poultry meat contains high amounts of polyunsaturated fatty acids, which makes it susceptible to oxidation (LUCERA *et al.*, 2009). Oxidation is one of the major causes of chemical spoilage, resulting in rancidity and deterioration of the nutritional quality, color, flavor, texture and safety of foods (ANTOLOVICH *et al.*, 2002). This irreversible change contributes to the development of unacceptable organoleptic characteristics in both fresh and frozen processed meat products. Oxidative processes are also associated with discoloration of meat products, as lipid oxidation results in the formation of pro-oxidants capable of reacting with oxymyoglobin, which leads to the development of metmyoglobin (LUCERA *et al.*, 2009).

There has been an increasing interest recently in the use of spices and aromatic herbs which, owing to their strong antioxidant and antimicrobial properties, are often considered superior to many of the currently used natural and synthetic antioxidants. The above properties result from the presence of vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens and minerals, and they render spices and some herbs or their antioxidant components as preservative agents in food (SUHAJ, 2006).

Rosemary *(Rosmarinus officinalis)* is a plant of the family *Labiatae,* whose major and most active components are carnosol, carnosic acid and rosmarinic acid. Rosematy is an important contributor to the oxidative stability of meat products (MARTÍNEZ *et al.*, 2006). Water-soluble rosemary extract is a natural product containing phenolic diterpenes, which suggests that it may possess antioxidant activity. However, the practical application of extracts is limited due to the strong flavor they impart to foods as well as to their interactions with some food ingredients (BURT, 2004). For these reasons the preservative effect of extracts may be achieved by using them in lower concentrations and in combination with other preservation technologies such as low temperature and vacuum or modified atmosphere packaging (LUCERA *et al.*, 2009). Several Authors reported that some compounds in rosemary extracts could have antibacterial activity (CUVELIER *et al.*, 1996; DEL CAMPO *et al.,* 2000)

Packaging methods including vacuum packaging (VP) and modified atmosphere packaging (MAP) have been investigated as technologies to increase the shelf-life of poultry products by inhibiting spoilage and the growth of pathogenic microorganisms (NTZIMANI *et al.*, 2008). Modified atmosphere packaging (MAP) has gained considerable popularity over the last decades as a modern non-thermal method of food preservation. The proper combination of gases (CO_2, N_2 and O_2) in the headspace of food packs results in suppression of the microbial flora of perishable foods such as meat products, developed under aerobic conditions, and it helps to maintain their sensory attributes (PATSIAS *et al.*, 2006).

MATERIALS AND METHODS

Materials

Thigh muscles of male British United BIG-6 turkeys (Frednowy, Poland) were purchased from the Indykpol Company (Olsztyn, Poland). Water-soluble rosemary extract (0791 Stabilotion WS) containing 9±1% phenolic diterpenes (carnosic acid, carnosol, rosmanol) was purchased from the RAPS GmbH &CO.KG Company (Kulmbach, Germany). Sodium erythorbate was supplied by the Parchn Group Company (Xingangshan Town, Dexingxing City, Jiangxi Province, China).

Preparation of samples, packaging and storage

Two different experiments were carried out, according to the following designs:

Experiment 1

After cutting and chilling, 1 kg portions of turkey meat were packaged in polyethylene bags and deep-frozen at –25°C at the "Indykpol" Poultry Processing Plant in Olsztyn. After three days, half of the material was placed in the refrigerator at 3°C, where it was defrosted over 20 hours and assigned to the first experiment. Defrosted meat was cut into 4-6 cm pieces and ground in a meat grinder, type MMU-10Z (Nakło, Poland) with a 4 mm mesh. Ground turkey meat was divided into four groups. Following the addition of wheat flour roll soaked in water (13%), beaten eggs (5%), flour (2%) and salt (1% in relation to total mass weight) to meat (80%), the mass was mixed in a multifunctional food processor (Bauknecht, Warsaw Poland). Experimental treatments were as follows: no additives (control - C), sodium erythorbate (SE) - 0.3 g/kg of total mass weight, water-soluble rosemary extract (WR) - 0.3 g/kg and a mixture of WR and

SE (MIX) - 0.15 and 0.15 g/kg, respectively. The amount of water-soluble rosemary extract (WS) added to turkey meatballs was determined based on the recommendations of the supplier regarding meat products, and previous research which investigated the sensory acceptability of the proposed amount in the final product. As a result, WS was added at the lowest concentration. The amount of sodium erythorbate was determined based on the recommendations of the supplier and the amount of WS, in order to compare their effects.

Water-soluble rosemary extract was mixed with part of the meat mass before mixing with the total mass. Sodium erythorbate was dissolved in water (20°C) in the amount of 15 g/100 mL, and it was slowly added to the meat mass while mixing. While preparing a mixture of SE and WR, the ingredients were added individually. Next the mass was formed into 90 g ± 1g meatballs (8 cm in diameter, 1 cm in thickness).

Eighty-four meatballs randomly selected from each treatment were placed on the oven tray (twelve meatballs at a time) and were cooked in the BECK FCV 4 EDS steam-convection oven (BECK GmbH, Jagsthausen, Germany) with a measuring probe. Steam and hot air were used for heat treatment (air temperature - 180°C, steam saturation - 30%). The treatment was continued until a temperature of 82°C was reached inside the product. The patties analyzed after cooking were stored overnight at 0°C. The remaining samples were cooled to room temperature and vacuum packaged (VP-Experiment 1) with the MULTIVAC A 300 packaging unit (Multivac, Wolfertschwenden Germany) into bags of a five-layer PE-LD/adh/PA/ADH/PE-PD film (total thickness – 0.08 mm, PA layer thickness – 0.024 mm, oxygen permeability - 40 $cm^3/m^{-2}24\ h^{-1}bar^{-1}$, water vapor permeability – 10 g $m^{-2}24\ h^{-1}bar^{-1}$). The samples were stored at 3° ± 1°C.

Experiment 2

After 14 days of storage, the other half of turkey meat was placed in the refrigerator at 3°C, where it was defrosted over 20 hours and assigned to the second experiment. Meatballs were prepared following the same procedure as in experiment 1. After cooking and cooling to room temperature, the samples were packaged under modified atmosphere (a gas mixture of 80% N_2 and 20% CO_2, MAP) with the MULTIVAC A 300 packaging unit (Multivac, Wolfertschwenden Germany) into identical bags as in experiment 1 and were stored at 3° ± 1°C.

Three randomly selected packages were collected every 5 days of storage from each experiment and from each treatment for an analysis of CIE L*, a* and b*, pH, AV, malondialdehyde (MDA) and water activity, and a sensory analysis. After 15 and 30 days of storage, one additional package was opened for a microbial analysis.

Analytical Methods

Lipid oxidation analysis

Lipid oxidation was assessed in 10 g of turkey meatballs by the 2-thiobarbituric acid (TBA) method of TARLADGIS *et al.* (1960) modified by PIKUL *et al.* (1989), after 0, 5, 10, 15, 20, 25 and 30 days of storage at 4°C. TBARS values were calculated from a standard curve of malondialdehyde and expressed as mg malondialdehyde (MDA) kg^{-1} product. TBARS determination was conducted in triplicate on each of three samples per treatment. The antioxidant potential, expressed in terms of the percentage of antioxidant activity, was calculated using the equation:

% AOA= [TBARS value of the control sample – TBARS of the experimental sample] x 100/ [TBARS value of the control sample]

Acid value (AV)

The acid value (AV) indicates how much fatty acid has accumulated as a result of lipolysis. It was determined in fat extracted from the products after 0, 5, 10, 15, 20, 25 and 30 days of storage at 4°C, in accordance with the Polish Standard PN-EN ISO 660 (Animal and vegetable fats and oils 2005). AV determination was conducted in triplicate on each of three samples per treatment.

pH

For pH determination, 10 g samples of the products stored for 0, 5, 10, 15, 20, 25 and 30 days at 4°C and 90 mL of distilled water were homogenized in a blender (type MPW-302) for 60 s. The pH of the mixture was measured using a pH-meter; model ATC PICCOLO 2 (Woonsocket USA), standardized at pH 4 and pH 7 (PEXARA *et al.,* 2002). pH was measured directly after homogenization and it was read after stabilization. pH determination was conducted in triplicate on each of three samples per treatment.

Water activity

An adequate amount of a ground sample was placed in a measuring container and three measurements were performed on the products after 0, 5, 10, 15, 20, 25 and 30 days of storage at 4°C, using a water analyzer (type AWC 203-C, Novasina, Pfäffikon, Switzerland), after reaching equilibrium at 20°C, according to the manufacturer's instruction. The samples were brought to room temperature prior to evaluation. Water activity determination was conducted in triplicate on each of two samples per treatment.

Color evaluation

The color of the samples was determined with a spectrophotometer (Dr Lange Spectro-color), d/8° with a 10 mm measuring aperture and SPECTRAL-QC computer software after calibration using Dr Langeblack and white standards. The results are given in the color-coordination system CIE Lab [where L* measures relative lightness (L* = 0 darkness, L* = 100 lightness), a* - relative redness (+60 = red, -60 = green) and b* - relative yellowness (+60 yellow, -60 = blue)]. Color was measured on the meat surface 30 min after the package had been opened. The measurement was repeated at six randomly selected points on each of three samples per treatment.

Microbiological analysis

A 10 g ground sample of each product was taken aseptically and placed in a sterile stomacher bag containing 90 mL of Ringer liquid. The sample in the bag was blended in a laboratory blender (Stomacher 400, Seward Laboratory, London, UK) for 2 min. and submitted to serial dilutions with the same diluents. For the determination of mesophilic and psychrotrophic bacteria, appropriate serial dilutions were plated on plate count nutrient agar, and were incubated at 30°C for 72 h (mesophilic bacteria) and at 6.5°C for 7 days (psychrotrophic bacteria) prior to enumeration. Coliforms were enumerated by plating on violet red bile lactose agar (Merck, Warsaw, Poland) and then they were incubated at 30°C for 48 h. The counts of fungi were determined on YGC – Agar (Merck) after 96 h incubation at 25°C. To determine the presence of sulfate IV reducing *Clostridium* sp., adequate dilutions were inoculated into a liquid meat-liver agar medium, were heated at 80°C for 15 minutes and incubated at 37°C for 48 h. All microbial counts were reported as a logarithmic value of colony forming units per gram of sample. Microbial populations in the samples were determined in triplicate before thermal processing, after thermal processing and after 15 and 30 days of storage at 3° ± 1° C. Samples taken from three randomly selected meatballs per treatment were homogenized.

Sensory quality

Meatballs were subjected to a sensory evaluation by the flavor profile method (MEILGAARD *et al.*, 1999). The sensory panel consisted of five panelists trained in accordance with the Polish Standard ISO 11035 (Identification and selection of descriptors, 1999). All judges were trained to be familiar with the flavor attributes to be measured (meaty, typical of poultry meat, aromatic, typical of roasted meat, spicy-rosemary, sour, WOF). The sensory assessment was performed after 0, 5, 10, 15, 20, 25 and 30 days of storage at 4°C. Two sensory evaluation sessions were conducted per day. The 2nd session comprised the replicates of the 1st session. The sessions were conducted at one-hour intervals. For flavor evaluation, two meatballs per treatment were cut into approximately uniform pieces. Samples were served in random order to each panelist on a white porcelain dish, coded with random numbers. At the beginning of each session, the panel was presented with the reference samples for the extremes of scales of the flavor attributes to be measured. The intensity of particular attributes was determined on a five-point scale: 0-lack of perception; 1-hardly noticeable perception; 2-slightly noticeable perception; 3-moderately noticeable perception; 4-strongly noticeable perception; 5-very strongly noticeable perception.

Statistical analysis

Both experiments were performed in a completely randomized 4 x 7 factorial design. The experimental factors included four additives and seven storage times. Data obtained for the initial characterization of the tested additives and storage time were subjected to a two-way analysis of variance (ANOVA), after the normality and homogeneity of variance had been confirmed. Since significant interactions were found, independent one-way ANOVA tests were carried out on each variable (additives and storage time). When the ANOVA showed the differences to be significant, the obtained values were evaluated by Tukey's test. Additive treatments were compared each day. The level of significance was determined at $P<0.05$. The relationships between the investigated properties were determined using Pearson's correlation. The correlations between sensory attributes and lipid changes (MDA, AV) were performed using Spearman's non-parametric rank correlation test. The statistical analysis was carried out using STATISTICA 7.1 software (StatSoft Inc. 2008).

RESULTS AND DISCUSSION

The effect of water-soluble rosemary extract and sodium erythorbate on lipid stability

Determination of lipid oxidation

An analysis of variance showed (Table 1) that the analyzed additives and storage time significantly ($P<0.001$) affected the MDA content in samples stored under vacuum and modified atmosphere conditions. Vacuum-packaged turkey meatballs (Table 1) without active compounds (C) showed the highest MDA values throughout storage. MDA concentrations in all samples decreased significantly after 5 days of storage, and during further storage MDA content changed

Table 1 - Effect of additives, storage time and packaging system on the malondialdehyde (MDA) content of cooked turkey meatballs and the antioxidant activity of the tested additives (mean±SD, n=6).

Method of packaging	Samples	Storage time [days]						Effect	p values
		0	5	10	15	20	25	30		
Malondialdehyde [mg/kg]										
Vacuum packaging	C	2.46±0.14^{cC}	0.93±0.01^{aD}	0.95±0.01^{aD}	0.92±0.02^{aD}	1.16±0.03^{bD}	0.87±0.02^{aD}	1.17±0.05^{bC}	Additives (A)	<0.001
	SE	0.53±0.02^{dA}	0.32±0.04^{aA}	0.40±0.01^{bA}	0.46±0.01^{cA}	0.46±0.01^{cA}	0.29±0.02^{aA}	0.43±0.02^{bcA}	Storage time (T)	<0.001
	WR	0.82±0.02^{dB}	0.44±0.01^{aB}	0.43±0.01^{aB}	0.56±0.02^{bB}	0.68±0.02^{cB}	0.59±0.02^{bB}	0.68±0.02^{cB}	AxT	<0.001
	MIX	0.79±0.01^{eB}	0.60±0.01^{bC}	0.47±0.01^{aC}	0.80±0.01^{eC}	0.88±0.02^{fC}	0.71±0.02^{dC}	0.66±0.01^{cB}		
Modified Atmosphere packaging (20% CO_2/ 80% N_2)	C	2.44±0.04^{eC}	1.33±0.02^{cD}	1.18±0.02^{bD}	1.04±0.06^{aC}	1.34±0.05^{cD}	1.26±0.03^{bcD}	1.72±0.06^{dB}	Additives (A)	<0.001
	SE	0.94±0.01^{fA}	0.49±0.02^{bA}	0.31±0.01^{aA}	0.56±0.01^{cA}	0.59±0.01^{cA}	0.63±0.01^{dA}	0.89±0.02^{eA}	Storage time (T)	<0.001
	WR	1.37±0.05^{dB}	0.93±0.03^{bB}	0.76±0.04^{aB}	0.86±0.01^{bB}	1.09±0.01^{cC}	0.85±0.02^{bB}	2.73±0.03^{eC}	AxT	<0.001
	MIX	1.28±0.03^{dB}	1.13±0.06^{cC}	0.86±0.02^{abC}	0.80±0.05^{aB}	0.92±0.04^{bB}	0.93±0.04^{bC}	3.03±0.03^{eD}		
Antioxidant activity [%]										
Vacuum packaging	SE	78.42±1.12^{dB}	65.62±3.51^{cC}	58.24±1.29^{bC}	50.17±1.37^{aC}	60.17±0.01^{bC}	66.81±1.95^{cC}	62.86±1.15^{bcB}	Additives (A)	<0.001
	WR	66.49±1.15^{dA}	52.54±1.19^{cB}	55.10±0.84^{cB}	38.99±075^{bB}	41.83±0.47^{bB}	32.05±1.96^{aB}	41.92±3.18^{bA}	Storage time (T)	<0.001
	MIX	67.93±2.35^{fA}	35.13±0.73^{cA}	50.88±0.81^{eA}	12.97±1.62^{aA}	24.32±2.55^{bA}	18.29±2.77^{aA}	43.07±2.67^{dA}	AxT	<0.001
Modified atmosphere packaging (20% CO_2/ 80% N_2)	SE	61.66±1.07^{cB}	62.98±1.73^{cC}	74.01±0.66^{dC}	46.07±2.39^{aB}	56.04±1.71^{bC}	50.11±1.37^{aC}	48.42±1.01^{aC}	Additives (A)	<0.001
	WR	42.19±2.80^{dA}	30.51±0.84^{cB}	35.33±1.88^{cdB}	16.85±4.47^{bA}	19.05±2.51^{bA}	32.40±3.32^{cB}	-59.02±4.03^{aB}	Storage time (T)	<0.001
	MIX	47.42±2.12^{dA}	15.23±4.45^{bA}	27.09±2.60^{cA}	22.76±0.93^{bcA}	31.46±3.78^{cB}	26.15±1.42^{cA}	-76.34±7.55^{aA}	AxT	<0.001

Samples: C- control, SE-with sodium erythorbate; WR-with water-soluble rosemary extract, MIX-with mixture of sodium erythorbate and water-soluble rosemary extract;
$^{a, b, c, d, e, f}$ mean values within the row followed by the different letters are significantly different at P<0.05;
$^{A, B, C, D}$ mean values within the column followed by the different letters are significantly different at P<0.05.

significantly, but the observed changes were ambiguous. The decrease in the MDA content noted after 5 days of storage could result from the fact that the rate of MDA reaction with protein was faster than the rate of MDA formation, or the formation of secondary products of lipid oxidation that do not react with TBA. ULU (2004) reported that malonaldehyde is highly reactive and remains bound to other food ingredients. After 30 days of vacuum storage, the MDA content of all samples was significantly higher, compared with the first period of storage. Similar changes in TBA values were also noted by NASSU *et al.* (2003) in a study investigating the effect of natural antioxidants in fermented goat sausages during a storage period of 80 days, and according to the Authors they were due to the fact that malonaldehyde did not accumulate as a stable end product.

Until day 25 of MA storage (Table 1), lipid oxidation in control (C) samples was more intense than in treated samples. At the last stage of storage, samples containing water-soluble rosemary extract and a mixture of sodium erythorbate and water-soluble rosemary extract were characterized by a higher MDA content, compared with C and SE samples. The inhibitory effect of water-soluble rosemary extract on lipid peroxidation in cooked turkey products might be due to the free radical scavenging and transition metal chelating activities of WR components (HUANG *et al.*, 1996). YU *et al.* (2002) found that water-soluble rosemary extract prevented lipid oxidation in cooked turkey products during refrigerated storage. FERNÁNDEZ-LÓPEZ *et al.* (2005) demonstrated that rosemary extracts (water- and oil-soluble) were effective in retarding lipid oxidation in cooked beef meatballs during 12 days of storage at 8°±1°C. According to BRAGAGNOLO *et al.* (2005, 2007), the addition of rosemary to minced chicken meat prior to high-pressure treatment was

effective in preventing the formation of "pressed-over-flavor" upon subsequent cooking and lipid oxidation during refrigerated storage. The positive effects of rosemary and sodium erythorbate used alone have been well documented with respect the prevention of lipid oxidation, whereas, to the Author's best knowledge, there are no reports on the combined use of rosemary and sodium erythorbate. In the present experiment, MDA content was lower in vacuum-stored turkey meatballs than in MA-stored samples. This could be caused by a higher amount of oxygen in MA-packaged samples, in comparison with vacuum-packaged ones, because complete removal of oxygen is extremely difficult. These findings agree with those reported by other Authors (NOLAN *et al.*, 1989; SMIDDY *et al.*, 2002). SHEARD *et al.* (2000) consider MDA concentrations higher than 0.5 mg/kg as threshold values for rancidity perception by consumers, whereas according to Ockerman (as cited in GEORGANTELIS *et al.*, 2007) meat products with MDA concentrations higher than 1 mg/kg are considered rancid. In the present study, control samples were perceived as rancid after 20 days of vacuum storage and throughout MA storage. The MDA content of all samples with additives and with sodium erythorbate was close to the threshold value during vacuum storage and during MA storage, respectively. In MAP samples with water-soluble rosemary extract and with a mixture water-soluble rosemary extract and sodium erythorbate, MDA content did not exceed 1 mg/kg until day 15 and 25 of storage, respectively.

Antioxidant activity

The individual and interactive effects of sodium erythorbate and water-soluble rosemary extract on the inhibition of lipid oxidation in turkey meatballs are showed in Table 1. Antioxidant activity was not stable during vacuum and MA storage. Samples containing sodium erythorbate showed higher antioxidant activity than those containing water-soluble rosemary extract, which could be caused by low phenol concentrations in this extract. It was found that sodium erythorbate used in combination with water-soluble rosemary extract showed lower antioxidant activity during vacuum storage than each of the additives applied alone. A similar tendency was observed during MA storage, except on day 15 and 20 when water-soluble rosemary extract combined with sodium erythorbate showed higher antioxidant activity than water-soluble rosemary extract applied alone. After 30 days of MA storage, water-soluble rosemary extract and a mixture of sodium erythorbate and water-soluble rosemary extract exhibited a prooxidative effect. As shown in Table 1, sodium erythorbate and water-soluble rosemary extract were more effective as individual antioxidants than their mixture. WILKINSON *et al.* (2001) reported that sodium erythorbate delayed lipid oxidation in freeze-dried beef and chicken. BALEV *et al.* (2005) demonstrated that the addition of sodium erythorbate suppressed the accumulation of secondary derivatives of lipid peroxidation in dry-fermented sausages, and that sodium erythorbate was more effective than rosemary. The prooxidative effect of the MIX additive observed at the final stage of storage in the present study could be caused by a too low concentration of sodium erythorbate in MIX samples, which therefore could show its prooxidative effect. Sodium erythorbate was probably utilized as a reducing agent and could be expected to behave similarly to its isomer, ascorbate. Ascorbate has been reported to act as an antioxidant or prooxidant depending on its concentration, the presence of metal ions, and tocopherol concentrations (SCHAEFER *et al.*, 1995). In consequence, the results of studies investigating the use of ascorbate as an antioxidant are ambiguous. DECKER and XU (1998) demonstrated that ascorbate can act as a prooxidant at low (0.02-0.03%) concentrations and as an antioxidant at high (0.5%) concentrations. The prooxidative effect of water-soluble rosemary extract could result from the interaction between phenol diterpens contained in this additive and other active components. According to HERNÁNDEZ-HERNÁNDEZ *et al.* (2009), antioxidant activity does not depend only on total phenol concentrations, but also on their polarity and molecular structure. MADSEN *et al.* (1995) reported that the effect of certain potential antioxidants might vary considerably depending on a complex interaction between various factors including the type and concentration of active compounds and the nature of the food system.

Hydrolytic changes

Both the additives and storage time significantly affected ($P<0.001$) the acid value of turkey meatballs (Table 2). During vacuum storage, acid value showed a rising tendency in C and WR samples, while changes in the acid value of SE and MIX samples were significant, but ambiguous. There was a significant additive x time interaction in AV ($P<0.001$). The smallest changes in acid value were noted in samples with sodium erythorbate, as compared with C, WR and MIX samples.

During storage, the rate of the hydrolytic process was slower in MAP turkey meatballs than in VP samples. After 10 days of MA storage, the acid value of control samples decreased significantly, and during subsequent storage periods it showed a rising tendency. The acid value of SE and MIX samples did not change significantly until day 20, after 25 days it increased significantly and remained at a similar level at the final stage of storage. The acid value of WR samples showed an ambiguous tendency during

Table 2 - Effect of additives, storage time and packaging system on the acid value and pH value of cooked turkey meatballs (mean±SD, n=6).

Method of packaging	Samples	Storage time [days]						Effect	p values
		0	5	10	15	20	25	30		
Acid value [mg/1 g of fat]										
Vacuum packaging	C	3.80±0.08^{aA}	3.85±0.10^{abA}	3.98±0.05^{bcB}	4.12±0.15^{cdA}	4.24±0.04^{dB}	4.10±0.08^{cdA}	4.51±0.06^{eB}	Additives (A)	<0.001
	SE	4.00±0.07^{bB}	4.19±0.05^{cB}	3.84±0.08^{aA}	4.08±0.11^{bcA}	3.98±0.05^{bA}	4.23±0.11^{cdA}	4.35±0.07^{dA}	Storage time (T)	<0.001
	WR	3.77±0.03^{aA}	4.19±0.14^{bB}	4.26±0.09^{bC}	4.24±0.08^{bA}	4.08±0.26^{bAB}	4.82±0.12^{cC}	4.72±0.05^{cC}	AxT	<0.001
	MIX	3.86±0.04^{aA}	3.84±0.24^{aA}	4.09±0.04^{bB}	4.41±0.06^{dB}	4.20±0.07^{bcAB}	4.56±0.11^{dB}	4.38±0.07^{cdA}		
Modified atmosphere packaging (20%CO_2/ 80% N_2)	C	3.76±0.03^{cC}	3.66±0.06^{bC}	3.56±0.06^{aBC}	3.54±0.04^{aBC}	3.65±0.07^{bC}	3.95±0.07^{dC}	3.90±0.05^{dB}	Additives (A)	<0.001
	SE	3.44±0.07^{aA}	3.53±0.08^{aAB}	3.49±0.08^{aAB}	3.51±0.05^{aB}	3.50±0.06^{aAB}	3.71±0.04^{bA}	3.71±0.05^{bA}	Storage time (T)	<0.001
	WR	3.67±0.05^{bB}	3.48±0.06^{aA}	3.39±0.05^{aA}	3.42±0.04^{aA}	3.40±0.08^{aA}	3.81±0.07^{cB}	3.73±0.03^{bcA}	AxT	<0.001
	MIX	3.63±0.03^{aB}	3.59±0.07^{aBC}	3.64±0.04^{aC}	3.59±0.04^{aC}	3.57±0.09^{aBC}	3.95±0.07^{bC}	3.99±0.09^{bB}		
pH										
Vacuum packaging	C	6.56±0.04^{bcd}	6.65±0.06^{d}	6.61±0.07^{d}	6.64±0.05^{d}	6.49±0.07^{bc}	6.46±0.03^{bB}	6.30±0.05^{aA}	Additives (A)	<0.001
	SE	6.55±0.05	6.65±0.06	6.61±0.08	6.65±0.05	6.55±0.05	6.53±0.03^{B}	6.54±0.05^{B}	Storage time (T)	<0.001
	WR	6.58±0.05^{b}	6.68±0.06^{b}	6.60±0.05^{b}	6.66±0.04^{b}	6.57±0.04^{b}	6.41±0.03^{aA}	6.31±0.03^{aA}	AxT	<0.001
	MIX	6.57±0.05^{ab}	6.67±0.06^{b}	6.60±0.03^{ab}	6.65±0.04^{ab}	6.57±0.05^{ab}	6.54±0.05^{aB}	6.54±0.05^{aB}		
Modified atmosphere packaging (20%CO_2/ 80% N_2	C	6.41±0.03^{a}	6.58±0.06^{b}	6.50±0.05^{ab}	6.55±0.05^{b}	6.54±0.03^{b}	6.55±0.02^{b}	6.52±0.05^{ab}	Additives (A)	NS
	SE	6.43±0.05	6.49±0.07	6.48±0.04	6.52±0.05	6.54±0.05	6.49±0.05	6.52±0.04	Storage time (T)	<0.001
	WR	6.43±0.09	6.50±0.07	6.49±0.04	6.50±0.05	6.50±0.05	6.46±0.04	6.49±0.05	AxT	NS
	MIX	6.42±0.10	6.52±0.05	6.51±0.02	6.53±0.02	6.51±0.04	6.47±0.04	6.50±0.04		

Samples: C- control, SE-with sodium erythorbate; WR-with water-soluble rosemary extract, MIX-with mixture of sodium erythorbate and water-soluble rosemary extract;
NS – not significant (p>0.05);
^{a,b,c,d,e} mean values within the row followed by the different letters are significantly different at P<0.05;
^{A,B,C} mean values within the column followed by the different letters are significantly different at P<0.05.

MA storage. These results are partly consisted with the findings of previous studies (BALEV *et al.*, 2005; KARPIŃSKA-TYMOSZCZYK, 2006). BALEV *et al.* (2005) found that the addition of sodium erythorbate slowed down lipolysis in dry-fermented sausages during the drying process. According to KARPIŃSKA-TYMOSZCZYK (2006), sodium erythorbate was a more effective inhibitor of hydrolytic changes in poultry meatballs than sage. FERNÁNDEZ-FERNÁNDEZ *et al.* (2002) reported that the hydrolytic process took place in chorizo sausages stored under modified atmosphere (100% CO_2, 100% N_2 and 50% CO_2/ N_2) and vacuum, but more slowly in MA-packaged samples.

pH

The effect of the analyzed additives on pH values in vacuum packaged turkey meatballs is presented in Table 2. In C and WR samples, pH values decreased with prolonged storage time. The decrease in pH values during storage could be caused by an increase in the quantity of free fatty acids as a result of hydrolytic changes. The pH of MIX samples showed small, but significant changes. At the final stage of storage (25-30 days), pH values were significantly lower (6.54) than after 5 days of storage (6.67), and in the remaining periods of storage pH values were similar (6.57 - 6.65). There was a significant additive x time interaction for pH values (P<0.001). At the final stage of storage (25-30 d), SE and MIX samples had higher pH values than WR and C samples.

In MA packaged samples, pH values were affected only by storage time (Table 2). The pH of samples with additives (SE, WR, MIX) was in the range of 6.42 - 6.54, and no significant differences were observed during storage. The pH values of samples without additives

increased significantly during the initial phase of storage, i.e. after 5 days (from 6.41 to 6.58), and during further storage they remained at a similar level (6.50 - 6.55). ROJAS and BREWER (2007) demonstrated that the pH values of cooked beef and pork patties during refrigerated storage were not affected by the antioxidants (oleoresin rosemary extract, grape seeds and water-soluble oregano extract). PEXARA *et al.* (2002) reported that the pH values of cooked sliced turkey fillets decreased during storage at 4°C, but to a lesser degree in VP than in MAP samples.

Water activity

Both the additives and storage time affected (P<0.05) water activity in vacuum-packaged turkey meatballs (data not shown). During 25 days of storage, water activity in control samples did not change significantly (0.954 - 0.964) and after 30 days of storage it was significantly higher (0.966) than after 10 days (0.954) and similar to the initial value (0 days- 0.962). Water activity in WR samples did not change significantly (0.956-0.969) and in MIX samples it decreased over the first 15 days of storage (from 0.973 to 0.951), after 20 days it increased significantly to 0.966, and during subsequent storage it remained at the same level (0.965 - 0.967). The most profound, but ambiguous changes were observed in water activity in SE samples (0.952 - 0.972). Significant differences in water activity between samples were observed only initially (0 days) and after 5 and 25 days of storage. Samples with additives showed higher water activity than control samples. In the experiment with MA stored samples, water activity was affected (P<0.05) only by storage time (data not shown) and it did not change significantly in samples with additives (0.964 - 0.978). Water activity in control samples decreased significantly after 5 days of storage (from 0.978 to 0.965) and during further storage it remained at the same level (0.965 - 0.967). Slighter changes in water activity were observed in MA-packaged turkey meatballs compared with vacuum-packaged samples. RUBIO *et al.* (2006) also found that water activity decreased during storage of sliced cured beef packaged under vacuum, but it did not vary in gas-packaged (20% CO_2/ 80% N_2) samples.

Color evaluation and the correlation between color and rancidity

Table 3 shows mean L*, a*, and b* values during storage of turkey meatballs packaged in vacuum and modified atmosphere. During vacuum storage, surface L* values (lightness) increased on day 30 in SE and MIX samples and on day 20 in WR samples, and they did not change in control samples. During MA storage, L* values did not change significantly in C samples and increased on day 10 and 20 in MIX and SE samples, respectively. Lightness (L*) values in WR samples increased significantly after 20 days of storage, and then they decreased to a similar level as immediately after cooking (0 days). The effect of the tested additives on the color lightness (L*) of cooked turkey meatballs was noted only in vacuum-packaged samples after 20 days of storage, and samples with water-soluble rosemary extract were characterized by the highest L* values. ANTON *et al.* (1993) reported that the increase in L* values could be related to the increase in metmyoglobin formation. Some Authors reported that color lightness in meat and meat products depends on several factors such as water-holding capacity (FERNÁNDEZ-LÓPEZ *et al.*, 2000) fat content (FISHER *et al.*, 2000), and free water content (JUDGE *et al.*, 1989).

Redness (a*) values were affected (P<0.001) by the additives in turkey meatballs packaged by both methods and by storage time only in MA packaged samples (table 3). During MA storage, the redness of control samples decreased gradually. In SE and MIX samples, a* values decreased significantly only after 10 days of storage. Initially (0 days) samples with water-soluble rosemary extract were characterized by the lowest redness and the value of this color component did not change significantly during storage. There was a significant interaction between the additive and storage time for a* values in both experiments. During vacuum storage, water-soluble rosemary extract applied alone and in combination with sodium erythorbate caused a decrease in the redness of turkey meatballs At the end of MA storage samples with additives showed higher values of redness than control samples. In the present study water-soluble rosemary extract protected turkey meatballs against redness fading during MAP storage. These results correspond with those reported by YU *et al.* (2002) who found that cooked turkey products containing water-soluble rosemary extract had higher a* values than of the control during refrigerated storage. A preserving effect of rosemary extract on red meat color has been demonstrated by other Authors (AKSU *et al.*, 2005; MANCINI *et al.*, 2005; SEYDIM *et al.*, 2006).

Storage time affected b* values (yellowness, P<0.05) only in VP samples (Table 3). In SE samples, yellowness decreased only during vacuum storage and this decrease was significant at the final stage of storage. The effect (P<0.01) of the tested additives was observed only in turkey meatballs stored under modified atmosphere. Samples with additives showed higher b* values than control samples, but a significant differences was found only after 10 days of storage. This difference may be explained by the effectiveness of the antioxidants added to turkey meatballs.

Microbial analysis

The results of a microbial analysis are presented in Table 4. In raw samples, total mesophilic bacteria counts in Experiment 1 (turkey meat-

Table 3 - Effect of additives, storage time and packaging system on the colorimeter values of turkey meatballs (mean±SD, n=9).

Specification	Samples	Storage time [days]				Effect	p values
		0	10	20	30		
Vacuum packaging							
Lightness (L*)	C	60.39±1.83	61.76±2.15	60.39±1.08^{A}	60.55±0.96	Additives (A)	<0.05
	SE	60.94±1.78^{ab}	59.92±2.54^{a}	59.53±2.14^{aA}	62.06±1.52^{b}	Storage time (T)	<0.05
	WR	59.68±1.94^{a}	60.88±1.30^{ab}	62.33±1.18^{bB}	61.47±1.65^{ab}	AxT	<0.01
	MIX	60.36±1.13^{ab}	59.62±2.05^{a}	59.87±1.00^{aA}	61.51±1.15^{b}		
Redness (a*)	C	2.98±0.43	2.84±0.30^{AB}	3.01±0.21^{BC}	3.13±0.27^{B}	Additives (A)	<0.001
	SE	2.88±0.55	3.09±0.34^{B}	3.10±0.40^{C}	2.64±0.32^{AB}	Storage time (T)	NS
	WR	2.60±0.86	2.77±0.35^{AB}	2.06±0.16^{A}	2.64±0.59^{AB}	AxT	<0.01
	MIX	2.78±0.45	2.52±0.30^{A}	2.73±0.31^{B}	2.48±0.43^{A}		
Yellowness (b*)	C	21.44±1.04	21.96±1.45	21.41±1.58	20.89±0.80	Additives (A)	NS
	SE	21.44±1.43^{ab}	22.31±1.12^{b}	22.02±1.33^{b}	20.74±1.25^{a}	Storage time (T)	<0.05
	WR	21.69±1.85	22.22±0.93	21.43±0.87	21.36±0.84	AxT	NS
	MIX	21.61±1.30	21.65±1.69	21.97±1.80	21.15±1.49		
Modified atmosphere packaging (20% CO_2/80% N_2)							
Lightness (L*)	C	59.22±0.94	60.35±1.51	59.04±2.44	60.72±2.10	Additives (A)	NS
	SE	58.53±2.06^{a}	60.32±1.73^{ab}	61.40±1.99^{b}	60.54±2.11^{ab}	Storage time (T)	<0.01
	WR	59.34±1.24^{a}	59.22±1.55^{a}	61.87±1.47^{b}	59.35±2.33^{a}	AxT	<0.05
	MIX	57.79±2.01^{a}	60.72±1.90^{b}	59.21±3.03^{ab}	59.83±0.83^{ab}		
Redness (a*)	C	3.87±0.35^{cAB}	3.28±0.34^{b}	3.31±0.28^{b}	2.68±0.31^{aA}	Additives (A)	<0.001
	SE	3.91±0.26^{bB}	3.28±0.48^{a}	3.39±0.39^{a}	3.57±0.29^{abB}	Storage time (T)	<0.001
	WR	3.68±0.44^{A}	3.30±0.44	3.35±0.20	3.15±0.78^{B}	AxT	<0.001
	MIX	3.77±0.50^{bAB}	3.10±0.34^{a}	3.01±0.34^{a}	3.39±0.26^{abB}		
Yellowness (b*)	C	22.15±1.37	21.89±0.64^{A}	22.14±0.81	22.48±1.30	Additives (A)	<0.01
	SE	22.52±1.20	22.85±1.08^{AB}	22.47±1.07	23.07±0.85	Storage time (T)	NS
	WR	22.43±1.41	23.33±1.06^{B}	23.34±1.26	23.69±1.94	AxT	NS
	MIX		22.65±1.88	23.24±2.02^{B}	22.55±1.74	23.72±1.13	

Samples: C- control, SE-with sodium erythorbate; WR-with water-soluble rosemary extract, MIX-with mixture of sodium erythorbate and water-soluble rosemary extract; NS – not significant (p>0.05); $^{a, b, c}$ mean values within the row followed by the different letters are significantly different at P<0.05; $^{A, B, C}$ mean values within the column followed by the different letters are significantly different at P<0.05

balls destined for VP) ranged from 5.5 x 10^3 to 1.1 x 10^5, and their counts were similar (around 10^4) in all samples in Experiment 2 (turkey meatballs destined for MAP). Thermal processing caused a reduction in the counts of mesophilic bacteria in all samples. After 15 days of vacuum storage, the counts of mesophilic bacteria did not change significantly only in WR samples (50 cfu/g) and they increased significantly in C (10^4), SE (10^4) and MIX samples (10^2). After 30 days of storage, the counts of mesophilic bacteria increased to 10^5 in samples with additives, and they remained at the same level as after 15 days of storage in control samples. During MA storage, the counts of mesophilic bacteria increased steadily in C and SE samples, from 10^2 to 10^5 cfu/g and from <100 to 10^5 cfu/g, respectively. The counts of mesophilic microorganisms in WR samples did not change after 15 days of storage and they increased to 10^5 cfu/g after 30 days. In MIX samples, the population size of mesophilic bacteria reached 60 cfu/g after 15 days and it increased to 10^2 cfu/g after 30 days of MA storage.

According to NTZIMANI *et al.* (2010), the limit of acceptability for poultry product is 10^7 cfu/g mesophilic microorganisms. The present results show that turkey meatballs stored under vacuum and modified atmosphere for 30 days were of an acceptable quality. The lowest counts of mesophilic microorganisms (10^2cfu/g) were noted in MIX samples stored under modified atmosphere. In control turkey meatballs, the counts of the above bacteria were lower in samples stored under vacuum than in those stored under modified atmosphere. Water-soluble rosemary extract had an inhibitory effect on mesophilic bacteria for 15 days of vacuum and MA storage, while a mixture of water-soluble rosemary extract and sodium erythorbate exerted an inhibitory effect for 15 days of MA storage.

The counts of psychrotrophic bacteria in raw turkey meatballs were 10^4 cfu/g in control samples and 10^3 cfu/g in samples with additives. Thermal processing reduced the counts of the above bacteria to below 10 cfu/g in samples assigned to VP and to 10^2 cfu/g in samples assigned to MAP. During vacuum and MA stor-

Table 4 - Effect of thermal processing and cold storage on the microbiological load [cfu/g] of turkey meatballs with different additives, vacuum and modified atmosphere packaging (n=3).

Microorganism	Vacuum packaging					Modified atmosphere packaging (20% CO_2/80% N_2)			
	Type of samples	Raw	After thermal processing	After 15 days of storage	After 30 days of storage	Raw	After thermal processing	After 15 days of storage	After 30 days of storage
Total mesophilic	Control	$1.1x10^5$	60	$1.1x10^4$	$2.9x10^4$	$5.0x10^4$	$2.1x10^2$	$6.6x10^3$	$2.2x10^5$
	SE	$4.0x10^4$	90	$1.7x10^4$	$2.3x10^5$	$3.0x10^4$	<100	$2.2x10^4$	$1.0x10^5$
	WR	$4.0x10^4$	20	50	$1.2x10^5$	$3.0x10^4$	$6.0x10^2$	$2.0x10^2$	$5.2x10^5$
	MIX	$5.5x10^3$	130	$9.2x10^2$	$7.0x10^5$	$3.0x10^4$	<100	60	$3.3x10^2$
Total psychrotrophic	Control	$3.0x10^4$	<10	$9.4x10^3$	$9.4x10^4$	$1.0x10^4$	$2.0x10^2$	$7.0x10^4$	$1.8x10^4$
	SE	$4.8x10^3$	<10	$1.8x10^3$	$1.8x10^3$	$9.6x10^3$	$1.0x10^2$	$2.1x10^2$	$4.0x10^3$
	WR	$9.1x10^3$	<10	20	$3.5x10^2$	$6.9x10^3$	$2.0x10^2$	$4.9x10^2$	$8.0x10^2$
	MIX	$3.8x10^3$	<10	50	$8.4x10^2$	$1.0x10^3$	$2.2x10^2$	90	$7.5x10^2$
Fungi	Control	700	<10	<10	<10	50	<10	<10	<10
	SE	500	<10	<10	<10	90	<10	<10	<10
	WR	400	<10	<10	<10	140	<10	<10	<10
	MIX	100	<10	<10	<10	30	<10	<10	<10
Coliforms	Control	200	<10	$1.2x10^4$	$5.8x10^4$	10	<10	<10	<10
	SE	60	<10	90	10	<10	<10	<10	<10
	WR	90	<10	<10	<10	<10	<10	<10	<10
	MIX	80	<10	<10	<10	17	<10	<10	<10
Presence of sulphate (IV)-reducing *Clostridium* sp.	Control	ND	ND	ND	ND	ND	ND	ND	ND
	SE	ND	ND	ND	ND	ND	ND	ND	ND
	WR	ND	ND	ND	ND	ND	ND	ND	ND
	MIX	ND	ND	ND	ND	ND	ND	ND	ND

[ND]- none detected; Samples: SE-with sodium erythorbate; WR-with rosemary water extract, MIX-with mixture of sodium erythorbate and rosemary water extract.

age, the levels of psychrotrophic bacteria increased in C and SE samples. The counts of psychrotrophic bacteria in WR samples remained at the same level during MA storage. After 15 days of vacuum storage. They reached 20 cfu/g and increased to 10^2 cfu/g after 30 days. In MIX samples, the numbers of psychrotrophic bacteria did not change after 15 days of vacuum storage and they decreased over the same period during MA storage to reach the final count of 10^2 cfu/g on day 30. The tested additives inhibited the growth of psychrotrophic bacteria during manufacturing and vacuum and MA storage. Water-soluble rosemary extract and its mixture with sodium erythorbate were more effective in this respect than sodium erythorbate applied alone.

Fungal contamination of raw turkey meatballs reached 100-700 cfu/g and 30-140 cfu/g in samples assigned to VP and MAP, respectively. Thermal processing decreased fungal counts to below 10 cfu/g and no fungal growth was noted in VP and MAP samples over refrigerated storage.

The total counts of coliforms in raw turkey meatballs were 60 - 200 cfu/g in samples assigned to VP and <10 - 17 cfu/g in samples assigned to MAP. It is possible that the bacteria were transmitted during the preparation of meatballs. They were completely destroyed during thermal processing. All additives inhibited the growth of coliforms during vacuum storage, while in control samples their counts increased to 10^4 cfu/g after 30 days. Turkey meatballs were free of sulfate reducing *Clostridium sp.*

To the author's best knowledge, there are no reports addressing the combined use of rosemary extract and sodium erythorbate in the preservation of meat products. An inhibitory effect on microbial growth has been observed in some meat products following the application of rosemary (DEL CAMPO *et al.*, 2000; DJENANE *et al.*, 2002,

2003; CAMPO *et al.,* 2003; FERNÁNDEZ–LÓPEZ *et al.,* 2005; ZHANG *et al.*, 2009) and sodium erythorbate (BARRINGER *et al.*, 2005; KARPIŃSKA-TYMOSZCZYK, 2006).

Sensory quality

After thermal processing all samples destined for vacuum packaging were characterized by similar intensity of meaty (3.8-4.0), typical of poultry meat (4.0-4.5), fatty (1.0) and typical of roasted meat flavors (3.5). During storage only in control samples significant decreased intensity of meaty (to 2.7), typical of roasted meat (to 2.0) and aromatic flavors (to 2.0). The typical of poultry meat flavor significantly decreased only in WR samples at the end of storage (to 3.0). Samples differed in scores for meaty flavor only on day 30, and for the typical of roasted meat flavor - on day 20, 25 and 30. The intensity of the above flavors was higher in samples with additives (2.5-3.0) than in control samples (2.0). Initially (storage time 0) all samples stored under modified atmosphere were characterized by similar intensity of meaty (3.7-4.0), typical of poultry meat (4.0-4.5), fatty (1.0) and typical of roasted meat flavors (3.5) and their intensity did not change significantly during storage.

Turkey meatballs with additives i.e. with water-soluble rosemary extract applied alone or in combination with sodium erythorbate, were characterized by higher intensity of the aromatic flavor (2.5-4.0 and 3.0 - 3.5 respectively in VP and 3.5-4.0 and 3.0 - 3.5 respectively in MAP) than control turkey meatballs (2.0-3.0 in VP and 2.5 - 3.0 in MAP) and samples with sodium erythorbate (2.8-3.0 in VP 2.5-3.0 in MAP). WR and MIX samples had a rosemary flavor of low intensity (1.7-2.5 and 1.0-1.5 respectively in VP and 1.7-2.5 and 1.0-1.5 respectively in MAP). WOF appeared during storage in all vacuum-packaged samples, after 15 days in C (1.0) and WR samples (0.5), after 20 days in SE samples (0.5) and at the end of storage in MIX samples (1.0). WOF intensity increased during subsequent storage, and after 30 days it was slightly noticeably only in control samples. WOF was detected at a later stage of storage under modified atmosphere in samples with additives (where it had lower intensity) and in control samples. Water-soluble rosemary extract and a mixture of sodium erythorbate and water-soluble rosemary extract were effective in slowing down WOF development in turkey meatballs during vacuum and MA storage.

Rosemary extract used as an antioxidant contributed to the maintenance of the sensory quality of turkey meatballs, during both VP and MAP storage. The effect of rosemary was determined by the type of packaging. Samples stored under modified atmosphere had better sensory quality than those stored under vacuum. This property of rosemary extract has been reported in other food products. The addition of rosemary in the formulation of turkey sausages also gave rise to an improvement of their sensory quality (BARBUT *et al.*, 1985). According to SÁNCHEZ–ESCALATE *et al.* (2001), beef patties with rosemary extract kept the characteristic fresh meat odor for a longer time than control samples. LIU *et al.* (2009) demonstrated that the addition of rosemary seemed not only to enhance the aroma of fresh chicken sausages, but also to retard the off-odor produced by fat oxidation.

The relationship between changes in lipids and sensory quality

In MAP samples, MDA content significantly affected WOF (r = 0.41). Similar results were obtained by others Authors (NOLAN *et al.*, 1989, AHN *et al.*, 2002, GIMÉNEZ *et al.*, 2004, MARTINEZ *et al.,* 2006). In vacuum-packaged samples, acid value was positively correlated with WOF (r = 0.52) and negatively with meaty flavor (r = -0.59), typical of poultry meat flavor (r = -0.63) and typical of roasted meat flavor (r = -0.57). The acid value of MAP samples was negatively correlated with aromatic flavor (r = -0.40) and typical of roasted meat flavor (r = -0.41). In samples packaged by both methods (VP and MAP), spicy flavor was positively correlated with aromatic flavor (r = 0.73 and r = 0.94, respectively) and negatively with meaty flavor only in MAP samples (r = -0.45). In VP samples, WOF was negatively correlated with meaty (r = -0.70), typical of poultry meat (r = -0.75), aromatic (r = -0.63) and typical of roasted meat (r = -0.87) flavors, while in MAP samples a negative correlation was observed between WOF and meaty flavor (r = -0.39).

CONCLUSION

The results of this study indicate that the addition of water-soluble rosemary extract (at a level found acceptable in a sensory analysis) to turkey meatballs retarded the formation of TBARS, but it was less effective than sodium erythorbate and offered protection against redness fading during MAP storage. Sodium erythorbate delayed the hydrolytic process in vacuum-packaged turkey meatballs. In MAP samples, a mixture of water-soluble rosemary extract and sodium erythorbate exerted a weaker antioxidant effect and a stronger antimicrobial effect, compared with the additives used alone. Turkey meatballs with water-soluble rosemary extract were characterized by the best sensory quality, in particular when stored under modified atmosphere. Further research could investigate the combined application of rosemary and sodium erythorbate in different meat products as well as the use of different quantities of the additives than those used in this study, with the aim to optimize their antioxidant and antimicrobial effects.

ACKNOWLEDGMENTS

The Author is grateful to Ania Fankidejska and Mariusz Szczepański for their technical assistance.

REFERENCES

Ahn J., Grün I.U. and Fernando L.N. 2002. Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. J. Food Sci. 67: 1364-1369.

Aksu M.İ. and Kaya M. 2005. The effect of α-tocopherol and butylated hydroxyanisole on the colour properties and lipid oxidation of kavurma, a cooked meat product. Meat Sci. 71: 277-283.

Animal and vegetable fats and oils. Determination of acid value and acidity. PN-EN ISO 660. 2005.

Antolovich M. Prenzler P.D., Patsalides E., McDonald S. and Robards K. 2002. Methods for testing antioxidant activity. Analyst, 127: 183-198.

Anton M., Salgues C., Gatellier P. and Renerre M. 1993. Ètude des relations oxidatives entre les lipids membranaires et la myoglobine in vitro. Sci. Aliment. 13: 261-274.

Balev D., Vulkova T., Dragoev S., Zlatanov M. and Bahtchevanska S. 2005. A comparative study on the effect of some antioxidants on the lipid and pigment oxidation in dry-fermented sausages. Int. J. Food Sci. Technol. 40: 977-983.

Barbut S., Josephson D.B. and Maurer A.J. 1985. Antioxidant properties of rosemary oleoresin in turkey sausage. J. Food Sci. 50: 1356-1363.

Barringer S.A., Abu-Ali J. and Chung H.-J. 2005. Electrostatic powder coating of sodium erythorbate and GDL to improve color and decrease microbial counts on meat. Innov. Food Sci. and Emerg. Technol. 6: 189-193.

Bragagnolo N., Danielsen B. and Skibsted L.H. 2005. Effect of rosemary on lipid oxidation in pressure-processed, minced chicken breast during refrigerated storage and subsequent heat treatment. Eur. Food Res. Technol. 223: 610-615.

Bragagnolo N., Danielsen B. and Skibsted L.H. 2007. Rosemary as antioxidant in pressure processed chicken during subsequent cooking as evaluated by electron spin resonance spectroscopy. Innov. Food Sci. Emerg. Technol. 8: 24-29.

Burt S. 2004. Essential oils: their antibacterial properties and potential application in foods. A review. Int. J. Food Microbiol. 94: 223-253.

Campo J.D., Nguyen-the C., Sergent M. and Amiot M.J. 2003. Determination of the most bioactive phenolic compounds from rosemary against *Listeria monocytogenes*: Influence of concentration, pH and NaCl. J. Food Sci. 68: 2066-2071.

Cuvelier M.E., Richard H. and Berset C. 1996. Antioxidative activity and phenolic composition of pilot plant and commercial extracts of sage and rosemary. J. Am. Oil Chem. Soc. 73: 645-652.

Decker E.A. and Xu Z.M. 1998. Minimizing rancidity in muscle foods. Food Technol. 52(10): 54-59.

Del Campo J., Amiot M.J. and Nguyen-The C. 2000. Antimicrobial effect of rosemary extract. J. Food Prot. 63: 1359-1368.

Djenane D., Sánchez-Escalante A., Beltrán J.A. and Roncalés P. 2002. Ability of α-tocopherol, taurine and rosemary, in combination with vitamin C, to increase the oxidative stability of beef steaks packaged in modified atmosphere. Food Chem. 76: 407-415.

Djenane D., Sánchez-Escalante A., Beltrán J.A. and Roncalés P. 2003. Extension of the shelf life of beef steaks packaged in a modified atmosphere by treatment with rosemary and displayed under UV-free lighting. Meat Sci. 64: 417-426.

Ferández-Fernández E., Vázquez-Odériz M.L. and Romero-Rodríguez M.A. 2002. Sensory characteristics of Galician chorizo sausage packed under vacuum and under modified atmospheres. Meat Sci., 62: 67-71.

Fernández -López J., Zhi N., Pérez-Alvarez J.A. and Aranda-Catalá V. 2000. Effect of mincing degree on colour properties in pork meat. Color Res. Appl., 25: 376-380.

Fernández -López J., Zhi N., Aleson-Carbonell L., Pérez-Alvarez J.A. and Kuri V. 2005. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. Meat Sci. 69: 371-380.

Fisher P., Hoffman L.C. and Mellet F.D. 2000. Processing and nutritional characteristics of value added ostrich products. Meat Sci. 55: 251-254.

Georgantelis D., Ambrosiadis I., Katikou P., Blekas G. and Georgakis S.A. 2007. Effect of rosemary extract, chitosan and α-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4°C. Meat Sci. 76: 172-181.

Giménez B., Roncalés P. and Beltrán J.A. 2004. The effect of natural antioxidants and lighting conditions on the quality characteristics of gilt-head sea bream fillets (*Sparus aurata*) packaged in a modified atmosphere. J. Sci. Food Agric. 84: 1053-1060.

Hernández-Hernández E., Ponce-Alquicira E., Jaramillo-Flores M.E. and Guerrrero Legareta I. 2009 Antioxidant effect of rosemary (*Rosmarinus officinalis* L.) and oregano (*Origanum vulgare* L.) extracts on TBARS and colour of model raw pork batters. Meat Sci. 81: 410-417.

Huang S., Frankel E.N., Schwarz K., Aeschbach R. and German J.B. 1996. Antioxidant activity of carnosic acid and methyl carnosate in bulk oil and oil- in- water emulsion. J. Agric. Food Chem. 44: 951-956.

Identification and selection of descriptors for establishing a sensory profile by a multidimensional approach. PN-ISO 11035. 1999.

Judge M., Aberele E., Forrest J., Hedrich H.Y. and Merkel R. 1989. Principles of Meat Science. Pp. 135-175. Dubuque: Kendall/Huny Publishing Co.

Karpińska-Tymoszczyk M. 2006. Effects of the addition of sage and sodium isoascorbate on the quality and shelf life of poultry meatballs during cold storage. Pol. J. Natur. Sc. 21(2): 1049-1063.

Liu D.-CH., Tsau R.-T., Lin Y.-CH., Jan S.-S. and Tan F.-J. 2009. Effect of various levels of rosemary or Chinese mahogany on the quality of fresh chicken sausage during refrigerated storage. Food Chem. 117: 106-113.

Lucera A., Mastromatteo M., Sinigaglia M. and Corbo M.R. 2009. Combined effects of thymol, carvacrol and grapefruit seed extract on lipid oxidation and colour stability of poultry meat preparations. Int. J. Food Sci. Technol. 44: 2256-2267.

Madsen H.L. and Bertelsen G. 1995. Spices as antioxidants. Trends Food Sci. Tech., 6: 271277.

Mancini R.A., Hunt M.C., Hachmeister K.A., Seyfert M.A., Kropf D.H., Johnson D.E., Cusik S. and Morrow C. 2005. The utility of lactate and *rosemary* in beef enhancement solutions: Effect on Londissimus color changes during display. J. Muscle Foods, 16: 27-36.

Martínez L., Cilla I., Beltrán J. and Roncalés P. 2006. Antioxidant effect of rosemary, borage, green tea, pu-erth tea and ascorbic acid on fresh pork sausages packed in a modified atmosphere: Influence of the presence of sodium chloride. J. Sci. Food Agric. 86: 1298-1307.

Meilgaard M., Civille G.V. and Carr B.T. 1999. Descriptive analysis techniques. In *Sensory evaluation techniques*. 3nd Ed., pp. 161-172, (New York, CRS Press).

Nassu R.T., Gonçalves L., A.G., Silva M.A.A.P. and Beserra F.J. 2003. Oxidative stability of fermented goat meat sausage with different levels of natural antioxidant. Meat Sci. 63: 43-49.

Nolan N.L., Bowers J.A. and Kropf D.H. 1989. Lipid oxidation and sensory analysis of cooked pork and turkey stored under modified atmospheres. J. Food Sci. 54: 846-849.

Ntzimani A.G., Paleologos E.K., Savvaidis I.N. and Kontominas M.G. 2008. Formation of biogenic amines and relation to microbial flora and sensory changes in smoked turkey breast fillets stored under various packaging conditions at 4°C. Food Microbiol. 25: 509-517.

Ntzimani A.G., Giatrakou V.I. and Savvaidis I.N. 2010. Combined natural antimicrobial treatments (EDTA, lysozyme, rosemary and oregano oil) on semi cooked coated chicken meat stored in vacuum packages at 4°C: Microbiological and sensory evaluation. Innov. Food Sci. Emerg., Technol. 11: 187-196.

Patsias A., Chouliara I., Badeka A., Sacvvaidis I.N. and Kontominas M.G. 2006. Shelf-life of a chilled precooked chicken products stored in air and under modified atmospheres: microbial, chemical, sensory attributes. Meat Sci. 23: 423-429.

Pexara E.S., Metaxopoulos J. and Drosinos E.H. 2002. Evaluation of shelf life of cured, cooked, sliced turkey fillets and cooked pork sausages – "piroski" – stored under vacuum and modified atmospheres at +4° and +10°C. Meat Sci. 62: 33-43.

Pikul J., Dennise E., LeszczyńskI E. and Kummerow F.A. 1989. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. J. Agric. Food Chem. 37: 1309-1313.

Rojas M.C. and Brewer M.S. 2007. Effect of natural antioxidants on oxidative stability of cooked refrigerated beef and pork. J. Food Sci. 72: S282-S288.

Rubio B., Martínez B., González-Fernández C., García-Cachán M.D., Rovira J. and Jaime I. 2006. Influence of storage period and packaging method of sliced dry-cured beef "Cecina de Leon": Effects on microbiological, physicochemical and sensory quality. Meat Sci., 74: 710-717.

Sánchez-Escalante A., Djenane D., Torrescano G., Beltrán J.A. and Roncalés P. 2001. The effects of ascorbic acid, taurine, carnosine and rosemary powder on colour and lipid stability of beef patties packaged in modified atmosphere. Meat Sci. 58: 421-429.

Schaefer D.M., Liu Q., Faustman C. and Yin M.C. 1995. Supranutritional administration of vitamin E and C improves oxidative stability of beef. Journal of Nutrition 125: 1792S-1798S.

Seydim A.C., Guzel-Seydim Z.B., Acton J.C. and Dawson P.L. 2006. Effects of rosemary extract and sodium lactate on quality of vacuum-packaged ground ostrich meat. J. Food Sci., 71: S71-S76.

Sheard P.R., Enser M., Wood J.D., Nute G.R., Gill B.P. and Richardson R.I. 2000. Shelf life and quality of pork and pork products with raised n-3 PUFA. Meat Sci. 55: 213-221.

Smiddy M., Papkovskaia N., Papkovsky D.B. and Kerry J.P. 2002. Use of oxygen sensors for the non-destructive measurement of the oxygen content in modified atmosphere and vacuum packs of cooked chicken patties; impact of oxygen content on lipid oxidation. Food Res. Intern. 35: 577-584.

StatSoft Inc. 2008. Data analysis software system. version 8.

Suhaj M. 2006. Spice antioxidants isolation and their antiradical activity: a review. J. Food Compost. Anal. 19: 531-537

Tarladgis B.G., Watts B.M. and Younathan M.T. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37: 44-48.

Ulu H. 2004. Evaluation of three 2-thiobarbituric acid methods for the measurement of lipid oxidation in various meats and meat products. Meat Sci., 67: 683-687

Wilkinson A.L., Sun Q., Senecal A. and Faustman C. 2001. Antioxidant effects on TBARS and fluorescence measurements in freeze-dried meats. J. Food Sci. 66: 20-24.

Yu L., Scanlin L., Wilson J. and Schmidt G. 2002. Rosemary extracts as inhibitors of lipid oxidation and color change in cooked turkey products during refrigerated storage. J. Food Sci. 67: 582-585.

Zhang H., Kong B., Xiong Y.L. and Sun X. 2009. Anti microbial activities of spice extracts against pathogenic and spoilage bacteria in modified atmosphere packed fresh pork and vacuum packed ham slices stored at 4°C. Meat Sci. 81: 685-692.

Paper received June 29, 2010 Accepted March 3, 2011

PAPER

REDUCING FAT UPTAKE DURING DEEP-FRYING OF MINCED CHICKEN MEAT-BALLS BY COATING THEM WITH DIFFERENT MATERIALS, EITHER ALONE OR IN COMBINATION

BASEM M. AL-ABDULLAH[b]*, MALAK M. ANGOR[a], KHALID M. AL-ISMAIL[b] and RADWAN Y. AJO[a]

[a]Al-Huson University College, Al-Balqa Applied University, Al-Huson, Jordan

[b]Department of Nutrition and Food Technology, Faculty of Agriculture, University of Jordan, Amman, Jordan

*Corresponding author: basem@ju.edu.jo

ABSTRACT

The main aim of this study was to investigate the influence of different coating materials, either alone or in combination, on fat uptake that occurred during deep-frying of minced chicken meat-balls. The data obtained showed that coatings made from whey protein isolate, carrageenan or starch all reduced the relative variation in fat-uptake and gave better results in this respect than a coating of breadcrumbs or a non-coated control. There was no effect of any coating material, or combination of materials, on the texture of the product or its density. All samples were found to be acceptable by a trained sensory panel, although there were small differences in sensory scores between some of the coating treatments that were statistically significant ($P \geq 0.05$).

- Key words: coating materials, fat uptake, minced chicken meat-balls, deep-fat frying -

INTRODUCTION

Deep-fat frying is a widely used method of cooking in commercial food processing. For the benefit of consumers, however, attention needs to be given to the resultant fat content of the product and its sensory acceptability. Foods of this kind should be crisp, with a smooth mouthfeel, while being palatable and having an acceptable colour. On the other hand, re-use of oil employed in deep-frying eventually leads to its deterioration and the changes that occur affect the quality of both the oil itself and the fried food. Whatever the quality of the oil, the cooking process will increase the fat content of the food and some fried foods may contain up to 50% fat. For example, the lipid content of French fries increases from 0.2 to 14% and may reach 40% in fried potato chips (PINTHUS *et al.,* 1993; AKDENZ *et al.,* 2006). Thus, fried foods have become a health concern and high consumption of lipids has been associated with obesity and other conditions, such as coronary heart disease. Furthermore, the frying process generates various lipid-oxidation products, some of which have been linked to premature aging, membrane damage, heart disease and cancer (ADDIS and WARNER, 1991).

Consumer awareness of the health implications of eating foods with a high fat content is on the increase (NGADI *et al.,* 2006). Therefore, there is much interest in reducing fat uptake during deep-fat frying. Several approaches have been proposed for reducing fat absorption and these include physical and chemical pre-treatments, such as pre-drying and pre-cooking the product, optimising processing conditions and modifying the product surface. Selection of an appropriate food coating is one possible means of solving this problem. These coatings can act as barriers to moisture loss, which is important commercially, and also reduce fat uptake during frying (SALVADOR *et al.,* 2008). The effectiveness of a coating material is determined by its mechanical and barrier properties, which depend on its composition and microstructure, and by the characteristics of the food product to which it binds (GARCÍA *et al.,* 2002; ADEDEJI *et al.,* 2009).

Over the past ten years, in particular, the American Heart Association and other public health organisations have recommended a reduction in the fat content of foods, so that fat accounts for less than 30% of the total calorie intake for most people. Saturated and trans fats are considered the most harmful (ALLAN, 2004). However, reductions in the oil content of fried foods are motivated by other factors as well. Oil is an expensive raw material and is an important determinant of product cost. Also, a high oil content often makes fried foods too oily or greasy (TRAN *et al.,* 2007).

Because the surface properties of a fried food have a significant effect on fat absorption, the application of a suitable coating is a promising way of reducing the subsequent oil content (NGADI *et al.,* 2006). The process of deep-fat frying involves the transfer of heat from the surrounding oil to the interior of the food, while also transferring oil and allowing water vapour to pass from the food to the oil (SALVADOR *et al.,* 2008). In practice, the barrier properties of the coating are important in controlling both oil uptake and moisture loss.

Incorporation of hydrocolloids in the batter used as a coating was found to reduce oil uptake, especially those hydrocolloids that formed methyl cellulose of hydroxypropyl methyl cellulose on heating (SALVADOR *et al.,* 2008). Of the two kinds of coating considered, those forming methyl cellulose were shown to be the more effective. Other hydrocolloids that reduced oil absorption during frying were gellan gum, carrageenan, karaya gum and guar gum, as demonstrated with a model deep-friend product made of chickpea flour (GARCÍA *et al.,* 2002).

Various coating materials have been used to reduce both fat uptake and moisture loss in different fried foods. These have included alginate, powdered cellulose, methyl cellulose, soy protein isolate and whey protein (MALLIKARJUNAN *et al.*, 1997). However, there is little published information on the effectiveness of combinations of such materials. Therefore, the aims of this study were to investigate the use of different coating materials (starch, carrageenan, whey protein and fine breadcrumb), both alone and in combination, for their ability to reduce fat uptake and moisture retention in minced chicken meat-balls. Effects of the coating treatments on texture, density and sensory properties of the product were also determined.

MATERIALS AND METHODS

Preparation of minced chicken meat-balls

Fresh chicken breast was obtained from a local market and the meat-balls were prepared at a nearby meat-processing factory. The breast portions were examined visually to avoid any showing damage or injury and the skin was removed. The meat was then ground coarsely in a grinding machine (LASKA 130, Austria), using a plate with holes of 5 mm diameter. To the ground meat were added sodium chloride (1.5%), garlic (0.28%), white pepper (0.38%) and ginger (0.38%), followed by blending for 2 min at low speed. Finally, sodium tripolyphosphate (0.5%) was added to the mixture as a binding agent and the meat-balls were formed in a commercial shaping machine (FORMAX 400, UK).

Coating materials

Solutions or suspensions of the coating materials in tap water were prepared in the laboratory and contained w/w starch (ST), 3%, whey protein isolate (WPI), 5%, carrageenan (CA), 3% or fine breadcrumbs (BC), 3%. Suitable combinations of these materials were also prepared for coating purposes, as shown in Table 1. Glycerol, 3% w/w was added to each preparation as a plasticiser. All preparations were refrigerated for 24 h before use.

Coating and frying of the meat-balls

Batches of five meat-balls were immersed in the appropriate coating preparations for 10 sec and then air-dried with a warm-air blower. The procedure was repeated to ensure a uniform coating of the product. A non-coated product was used as a control.

All samples were cooked for 4 min in an electric deep-fat fryer (Moulinex, Model SERIEF 37-R, France) containing corn oil (Golden Corn, UMIC, Jordan) at 180° ± 2°C, with the lid closed. At this point, the meat-balls had a brown, cooked colour and an internal temperature of 90°C, according to a thermocouple (Digitec, Italy) placed at the geometric centre of a representative sample. The oil was replaced after every four batches had been cooked. On completion of the cooking process, each meat-ball was wrapped in absorbent paper and held for 5 min prior to cooling and testing (SOUTHERN *et al.*, 2000). Cooling was achieved by holding the samples under ambient conditions for 1 h. A portion of each sample was taken immediately for sensory evaluation, while the remainder was weighed, placed in a plastic bag and held frozen at -18°C until required for chemical and physical analysis.

Table 1 - The different coating treatments studied*.

Treatment no.	Material g (L)			
	starch	whey protein	carrageenan	breadcrumb
1	30	-	-	-
2	-	50	-	-
3	-	-	30	-
4	-	-	-	30
5	15	25	-	-
6	15	-	15	-
7	15	-	-	15
8	-	25	15	-
9	-	25	-	15
10	-	-	15	15
11	10.5	17.5	10.5	-
12	7.5	12.5	7.5	7.5
13	-	-	-	-

*All preparations contained 30 g/L glycerol.
Treatment 13 was a non-coated control.

Sample analyses

Chemical methods

Samples were analysed for moisture and fat content according to AOAC (2000) and each analysis was carried out in duplicate.

Moisture content (MC) was determined by an oven-drying method in which minced samples (5 g) were placed in dishes and held in an oven (Memmert, 854, Schwapach, Germany) at 102°C for 18 h.

Fat content (FC) was determined by the Soxhlet method, which involved continuous vaporisation and condensation of petroleum ether during passage through a 5 g, moisture-free sample placed in the extraction thimble and covered with cotton wool. After 16 h, the extract was collected and subjected to distillation in order to collect the crude fat, which was then dried and weighed.

The data obtained for fat and moisture were used to calculate the relative variation in fat uptake (FU) and moisture retention (MR) respectively, relative to the non-coated product, as described by GARCÍA *et al.* (2002):

$$FU = \left(\frac{\text{FC coated}}{\text{FC non} - \text{coated}} - 1 \right) \text{x } 100 \qquad \text{Equation 1}$$

$$MR = \left(\frac{\text{MC coated}}{\text{MC non} - \text{coated}} - 1 \right) \text{x } 100 \qquad \text{Equation 2}$$

Physical methods

Mechanical properties of the fat film are important in relation to its retention during product handling and mouth-feel, following ingestion. The texture of the fried meat-balls was determined by a penetration test (Ateba and Mittal, 1994), using a pentrometer (Petrotest, PNR 10, Dahlewitz, Germany) and a needle plunger (SETAA5T, MIP). A reading was taken 5 sec after the sample had been penetrated (5O N load).

The volume of each sample was determined by immersion in a graduated cylinder filled with paraffin oil. Sample density was calculated by dividing the sample weight by the volume of paraffin displaced (AKDENZ *et al.*, 2006).

Sensory evaluation

Samples from each treatment were evaluated by an 11-member panel, chosen from the employees and students of the Department of Nutrition and Food Technology, Al-Balqa University. The panellists were of both sexes and different ages. Before evaluating the samples, they were familiarised with the test procedure and use of the rating system.

Each sample was evaluated for appearance,

colour, flavour, taste and overall acceptability, using a five-point hedonic scale on which 5 was "like extremely" and 1 was "dislike extremely".

Small amounts of bread and water were used between samples to neutralise any lingering taste from the previous sample.

All treatments were evaluated in duplicate on separate occasions.

Statistical analysis

Data were analysed using a computer package providing a Statistical Analysis System (SAS Inc., 1997). This involved a complete randomised design to study treatment effects on fat and moisture, FU, MU, surface penetration, product density and sensory attributes.

RESULTS AND DISCUSSION

Values for fat and moisture content of the meat-balls, both coated and non-coated, are given in Table 2. For fat, the lowest value, 14.82%, was obtained with the WPI-coated product, while the highest value, 28.60%, was associated with the non-coated control. In the case of moisture content, the situation was reversed and the non-coated control gave the lowest value (46.90%). The highest value of 59.63% was obtained from the CA-coated product.

Statistical analysis of the data revealed significant differences between the different coating treatments with respect to both moisture and fat content. For moisture, it was anticipated that the greatest loss would be found in the non-coated control and the value obtained was significantly greater ($P \leq 0.05$) than those of all other treatments. Similarly, the higher fat content of the control product was significantly different ($P \leq 0.05$) from that of all coated products, thus confirming BALASUBRAMANIAN *et al.* (1997). In practice, fat absorption occurs as moisture is drawn off from the food during the frying process (SAGUY and PINTHUS, 1995).

The effect of coating type on fat uptake is shown in Table 3. The FU values obtained were always negative, because the lipid content of the coated product was lower in all cases than that of the non-coated control (GARCÍA *et al.,* 2002). As expected, the FU value, calculated according to Equation 1, was highest for the non-coated control. The different coatings, on the other hand, showed varying effects, with CA and WPI yielding the lowest values that were significantly different ($P \leq 0.05$) from all other coatings studied. The efficacy of a whey protein film in reducing fat absorption by a fried cassava product was observed by FREITAS *et al.* (2009) and for a pre-formed potato item by ALBERT and MITTAL (2002). Of the other coatings, only ST approached the efficacy of CA and WPI in diminishing fat uptake. Values for the remaining treatments were significantly inferior ($P \leq 0.05$), although mixtures of ST, WPI and CA, and WPI and CA gave the lowest values in this group. Interestingly, combining coating ingredients for those that were most successful individually reduced rather than enhanced the reduction in FU obtained.

In the present study, no significant differences were observed between treatments involving BC (except the combination of ST + WPI + CA + BC), but the FU values were higher than those of other coatings and this may have been due to the fact that fat is a normal component of pan breadcrumbs (KULP, 1991).

With regard to MR, the carrageenan coating was associated with the highest value obtained (Table 3) and this was significantly different ($P \leq 0.05$) from the values for all other product coatings. It has been reported (MARRS, 1998) that carrageenan particles not only have a high affinity for water, but also have a structural "memory". This means that when water is added to the

Table 2 - Effect of coating treatment on fat and moisture content of the end-product.

Treatment no.	Treatment*	Fat (%)	Moisture (%)
1	ST	16.72 ^{fg}	52.03 ^{e}
2	WPI	14.82 ^{h}	54.29 ^{d}
3	CA	16.64 ^{gh}	59.63 ^{a}
4	BC	20.06 ^{bcd}	55.30 ^{cd}
5	ST + WPI	18.83 ^{bcde}	56.44 ^{bc}
6	ST + CA	18.56 ^{cdef}	55.84 ^{bcd}
7	ST + BC	20.17 ^{bc}	49.98 ^{f}
8	WPI + CA	18.28 ^{defg}	56.52 ^{bc}
9	WPI + BC	17.81 ^{efg}	54.85 ^{cd}
10	CA + BC	20.18 ^{bc}	57.20 ^{b}
11	ST + WPI + CA	20.45 ^{b}	54.65 ^{d}
12	ST + WPI + CA + BC	18.77 ^{bcde}	56.45 ^{bc}
13	control	28.60 ^{a}	46.90 ^{g}

Within the same column, values followed by a different superscript are significantly different ($P \leq 0.05$).
*ST: Starch; WPI: whey protein isolate; CA: carrageenan; BC: breadcrumb.

Table 3 - Relative variation in fat uptake and moisture retention during frying of chicken meat-balls.

Treatment no.	Treatment*	Relative variation in fat uptake (FU%) {Eq. 1}	Relative variation in moisture retention (MR%) {Eq. 2}
1	ST	-41.57 ^{e}	13.00 ^{cd}
2	WPI	-47.73 ^{f}	15.78 ^{bc}
3	CA	-41.87 ^{ef}	27.21 ^{a}
4	BC	-29.91 ^{bc}	18.01 ^{bc}
5	ST + WPI	-34.17 ^{bcd}	20.37 ^{b}
6	ST + CA	-35.14 ^{cd}	19.10 ^{bc}
7	ST + BC	-29.48 ^{bc}	6.61 ^{d}
8	WPI + CA	-36.36 ^{de}	20.51 ^{b}
9	WPI + BC	-29.70 ^{bc}	16.97 ^{bc}
10	CA + BC	-28.49 ^{b}	20.10 ^{b}
11	ST + WPI + CA	-36.90 ^{de}	16.59 ^{bc}
12	ST + WPI + CA + BC	-33.49 ^{bcd}	20.40 ^{b}
13	control	0.00 ^{a}	0.00 ^{e}

Within the same column, values followed by a different superscript are significantly different ($P \leq 0.05$).
*ST: Starch; WPI: whey protein isolate; CA: carrageenan; BC: breadcrumb.

Table 4 - Density and penetration-point values for coated and non-coated chicken meat-balls.

Treatment no.	Treatment*	Density values (g/mL)	Penetration-point values (mm/5 sec)
1	ST	0.82 ^{a}	8.56 ^{a}
2	WPI	0.85 ^{a}	8.60 ^{a}
3	CA	0.85 ^{a}	8.62 ^{a}
4	BC	0.83 ^{a}	8.65 ^{a}
5	ST + WPI	0.84 ^{a}	8.75 ^{a}
6	ST + CA	0.82 ^{a}	8.55 ^{a}
7	ST + BC	0.84 ^{a}	8.55 ^{a}
8	WPI + CA	0.85 ^{a}	8.65 ^{a}
9	WPI + BC	0.84 ^{a}	8.65 ^{a}
10	CA + BC	0.84 ^{a}	8.60 ^{a}
11	ST + WPI + CA	0.84 ^{a}	8.65 ^{a}
12	ST + WPI + CA + BC	0.82 ^{a}	8.75 ^{a}
13	control	0.85 ^{a}	8.65 ^{a}

Within the same column, values with the same superscript are not significantly different.
*ST: Starch; WPI: whey protein isolate; CA: carrageenan; BC: breadcrumb.

particle, it is absorbed into the structure, but the particle always returns to something approaching its original shape and size. Not surprisingly, carrageenan has strong water-binding properties. When the meat product has been cooked, the carrageenan remains hydrated and continues to bind water, but the meat protein forms a gel, which effectively traps the carrageenan particles. As a result, moisture loss during cooking is minimised and eating quality is improved.

The non-coated control had a significantly lower MR value ($P \leq 0.05$) than any coated product. This may be due to the high fat absorption, in accordance with the relationship between FU and moisture loss during deep-frying that was reported by TRAN *et al.* (2007). For the other coatings studied, there were no significant differences between them (Table 3).

Data given in Table 4 show the effect of coating type on the density of the product and also its texture, as measured by the penetration test. None of the coatings showed any significant differences among themselves or from the non-coated control, indicating a lack of any coating effect on these particular parameters. RAYNER *et al.* (2000) also reported no significant differences between coated and non-coated products in the penetration test, but highlighted the advantage in using coatings that did not interfere significantly with product texture. A similar lack of coating effect on the texture of fried foods has been reported by GARCÍA *et al.* (2002).

Results for the sensory evaluation of the different products are presented in Table 5, and cover appearance, colour, flavour, taste and overall acceptability. In relation to the last-

Table 5 - Means of sensory scores for coated and non-coated chicken meat-balls.

Treatment no.	Treatment *	Appearance	Colour	Flavour	Taste	Overall acceptability
1	ST	4.73 ^{a}	4.73 ^{a}	4.36 ^{a}	4.27 ^{a}	4.73 ^{a}
2	WPI	4.27 ^{abc}	3.73 ^{bcd}	4.09 ^{abc}	4.09 ^{a}	3.82 ^{abcd}
3	CA	2.18 ^{e}	2.82 ^{e}	3.27 ^{cd}	3.36 ^{ab}	2.36 ^{e}
4	BC	4.55 ^{ab}	3.82 ^{bcd}	4.09 ^{abc}	3.82 ^{ab}	3.82 ^{abcd}
5	ST + WPI	3.82 ^{bcd}	4.27 ^{ab}	4.09 ^{abc}	3.45 ^{ab}	4.00 ^{abc}
6	ST + CA	2.36 ^{e}	2.82 ^{e}	3.45 ^{bcd}	3.00 ^{b}	3.09 ^{cd}
7	ST + BC	4.36 ^{abc}	4.27 ^{ab}	4.27 ^{ab}	3.73 ^{ab}	3.73 ^{bcd}
8	WPI + CA	3.00 ^{de}	3.09 ^{cde}	3.36 ^{cd}	3.64 ^{ab}	3.09 ^{cd}
9	WPI + BC	3.64 ^{cd}	4.09 ^{ab}	3.64 ^{abcd}	3.45 ^{ab}	4.00 ^{abc}
10	CA + BC	3.64 ^{cd}	3.00 ^{de}	3.91 ^{abcd}	4.18 ^{a}	3.73 ^{bcd}
11	ST + WPI + CA	4.09 ^{abc}	3.55 ^{bcde}	3.18 ^{d}	3.00 ^{b}	4.00 ^{abc}
12	ST + WPI + CA + BC	3.73 ^{bcd}	3.00 ^{de}	3.73 ^{abcd}	3.64 ^{ab}	3.45 ^{bcd}
13	control	4.36 ^{abc}	3.91 ^{abc}	3.82 ^{abcd}	3.45 ^{ab}	3.10 ^{cd}

Within the same column, values followed by a different superscript are significantly different ($P \leq 0.05$).
*ST: Starch; WPI: whey protein isolate; CA: carrageenan; BC: breadcrumb.
Means are the average of 11 readings, where 5 refers to "like extremely" and 1 refers to "dislike extremely".

mentioned, only the starch and carrageenan coatings showed any significant treatment effects. These appear to have been associated with product colour. The starch coating yielded the highest overall-acceptability rating and was brown in colour, while the carrageenan-coated product had the lowest rating, being bright yellow. Surface colour is said to be the first quality parameter that consumers consider in foods such as fried products, and is critical to product acceptance prior to ingestion (PEDRESCHI *et al.,* 2004).

The effects of the coatings on the taste score (Table 5) were generally small, the highest scores being associated with ST, WPI and CA + BC, and the lowest with ST+ CA and ST + WPI + CA, which were significantly different ($P \geq 0.05$). This shows that even the more favourable coating materials performed less well in some combinations. Flavour scores revealed a similar situation, involving different combinations, but differences between the control and any of the coated products were generally small, except in the case of CA, ST + CA and ST + WPI + CA, where the lower ratings appear to be due to the intrinsic flavour of CA. Previous studies (MALLIKARJUNAN *et al.,* 1997) reported that HPMC coatings improved the sensory attributes of fried chicken nuggets and marinated chicken strips, even more so when HPMC was incorporated into a breading mix. In the present study, all samples were found acceptable by the panellists, despite a clear colour difference between coated and non-coated samples. Also, the application of a coating had little or no effect on the texture attributes of the fried product. Such a finding, which was extended here to other coating materials, was in accordance with our goal of finding suitable coating materials to reduce oil uptake during deep-frying, without any deleterious effect on the sensory characteristics of the final product.

CONCLUSIONS

Various coating materials, either alone or in combination, were tested for their suitability in coating pre-formed chicken meat-balls prior to deep-drying. The resultant products were evaluated in relation to fat and moisture content, and sensory characteristics. They were studied primarily as a means of reducing fat absorption during the frying process and maximising moisture retention and product yield. The results showed that these objectives could be met readily with the coating materials used, but the best results were obtained with ST, WPI and CA. There were no significant effects of any of the materials on the density or texture of the final product.

All samples were found to be acceptable by the sensory panel and any differences in sensory rating between coated and non-coated products were generally small. Thus, the goal of reducing uptake of frying oil, while maximising moisture retention, without adverse sensory effects, was achieved.

REFERENCES

Addis P. and Warner G. (1991). The potential gerbil aspects of lipid oxidation products in foods. In: O.I. Arouma and B. Halliwel. Taylor and Fracis (Editors), Free Radicals and Foods Additive. London, chapter 5, pp. 77-119.

Adedeji A., Ngadi M. and Raghavan G. (2009). Kinetics of mass transfer in microwave precooked and deep-fat fried chicken nuggets. Journal of Food Engineering, 91, 146 -153.

Akdenz N., Sahin S. and Sumnu G. (2006). Functionality of batters containing different gums for deep-fat frying of carrot slices, Journal of Food Engineering, 75, 522-526.

Albert S. and Mittal G.S., (2002). Comparative evaluation of edible coatings to reduce fat uptake in a deep-fat fried cereal product. Food Research International 35, 445-458.

Allan B. (2004). More fat with that? Consumer, 433, 10-11.

AOAC (2000). Official Methods of Analysis (17th Ed). Washington, DC: Association of Official Analytical Chemists.

Ateba P. and Mittal G. (1994). Modeling the deep-fat frying of beef meatballs. International Journal of Food Science and Technology, 29, 429-440.

Balasubramaniam V., Chinnan M., Mallikarjunan P. and Phillips R. (1997). The effect of edible film on oil uptake fried poultry product and moisture retention of a deep-fat. Journal of Food Process Engineering, 20 (1), 17-29.

Freitas D., Berbari S., Prati P., Fakhouri F., Queiroz F. and Vicente E. (2009). Reducing of fat uptake in cassava product during deep-fat frying, Journal of Food Engineering, 94, 390-394.

García M., Ferrero C., Bértola N., Martino M. and Zaritzky N. (2002). Edible coatings from cellulose to reduce oil uptake in fried products, Innovative Food Science and Emerging Technologies, 3, 391-397.

Kulp K. (1991). Bread and Yeast-Leavened Bakery Foods. In: Lorena and Kulp (Eds). Handbook of Cereal Science and Technology. Marcel Dekker Inc., New York, Basel Hong Kong.

Mallikarjunan P., Chinnan M., Balasubramaniam V. and Phillips R.D. (1997). Edible coatings for deep-fat frying of starchy products. Lebensmittle-Wissenschaft and Technology, 30, 709-714.

Marrs W.M. (1998). The stability of carrageenans to processing. In: P.J. Williams and G.O. Phillips (Eds), Gums and Stabilisers for the Food Industry 9. The Royal Society of Chemistry, Cambridge, pp. 345-357.

Ngadi M., Dirani K. and Oluka S. (2006). Mass transfer characteristics of chicken nuggets. International Journal of Food Engineering, 2(3), 1-16.

Pedreschi F., Moyano P., Kaack K. and Granby K. (2004). Color changes and acrylamide formation in fried potato slices. Food Research International, 39(1): 1-9.

Pinthus E.J., Weinberg P., and Saguy I.S. (1993). Criterion for oil uptake during deep-fat frying. Journal of Food Science, 58, 204-205, 222.

Rayner M., Ciolfi V., Maves B., Stedman P. and Mittal G. (2000). Development and application of soy-protein films to reduce fat intake in deep-fried foods. Journal of the Science of Food and Agriculture, 80, 777-782.

Saguy I.S. and Pinthus E.J. (1995). Oil uptake during deep-fat frying: factors and mechanism. Food Technology, 49(4), 142-145, 152.

Salvador A., Sanz T. and Fiszman S. (2008). Performance of methyl cellulose in coating batters for fried products. Food Hydrocolloids, 22, 1062-1067.

SAS (1997). User's Guide in Statistics, (7th Ed.). SAS Institute Inc., Cary, NC, USA.

Southern C.R., Chen X.D., Farid M.M., Howard B. and Eyres L. (2000). Determining internal oil uptake and water content of fried thin potato crisps. Transactions of IChemE Part C: Food and Bioproduct Processing, 78, 119-125.

Tran T., Chen X. and Southern C. (2007). Reducing oil content of fried potato crisps considerably using a sweet pretreatment technique. Journal of Food Engineering, 80, 719-726.

Paper received March 22, 2010 Accepted December 27, 2010

SHORT COMMUNICATION

SOME PHYTOCHEMICAL CHARACTERISTICS OF TURNIP (*BRASSICA RAPA* VAR. *RAPA* L.) ROOTS

MEMNUNE SENGUL[1], HILAL YILDIZ[1], SEZAI ERCISLI[2]*, ERTAN YILDIRIM[2], METIN TURAN[3], OZLEM OZDEMIR[2] and DERYA SENER[1]

[1]Department of Food Science, Faculty of Agriculture, Ataturk University 25240 Erzurum, Turkey
[2]Department of Horticulture, Faculty of Agriculture, Ataturk University 25240 Erzurum, Turkey
[3]Department of Soil Science, Faculty of Agriculture, Ataturk University 25240 Erzurum, Turkey
*Corresponding author: Fax+90 442 2360958,
email: sercisli@hotmail.com

ABSTRACT

Vegetables of the family *Brassicaceae*, including turnip (*Brassica rapa* var. *rapa*), are used extensively for food throughout the Eastern Anatolia region in Turkey. Wide morphological diversity is evident among turnip genotypes grown in Turkey. In this study, we sampled seven morphologically diverse turnip (*Brassica rapa* var. *rapa*) genotypes in the Erzurum region. The roots of these plants were analyzed for moisture (%), titratable acidity (%), pH, SSC (soluble solid content, %), crude protein (%), vitamin C (mg/100 g), minerals (N, P, K, S, Ca, Mg, Na, Fe, Zn, Mn, B and Cu), total phenolic content and total antioxidant capacity. The results of the study revealed a wide variation among genotypes for all the parameters investigated. Antioxidant activity, determined by β-carotene-linoleic acid and 2-diphenyl-1-picrylhydrazyl (DPPH) free-radical-scavenging assays, ranged from 88.42 to 96.92% and 23.07 to 62.71 μg extract/mL, respectively. The total phenolic content of seven turnip genotypes varied from 16.59 to 70.23 mg gallic acid equivalent on a basis of 100 g fresh weight. This result shows that turnip roots are an important antioxidant source.

- Key words: antioxidants, biofactor, minerals, total phenolics, turnip -

ÌINTRODUCTION

Turkey is located on the border of two main gene centers, the Mediterranean and the Near East. Its location has fostered the growth of a variety of cultivated and indigenous horticultural crops. It is estimated that over 100 fruit taxa and nearly 50 vegetable taxa are grown successfully in the country (ERCISLI, 2004). Indigenous plants are found mostly in the rural areas of Turkey, where the occurrence of exotic species is limited. These indigenous plants are valuable sources of vitamins and minerals. They also provide a part of the nutrient requirements of the local communities.

Preventive medicine and the food industry have shown considerable interest in the development of natural antioxidants obtained from botanical sources (KAUR and KAPOOR, 2002; DJILAS *et al.*, 2007). The search for potential sources of antioxidant compounds has included several types of plant materials, including vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs (RAMARATHNAM *et al.*, 1995).

Guidelines for healthy diets have also encouraged the general public to adopt a more balanced diet rich in whole-grain cereals, nuts, seeds, fruits and vegetables. A diet of this type provides essential vitamins, minerals, antioxidants and other nutrients that reduce the risk of cardiovascular disease and cancer (SPARLING and ANDERSON, 2009).

Turnip (*Brassica rapa* var. *rapa* L.) is one of the oldest cultivated vegetables and has been used for human consumption since prehistoric times (LIANG *et al.*, 2006). This species is particularly popular in Europe, including Turkey, especially in the colder regions. It grows well in cold climates and can be stored several months after harvest. In Eastern Anatolia, together with other *Brassica* species, it provides a unique supply of vegetables during the winter (OKCU, 2003).

The turnip (*Brassica rapa* var. *rapa*) is a root vegetable commonly grown for its white, bulbous taproot. Small, tender varieties are grown for human consumption, whereas larger varieties are grown as feed for livestock. The domestication of the turnip has very ancient origins. The word laptu in ancient Assyrian dates back to 1800 B.C. (OPPENHEİM *et al.*, 1973). The turnip is also a very well-known vegetable in the entire Middle East (Arab: lift, Persian and Turkish: salgham and salgam).

Most of the indigenous vegetables are edible, and they play an important role in the diet, particularly in rural areas. Information on the chemical composition of these vegetables is limited. This study was therefore undertaken to obtain some data on moisture, acidity, pH, SSC (soluble solid content), crude protein, vitamin C, minerals (N, P, K, Ca, Mg, Na, Fe, B, Cu, Na, Zn, Mn), the total phenolic content and the total antioxidant capacity of seven turnip genotypes.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were purchased from the Sigma & Aldrich Company and were of analytical grade.

Collection and preparation of turnip roots

A total of seven turnip (*Brassica rapa* var. *rapa*) genotypes were sampled from Erzurum, located in the Eastern Anatolia region of Turkey. Fully mature roots were harvested by hand and brought to the laboratory for phytochemical analysis. Samples were frozen immediately and then stored in approximately 100 g batches at -30°C prior to analysis.

Determination of moisture, titratable acidity, pH, SSC (soluble solid content), crude protein (%) and vitamin C (mg/100 g) in turnip roots

For each genotype, a total of 10 roots were thawed at room temperature and homogenized in a standard food blender. Homogenates were assayed for titratable acidity (%), pH, SSC (soluble solid content, %) and vitamin C (mg/100 g) as ascorbic acid. Moisture (%) and crude protein (%) were determined on intact roots. Soluble Solid Contents (SSC) were determined by a digital refractometer (Model RA-250HE, Kyoto Electronics Manufacturing Co. Ltd., Japan) at 22°C. Moisture (%), crude protein (%) and pH were determined by AOAC (1984). The Kjeldahl method (BREMNER, 1996) and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N. Ascorbic acid (Vitamin C) of samples was quantified with a Merck reflectometer set (Merck RQflex).

Determination of total phenolics and antioxidant activity in turnip roots

Fruit homogenates obtained with a blender were extracted with acetone, water, and acetic acid (70:29.5:0.5, v/v/v) for 1 h in darkness (SINGLETON and ROSSI, 1965). This extract was filtered and used for phytochemical analysis.

Total phenolics were determined colorimetrically using Folin-Ciocalteu reagent as described by SLINKARD and SINGLETON (1977). Gallic acid was used as the standard, and results were expressed as mg gallic acid equivalents per 100 g fresh weight basis.

Total antioxidant capacity of samples was determined by β-carotene bleaching and 2,2-Di-

phenyl-1-picrylhydrazyl radical (DPPH•) assays.

In the β-carotene bleaching assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (KAUR and KAPOOR, 2002). Antioxidant capacities of the samples were compared with those of the synthetic antioxidant butylated hydroxyanisole (BHA) and the blank.

In the DPPH assay, 50 μL of various concentrations of the extracts in methanol were added to 5 mL of a 0.004% methanol solution of DPPH·. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. DPPH in percent (%) was calculated as follows: $DPPH\% = (A_{blank}-A_{sample}/A_{blank})$ x100; where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. EC_{50} is the effective concentration in μg extract/mL (μg extract for EC_{50} in 1 mL of DPPH solution) that inhibits the DPPH activity by 50%. EC_{50} is calculated from the plot of scavenging activity against extract concentration and represents the amount of extract necessary to decrease the initial DPPH concentration by 50%. Tests were carried out in triplicate. Results were expressed as (EC_{50}) (BURITS and BUCAR, 2000).

Determination of mineral elements

Samples were oven-dried at 68°C for 48 h and ground to pass a 1-mm sieve. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N (*Bremner*, 1996). Macro- (P, S, K, Ca, Mg and Na) and micro-elements (Fe, Mn, Zn, Cu, and B) were determined after wet digestion of dried and ground subsamples using a HNO_3-H_2O_2 acid mixture (2:3 v/v) with three steps (first step: 145°C, 75%RF, 5 min; second step: 180°C, 90%RF, 10 min and third step: 100°C, 40%RF, 10 min) in a microwave (Bergof Speedwave Microwave Digestion Equipment MWS-2) (MERTENS, 2005a). Tissue P, K, S, Ca, Mg, Na, Fe, Mn, Zn, Cu and B were determined using an Inductively Coupled Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) (MERTENS, 2005b).

Statistical analysis

The experiment was a completely randomized design with 5 replications. Data were subjected to analysis of variance (ANOVA), and means were compared using Duncan's multiple range test at the $P<0.05$ significance level.

RESULTS AND DISCUSSION

Moisture, titratable acidity, pH, SSC (soluble solid content), crude protein and vitamin C in turnip roots

Statistically significant differences were found among genotypes for all of the above parameters (Table 1). The SSC content of turnip genotypes ranged from 5.03 to 7.70% (Table 1). The genotype ETS1 was notable for its relatively higher soluble solid content. Among the genotypes, vitamin C, pH and titratable acidity ranged from 12.56 to 15.48 mg/100 g, 6.33 to 6.91% and 0.26 to 0.52%, respectively (Table 1). The mean vitamin C content of the turnip genotypes was 14.41 mg/100 g. The genotype-dependent moisture and crude protein content of turnip roots ranged between 88.44-90.73% and 13.19-15.75%, respectively (Table 1). Previously reported moisture, protein and pH values for turnip roots have ranged from 89.9-93.0, 10.0-14.0 and 5.75-6.35, respectively (UNDERSANDER *et al.*, 1991; OKCU, 2003; MATAIX *et al.*, 2007).

The variation of SSC, vitamin C, pH and crude proteins in turnip roots can be influenced by various factors such as varieties, climatic conditions, cultural practices, maturity at harvest, and storage conditions (PODSEDEK, 2007).

Table 1 - Moisture, pH, soluble solid content, crude protein, vitamin C and acidity of samples.

Genotypes	Moisture (%)	pH	SSC (%)	Crude protein (%)	Vitamin C (mg/100 g FW)	Acidity (%)
ETS1	88.44bc	6.88cd	7.70a	15.75a	15.48ab	0.38c
ETS2	90.73a	6.78e	5.03f	14.63ab	13.33ab	0.26ef
ETS4	90.21a	6.73f	5.10f	15.88a	13.78ab	0.26ef
ETS8	88.78bc	6.91bc	6.50c	13.19b	16.24a	0.28def
ETS9	89.18bc	6.84d	5.90d	13.69b	12.56b	0.32d
ETS10	89.50b	6.33i	5.60e	14.63ab	14.45ab	0.46b
ETS11	89.01bc	6.50h	5.83d	14.75ab	15.02ab	0.52a
Average	89.41	6.71	5.95	14.65	14.41	0.35

*Different letters indicate the statistical difference within same column among genotypes at 5% level.

Total phenolics and antioxidant activity in turnip roots

The total phenolic content of the turnip roots varied from 16.59 (ETS 4) to 70.22 (ETS1) mg GAE/100 g fw (Table 2). These results indicate a wide variability among genotypes. A previous study has shown that phenolic content of *Brassica* vegetables ranged from 15 mg/100 g fw in white cabbage to 337.0 mg/100 g fw in broccoli. Phenolic level in broccoli cultivars varied from 35 to 337 mg/100 g (PODSEDEK, 2007).

The total antioxidant capacity of the turnip genotypes is shown in Table 2. The genotype seemed to influence the extent of antioxidant activity in turnip roots.

Determination of antioxidant activities by β-carotene-linoleic acid and 2-diphenyl-1-picryhydrazyl (DPPH) free-radical-scavenging assays resulted in average values of 93.34% and 45.07 μg extract/mL for EC_{50}, respectively.

The DPPH scavenging capacities of the 7 turnip genotypes are summarized in Table 2. Significant differences in DPPH scavenging activity were found among the 7 turnip genotypes ($p<0.05$) (Table 2). ETS8, with an estimated EC_{50} value of 23.07 μg extract/mL, possessed the highest DPPH scavenging activity, followed by ETS1 (28.31), ETS4 (43.62), and ETS9 (50.32).

In the β-carotene-linoleic acid assay, the value of antioxidant activity varied between 88.42-96.92% for the genotypes tested. ETS11 had the highest antioxidant capacity in the β-carotene-linoleic acid assay (96.92%), whereas the genotype ETS9 showed the lowest antioxidant activity (88.42%). Relative to BHA in the β-carotene-linoleic acid assay, the antioxidant activity of all turnip extracts except ETS9 was higher than the BHA value (Table 2).

Growing evidence suggests that both genotype and growing conditions may alter the antioxidant composition and properties of a selected agricultural crop. For example, the highest day/night temperature has found to result in the greatest phenolic content and the strongest antioxidant activity (WANG and ZHENG, 2001).

In the study cited, according to a linear correlation model, the total phenolic content was not strongly correlated with the DPPH scavenging ability of turnip samples (R^2 =0.46). Different phenolic compounds are known to exhibit different responses in the Folin-Ciocalteau method. Similarly, the molecular antioxidant response of phenolics in methyl linoleate varies remarkably, depending on their chemical structure (STATUE-GRACIA *et al.*, 1997). In addition, phenolics are probably not the only major antioxidants in turnip extract. Other major antioxidant components, including ascorbic acid, are present as well.

The results of this study indicate that turnip roots are important sources of natural antioxidants. These results agree with previous reports that *Brassica* group vegetables, including the turnip, are effective antioxidants (CHU *et al.*, 2002; PODSEDEK, 2007). Antioxidants help to maintain the oxidant-antioxidant balance and inhibit the damage caused by reactive oxygen species. The antioxidant capacity of vegetables has been analyzed in some previous studies (CAO *et al.*, 1996; OU *et al.*, 2002; WU *et al.*, 2004). These studies have demonstrated that the common vegetables exhibit a wide diversity of antioxidant capacities.

A wide diversity among turnip genotypes in Turkey, presumably the one of the center of origin and diversity of *Brassica rapa* var. *rapa*, creates the opportunity to select superior genotypes for use. The results of this study also imply that dietary polyphenolic phytochemicals from turnip roots may supply substantial amounts of antioxidants. In turn, these antioxidants may provide health-promoting effects to consumers.

Mineral element contents of turnip root

The mineral element contents of turnip genotypes are shown in Table 3. Statistical differences between the genotypes were observed for the values of N, P, K, S, Mg, Cu, Mn and Fe content (Table 3). The average N, P, K, S, Mg,

Table 2 - Total phenolic content (TPC), antioxidant activity (β-carotene) and free radical scavenging capacity (DPPH) of samples.

Genotypes	TPC (mg GAE/100 g FW)	EC50	β-carotene bleaching assay (%)
ETS1	70.22a	28.31e	92.11c
ETS2	34.91d	62.71a	93.35bc
ETS4	16.59f	43.62d	95.35ab
ETS8	23.19e	23.07f	94.37b
ETS9	37.78c	50.32c	88.42d
ETS10	25.18e	54.20b	92.89c
ETS11	40.74b	53.28b	96.92a
BHA			89.01d

*Different letters indicate the statistical difference within same column among genotypes at 5% level.

Table 3 - Mineral content of turnip genotypes.

Minerals	Genotypes						
	ETS1	ETS2	ETS4	ETS8	ETS9	ETS10	ETS11
				% dw			
N	2.52a	2.34ab	2.54a	2.11b	2.19b	2.34ab	2.36ab
P	0.64c	0.26d	0.26d	0.84a	0.85a	0.65c	0.75b
K	3.11d	3.78d	1.91e	4.08c	4.91b	4.09c	6.63a
S	3.59a	1.09e	1.13e	2.85b	1.68d	1.90c	3.14a
Ca	1.49a	1.26ab	1.31a	1.37a	1.58a	1.44a	1.56a
Mg	0.34a	0.24b	0.09d	0.26b	0.17c	0.15c	0.32a
				mg kg^{-1} dw			
B	47.42ab	27.43c	51.00a	59.27a	36.64b	40.70b	55.20a
Cu	7.21a	6.69ab	6.52ab	6.02b	6.13b	6.12b	8.42a
Fe	75.76e	171.97c	81.46e	355.47a	215.63b	95.00e	164.67d
Zn	46.22a	25.96c	21.58c	34.93b	34.69b	36.79b	48.10a
Mn	44.96a	49.37a	29.77b	24.85b	19.85b	17.41b	59.59a
Na	0.12a	0.10a	0.07a	0.09a	0.15a	0.10a	0.16a

*Different letters indicate the statistical difference within same row among genotypes at 5% level.

B, Cu, Mn and Fe values of turnip genotypes were 2.3, 0.6, 4.0, 2.2, 0.2%, 45.4, 6.7, 35.11 and 165.7 mg/kg (Table 3), respectively. The data obtained from turnip genotypes exhibited statistically significant relationships between total phenolic content (TPC) and P (r^2=0.89), Mg (r^2=0.93), B (r^2=0.98), and Ca (r^2=0.77). Likewise, the relationship of DPPH to K (r^2=0.86) in the plants was statistically significant. The highest values of K, Zn, and Mn content were found in the ETS11 genotype, whereas the highest value of Ca content was found in ETS8 (Table 3). The turnip genotypes analyzed in this study have very high nutritional potential, particularly for Ca, Fe, P, K, and Mg, and their nutritional value is greater than those of some cultivated vegetables shown in Table 4 (HOLLAND *et al.*, 1992). Macro- and trace elements play an important role in many metabolic processes and functions throughout the life cycle. Human as well as animal studies have shown that optimal intake of elements such as potassium, magnesium, calcium, sodium, manganese, copper and zinc could reduce individual risk factors, including those related to cardiovascular disease (MERTZ, 1982). The Ca and Fe content of the turnip genotypes analyzed in this study may offer superior nutritional potential. The high K, P and Mg content of the turnip genotypes studied suggests that these genotypes could be used to meet the daily K, P and Mg requirements of an adult.

The results of this study suggest that turnips may serve as a potential dietary source of natural phenolic antioxidants. These findings also confirm the great interest of the nutraceutical sciences in extracts of *Brassica rapa* var. *rapa*. The phytochemistry and phytopharmacology of turnips should be investigated further to detect potential phytotherapeutic applications for the prevention of age-related and other types of diseases.

Table 4 - Protein and mineral contents of some selected vegetables (Holland *et al.*, 1992).

Species	Protein g 100 g^{-1}	N %	P	K	Ca	Mg	Fe	Cu	Zn	Mn
						mg 100 g^{-1} Dw				
Spinach	2.2	0.35	28	230	160	34	1.6	0.01	0.5	0.5
Radish	3.5	0.57	44	370	100	26	3.8	0.01	0.5	0.5
Pepper	5.5	0.46	80	220	30	24	1.2	0.01	0.4	0.5
Lettuce	0.8	0.13	28	220	28	6	0.7	0.01	0.2	0.3
Cabbage	1.0	0.16	25	120	33	4	0.3	0.01	0.3	0.2
Broccoli	3.1	0.50	57	170	40	13	1.0	0.02	0.4	0.2
Celery	0.9	0.15	63	330	40	63	0.7	0.03	0.3	0.1
Asparagus	1.6	0.26	50	220	25	13	0.6	0.08	0.08	0.2

REFERENCES

AOAC. 1984. Officials methods of analysis (14th Ed.). VA, USA: Association of Official Analytical Chemist, Arlington.

Bremner J.M. 1996. Nitrogen-Total. p. 1085-1122. In: D.L. Sparks (Ed.) Methods of soil analysis. Part 3. SSSA Book Ser. No. 5. SSSA, Madison, WI. (1996).

Burits M. and Bucar F. 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy Res. 14:323.

Cao G., Sofic E. and Prior R.L. 1996. Antioxidant capacity of tea and common vegetables. J. Agric. Food Chem. 44:3426.

Chu Y.F., Sun J., Wu X. and Liu R.H. 2002. Antioxidant and antiproliferative activities of common vegetables. J. Agric. Food Chem. 50:6910.

Djilas S.M., Canadanovic-Brunet J.M., Cetkovic G.S. and Tumbas V.T. 2003. Antioxidative activity of some herbs and spices- review of ESR studies. In: Magnetic Resonance in Food Science (edited by P.S. Belton, A.M. Gill, G.A. Webb & D. Rutledge). Pp. 110-120. Cambridge, UK: The Royal Society of Chemistry.

Ercisli S. 2004. A short review of the fruit germplasm resources of Turkey. Genet. Res. and Crop Evol. 51:419.

Holland L., Unwin D. and Buss D.H. 1992. Fruit and nuts the composition of food the Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.

Kaur C. and Kapoor H.C. 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. Int J Food Sci Technol. 37:153.

Liang Y.S., Kim H.K., Lefeber A.W.M., Erkelens C., Choi Y.H. and Verpoorte R. 2006. Identificaion of phenylpropanoids in methyl jasmonate treated *Brassica rapa* leaves using two-dimensional nuclear magnetic resonance spectroscopy. J. Chromatography A 1112: 148-155.

Mataix J., Manas M., Llopis J., Martinez E.J., Sanchez J. and Borregon A. 1998. Tabla de composicion de alimentos espanoles. Granada: Universidad de Granada.

Mertens D. 2005a. AOAC Official Method 922.02. Plants Preparation of Laboratuary Sample. Official Methods of Analysis, 18th Ed. Horwitz, W., and G.W. Latimer (Eds.). Chapter 3, pp. 1-2, AOAC-International Suite 500, 481. North Frederick Avenue, Gaitherburg, Maryland 20877-2417, USA.

Mertens D. 2005b. AOAC Official Method 975.03. Metal in Plants and Pet Foods. Official Methods of Analysis, 18th Ed. Horwitz, W., and G.W. Latimer (Eds). Chapter 3, pp. 3-4, AOAC-International Suite 500, 481. North Frederick Avenue, Gaitherburg, Maryland 20877-2417, USA.

Mertz W. 1982. Trace minerals and atherosclerosis, Federation Proceedings 41, 2807-2812.

Okcu Z. 2003. Change occuring in peroxidase activity during the preservation of some vegetables. MS thesis. Ataturk University, 64 p.

Oppenheim A.L., Reiner E., Biggs R.D., Renger J.M. and Stol M. 1973. Editors of The Assyrian Dictionary of the Oriental Institute of the University of Chicago Volume 9, L, page 96 "laptu A" (Roth M.T. present Ed.), Augustin Verlagsbuchhandlung Glückstadt, Germany.

Ou B., Huang D., Hampsch-Woodill M., Flanagan J.A. and Deemer E.K. 2002. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. J. Agric. Food Chem. 50:3122.

Podsedek A. 2007. Natural antioxidants and antioxidant capacity of *Brassica* vegetables: A review. LWT 40:1.

Ramarathnam N., Ochi H. and Takeuchi M. 1995. Antioxidant defense system in vegetable extracts. In Natural Antioxidants: Chemistry, Health Effects, and Applications; Shahidi F. Ed.; AOCS Press: Champaign, IL, pp 76Singleton V.L. and Rossi J.L. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. 16:144.

Slinkard K. and Singleton V.L. 1997. Total phenol analyses: automation and comparison with manual methods. Am. J. Enol. Viticult. 28:49.

Sparling M. and Anderson J. 2009. The Mediterranean diet and cardiovascular diseases: translating research findings to clinical recommendations. Nutrition Today 44:124.

Statue-Gracia M.T., Heionen M. and Frankel E.N. 1997. Antioxidant activity of anthocyanin in LDL and lecithin liposome systems. J. Agric. Food Chem. 5:3362.

Undersander D.J., Kaminski A.R., Oelke E.A., Smith L.H., Doll J.D., Schulte E.E. and Oplinger E.S. 1991. Turnip. Alternative Field Crops Manual. University of Wisconsin, Extension Service.

Wang SY. and Zheng W. 2001. Effect of plant growth temperature on antioxidant capacity in strawberry. J. Agric. Food Chem. 49:4977.

Wu X., Beecher G.R., Holden J.M., Haytowita D.B., Gebhardt S.E. and Prior R.L. 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J. Agric. Food Chem. 52:4026.

Paper received January 26, 2010 Accepted February 4, 2011

GUIDE FOR AUTHORS

ITALIAN JOURNAL OF FOOD SCIENCE -IJFS

1. Manuscript Submission

Manuscripts must submitted as an electronic version by e-mail to paolofan@unipg.it. The word processor used to generate the file should be indicated and the files should be saved in format "Text only"; **graphs, pictures and diagrams must be saved at 300 dpi in TIF, JPG or EPS formats** (not included in MsWord documents).

Manuscripts must be typed, double-spaced and pages should be in A4 format using Times New Roman 12 pt as the advised font. Top, bottom and side margins should be 25 mm. Pages and lines on all pages, including those for References and figure legends, **must be electronically numbered in the left margin.**

English is the official language. The Editor-in-Chief and/or Co-Editors reserve the right to make literary corrections and to make suggestions to improve brevity, but **the paper must be previously revised for English by the authors**. Authors who are not fluent in written English **must seek** help from one of the following agencies (or other similar official agencies):

www.journalexperts.com
www.sciencedocs.com
www.internationalscienceediting.com
www.writescienceright.com
www.genedits.com

2. Manuscript Preparation

(1) The paper should be divided under the following headings in this order:

Title. Informative of the content of the article (<50 characters + spaces).

Author(s). Initials and Surname, omit professional and official titles. The institute and address where the research was carried out and the current address of each author should be given on the title page.

Abstract. Clearly state the objective of the study, give a concise description of experiment(s), observations, results and conclusions. No references should be cited. **Do not exceed 100 words**.

Key words. Up to six words, in alphabetical order, which describe the document must be given to aid data retrieval and indexing.

Introduction. Review pertinent previous work and cite appropriate references. State the purpose of the investigation.

Materials and Methods. Indicate apparatus, instruments, reagents, etc., giving sufficient detail to allow the work to be repeated.

Results and Conclusions. Results and Conclusions may be presented together or separately. Concisely present results using tables and figures to help justify conclusions (do not present the same information in both forms). Use statistical analysis when appropriate. Unsupported hypotheses should be avoided. Conclusions should point out the significance of the findings and, if possible, relate the new findings to some problem in Food Science and Technology.

Acknowledgments. Acknowledgments of assistance are appropriate provided they are not related to analyses or other services performed for a fee. Financial support, thanks for assistance, artic1e number or thesis fulfilment may be included.

Units. A list of units particular to the paper may be included.

References. References in the Reference list should be arranged alphabetically (initials of first name, only), and, for the same author, should be arranged consecutively by year, typed double-spaced. Each individual reference should begin flush left (no indentation). Refer to attached examples taken from "Style Guide for Research Papers" by the Institute of Food Technologists (Chicago - I1linois - USA). Literature citations in the text should be referred to by Surname and year in parentheses. If there are more than two authors, give the surname of the first author and add et al. and the year in parentheses. Examples: (SMITH, 2007), (SMITH and JONES, 2008) (SMITH et al., 2008).

(2) Tables should be as few and as simple as possible and include only essential data. Each table must be saved and printed on a separate sheet and have an Arabic number, e.g. Table 4 NOT Tab.

4. Legends must be self-explanatory and on a separate sheet. Use lower-case letters for footnotes in tables and explain below the table in the order in which they appear in the table.

(3) Figures must be prepared and saved separately in **TIF, JPEG, EPS (300 dpi resolution).** They should be prepared so that on 50% reduction, lines, figures and symbols will be clearly legible and not overcrowded. All figures must be given Arabic numbers, e.g. Fig. 3. Legends for figures must be self-explanatory and should be typed on a separate sheet under "Legends to Figures".

(4) Standard Usage, Abbreviations and Units. The Concise Oxford and Webster's English Dictionaries are the references for spelling and hyphenation. Statistics and measurements should always be given in figures, e.g. 10 min, except when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelled out unless it is 100 or greater. Abbreviations should be used sparingly, only when long or unwieldy names occur frequently, and never in the title; they should be given at the first mention of the name. International Standard abbreviations should generally be used except where they conflict with current practice or are confusing. For example, 3 mm rather than $3x10^{-3}$ m. Abbreviations should be defined the first time they are used in the text and they should be used consistently thereafter. Temperatures should be expressed in the Celsius (centigrade) scale. Chemical formulae and solutions must specify the form used, e.g. anhydrous or hydrated, and the concentration must be in c1early defined units.

Common species names should be followed by the Latin binomial (italics) at the first mention. For subsequent use, the generic name should be contracted to a single letter if it is unambiguous.

3. Editorial and Review Policy

Scientific contributions in one of the following forms may be submitted:

Reviews –They can be submitted directly to the Editor-in-Chief or articles can be requested directly by the Editor-in-Chief.

Short Communications, Surveys and Opinions – They do not need to have the formal organization of a research paper; they will receive priority in publication; maximum of five pages allowed.

Papers – The paper must follow the guidelines as specified under the section Manuscript Preparation.

Reviews, Papers, Short Communications and Surveys will be **subjected to critical review by referees.**

(1) Manuscripts will be processed in the order received. The Editor-in-Chief will select papers to enter into the reviewing system based on originality and innovation. A letter will be sent to the authors acknowledging receipt of the manuscript along with a Declaration form stating that it has NOT been previously published, accepted or submitted for publication elsewhere and agreeing to the page charges upon acceptance of the paper. On receipt of the signed Declaration form, the Editor-in-Chief will send the manuscript to a Co-Editor and/or referees for evaluation.

(2) Referees may not be from the same institution as the author. Referees should make their comments and questions in detail and return the paper to the Editor-in-Chief and/or Co-Editor as soon as possible, usually within two weeks. The identity and report of the referees are made know to the Editor-in-Chief, but only the anonymous referee report is sent to the author(s). If all referees recommend acceptance or rejection, the decision stands. If the opinions of the referees tie, the Editor-in-Chief and/or Co-Editors have the freedom to decide upon acceptance or rejection of the paper.

(3) The results of the refereeing process, accompanied by a letter from the Editor-in-Chief or the Co-Editor, will be sent to the author(s). Papers needing revision must be returned to the Co-Editor within the timeframe suggested, otherwise the paper will be considered as

withdrawn. A letter announcing acceptance of the manuscript will be sent to the author(s) upon acceptance by the referees.

(4) The authors **will receive galley proofs** of the manuscript along **with the invoice** for the page charges (stated on the first page of each issue) which **must be paid in order to allow for publication**. The proofs will be sent to the corresponding author as a PDF file by e-mail, only. A hard copy will be sent by mail only if the author makes this request when the paper is accepted for publication.

The revised galley proofs must be returned by fax or mail to Chiriotti Editori – 10064 Pinerolo (TO) – Italy –Fax: +39 0121 794480; e-mail: info@chiriottieditori.it

REFERENCE EXAMPLES

EXAMPLES of use in a Reference list are given below. The bold-faced parenthetical type of citation above the example is indicated ONLY for information and is NOT to be included in the reference list.

(Anonymous)

Anonymous. 1982. Tomato product invention merits CTRI Award. Food Technol. 36(9): 23.

(Book)

AOAC. 1980. "Official Methods of Analysis" 13th ed. Association of Official Analytical Chemists, Washington, DC.

Weast, R.C. (Ed.). 1981 "Handbook of Chemistry and Physics" 62nd ed. The Chemical Rubber Co. Cleveland, OH.

(Bulletin, circular)

Willets C.O. and Hill, C.H. 1976. Maple syrup producers manual Agric. Handbook No. 134, U.S. Dept. of Agriculture, Washington, DC.

(Chapter of book)

Hood L.F. 1982. Current concepts of starch structure. Ch. 13. In "Food Carbohydrates". D.R. Lineback and G.E. Inglett (Ed.), p. 217. AVI Publishing Co., Westport, CT.

(Journal)

Cardello A.V. and Maller O. 1982. Acceptability of water, selected beverages and foods as a function of serving temperature. J. Food Sci. 47: 1549.

IFT Sensory Evaluation Div. 1981a. Sensory evaluation guide for testing food and beverage products. Food Technol. 35 (11): 50.

IFT Sensory Evaluation Div. 1981b. Guidelines for the prepaation and review of papers reporting sensory evaluation data. Food Technol. 35(4): 16.

(Non-English reference)

Minguez-Mosquera M.I., Franquelo Camacho A, and Fernandez Diez M.J. 1981. Pastas de pimiento. Normalizacion de la medida del color. Grasas y Aceites 33 (1): 1.

(Paper accepted)

Bhowmik S.R. and Hayakawa, K. 1983. Influence of selected thermal processing conditions on steam consumption and on mass average sterilizing values. J. Food Sci. In press.

(Paper presented)

Takeguchi C.A. 1982. Regulatory aspects of food irradiation. Paper No. 8, presented at 42nd Annual Meeting of Inst. of Food Technologists, Las Vegas, NV, June 22-25.

(Patent)

Nezbed R.I. 1974. Amorphous beta lactose for tableting U.S. patent 3,802,911, April 9.

(Secondary source)

Sakata R., Ohso M. and Nagata Y. 1981. Effect of porcine muscle conditions on the color of cooked cured meat. Agric. & Biol. Chem. 45 (9): 2077. (In Food Sci. Technol. Abstr. (1982) 14 (5): 5S877).

Wehrmann K.H. 1961. Apple flavor. Ph. D. thesis. Michigan State Univ., East Lansing. Quoted in Wehrmann, K.H. (1966). "Newer Knowledge of Apple Constitution", p. 141, Academic Press, New York.

(Thesis)

Gejl-Hansen F. 1977. Microstructure and stability of Freeze dried solute containing oil-in-water emulsions Sc. D. Thesis, Massachusetts Inst. of Technology, Cambridge.

(Unpublished data/letter)

Peleg M. 1982. Unpublished data. Dept. of Food Engineering., Univ. of Massachusetts, Amherst.

Bills D.D. 1982. Private communication. USDA-ARS. Eastern Regional Research Center, Philadelphia, PA.

CONTRIBUTORS

Gratitude is expressed to the following entities for contributing to the realization of the Journal by being supporting subscribers for 2010.

ASSOCIATIONS and COMPANIES

Associazione Italiana di Tecnologia Alimentare (A.I.T.A.) - Milano	Fax +39-02-2365015 www.aita-nazionale.it
Società Italiana di Scienze e Tecnologie Alimentari (S.I.S.T.Al) - Perugia	Fax +39-075-5857939 www.sistal.org
Soremartec Italia srl - Alba	Fax +39-0173-313966

RESEARCH INSTITUTES

Dipartimento di Scienze Tecnologie Agroalimentari (D.I.S.T.A.),
Facoltà di Agraria,
Università degli Studi della Tuscia, Viterbo — Fax +39-0761-357498

Dipartimento di Scienze degli Alimenti,
Università di Udine, Udine — Fax +39-0432-558130

Dipartimento di Scienze e Tecnologie Agroalimentari e
Microbiologiche (DI.S.T.A.A.M.),
Università del Molise, Campobasso — Fax +39-0874-404652

Dipartimento di Scienze e Tecnologie Alimentari
e Microbiologiche (DI.S.T.A.M.), Università di Milano, Milano — Fax +39-02-50316601

Dipartimento di Valorizzazione e Protezione delle Risorse
Agroforestali (DI.VA.P.R.A.), Sezione Microbiologia
ed Industrie Agrarie, Università di Torino, Grugliasco — Fax +39-011-6708549

ITALIAN JOURNAL OF FOOD SCIENCE
Rivista Italiana di Scienza degli Alimenti
DIRETTORE RESPONSABILE: Alberto Chiriotti
AUTORIZZAZIONE: n. 3/89 in data 31/1/1989
del Tribunale di Perugia
Proprietà dell'Università di Perugia
TIPOGRAFIA Giuseppini - Pinerolo
Una copia € 20.00

ISSN 1120-1770 © 2011

CHIRIOTTI EDITORI srl - 10064 Pinerolo - Italy

publishes the technical magazines:



VOLUME XXIII IJFS No. 3, 2011

CONTENTS

OPINION PAPER

Technological Parameters Affecting Aging and Refining of Balsamic Vinegar from Modena in Wood Receptacles Related to the New EC Regulation N. 583/2009
A. Del Signore **228**

PAPERS

Do Monoacylglycerols Act as Pro-Oxidants in Purified Soybean Oil? Evidence of a Dose-Dependent Effect
F. Caponio, V.M. Paradiso, G. Bruno, C. Summo, A. Pasqualone and T. Gomes **239**

LC-ESI-MS/MS Identification of Oleuropein as Marker of Olea Europaea L. Leaves Used as a Bulking Agent in Ground Oregano and Sage
M. Bononi and F. Tateo **245**

"Total Flavonoid" Content Assessment Via Aluminum [AL(III)] Complexation Reactions. What We Really Measure?
V.T. Papoti, S. Xystouris, G. Papagianni and M.Z. Tsimidou **252**

Irradiation of Ready-To-Eat Sausages Containing Lycopene
M.C. Gámez, M.L. Garcìa, M.D. Selgas and M.M. Calvo **260**

Content of Selected Nutrients in Sour Cherries, Blackcurrants and Apples Osmodehydrated in Reused Fructooligosaccharide Concentrate
M. Uczciwek, E. Piasecka, R. Klewicki, D. Konopacka, M. Mieszczakowska-Frąc and C. Bonazzi **270**

Oven Cooking of Turkey Meat at Different Relative Humidities
B. Mora, D. Barbanti, G. Betta and F. Bozzoli **279**

Biolog Methodology for the Antimicrobial Activity Evaluation of Essential Oils' Volatile Compounds Against Foodborne Microflora of Fresh-Cut Salad
E. Bertolone, J.L. Minati, B. Zanoni and R. Ambrosoli **289**

Stability of Invertase Immobilized of Three Different Supports
Taiseer M. Abou Baker, Samir M. El-Iraqi, Laila A. Shekib and Hanaa A. Abdellah **302**

Diet Fat Level and Season Effect on Heavy Pig Lipid Profile
L. Rotolo, K.J. Guo and I. Zoccarato **310**

The Effect of Water-Soluble Rosemary Extract, Sodium Erythorbate, Their Mixture and Packaging Method on the Quality of Turkey Meatballs
Mirosława Karpińska-Tymoszczyk **318**

Reducing Fat Uptake During Deep-Frying of Minced Chicken Meat-Balls by Coating Them with Different Materials, Either Alone or in Combination
Basem M. Al-Abdullah, Malak M. Angor, Khalid M. Al-Ismail and Radwan Y. Ajo **331**

SHORT COMMUNICATIONS

Some Phytochemical Characteristics of Turnip (Brassica Rapa Var. Rapa L.) Roots
Memnune Sengul, Hilal Yildiz, Sezai Ercisli, Ertan Yildirim, Metin Turan, Ozlem Ozdemir and Derya Sener **338**

GUIDE FOR AUTHORS **344**
CONTRIBUTORS **347**