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Editor-in-Chief:
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Dipartimento di Scienze degli Alimenti, Università di Perugia, S. Costanzo, I-06126 Perugia, Italy
Tel. +39 075 5857910 - Telex 662078 UNIPG - Telefax +39 075 5857939-5852067
E-mail: paolofan@unipg.it

Scientific Editor:
S. Mary F. Traynor, F.S.E.
Dipartimento di Scienze degli Alimenti, Università di Perugia, S. Costanzo, I-06126 Perugia, Italy
Tel. +39 075 5857912 - Telex 662078 UNIPG - Telefax +39 075 5857939-5852067
E-mail: ijfs@unipg.it

Publisher:
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Chiriotti Editori s.p.a., Viale Rimembranza 60, I-10064 Pinerolo, Italy
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OPINION PAPER

INFORMATION ON FOOD, HEALTH AND THE CONSUMER

The following pages deal with the topic "Nutrition-Information vs Education" discussed at the European CIAA (Confedération des Industries Agro-Alimentaires de l'UE) Congress 2004 "Food Futures: Eating Well, Feeling Good", held in Brussels from September 30 to October 1, 2004.

The choice of food has become more and more complex, particularly over the last few years, due to the appearance of new food products that promise various health benefits in addition to the traditional supply of nutrients. These food products are described as natural, integral, low and light, fortified, functional and more recently as novel foods.

Such a rapid release of new foodstuffs on the market is causing misleading information due to concomitant claims of their health benefits based on the exploitation of research often validated only with *in vitro* tests. Therefore, the work of nutritionists to educate people about correct and healthy eating has become more difficult (CANNELLA and CARRADA, 1999).

In the past, nutrition information and food habits were acquired daily in the family kitchen, but today this happens more often in coffee shops and fast food restaurants. Foodstuff producers, besides preparing continually new temptations to stimulate our appetite, are also using advertising to send out seductive messages, emphasizing the supposedly "health" benefits or the "biological/natural" origin of the food product. They are persuading the consumer to choose foodstuffs by mixing well-known stereotypes with a clever use of communication strategies and old popular "myths" with health claims.

An example could be the slogan "the brain needs sugar"! The business-related claim emerges from the need to re-qualify this particular food product negatively labelled for years as "the white poison". The brain needs glucose and does not care if it comes from sucrose or from starch. In contrast food containing starch, such as bread or pasta, was proposed as taboo for people wishing to lose weight by means of a diet… but without promoting the need to exercise.

Another example is the corn seed oil claimed to be produced with the "heart" of the seed (when it is really made with the germ), but it is correlated with a claim about its properties to protect from cardiovascular diseases. It is presented as healthy, light and dietetic, without actually having any substantial differences from other seed oils apart from a modest addition of vitamins which serves to prevent oxidation of EFA. While butter, even though it is one of the few foods with short chain fatty acids and with a high content of conjugated linoleic acid (CLA: a natural anti-cancer agent), is strongly penalized by the presence of cholesterol and by its animal origin… in comparison with 100% vegetable margarine, despite the fact that the latter is sometimes produced by the hydrogenation of unspecified seed oils.

There are many other unclear questions: which is better the white bread or brown? to eat the fruit with the peel or without? In athletes' diets: steak or nutritional sup-

plements (branched-chain amino-acids and carnitine)? Is the Mediterranean diet a healthy way to eat since it includes phytochemicals in a large variety of vegetables or do we need functional and novel foods? What is the best choice: foods fortified with PUFA or fish? What can be said to reassure the consumer about the addition of vegetable oils to "authentic" chocolate? Traditional or functional foods, who is receiving more benefit the consumers or the producers?

A nutrition expert should be able to give an answer and clarify many of these misleading questions to properly educate the consumer. Information on food is usually directly linked to the marketing of food products, but, it should be based on science rather than on producers' interests. It should drive and enlighten the choice of food not just increase consumeristic behaviour! Scientific and technological progress and the release of available knowledge are increasing the importance of problems related to the collection, selection and recording of information and it is becoming increasingly important that information be supported by documentary evidence.

In nutrition, information comes from food producers and in a smaller percentage of cases from nutrition researchers, while the consumer is completely inactive. This unbalanced situation can cause a distortion of information that produces confusion for the consumer. Sometimes the result of one study, even though perfectly valid in itself, is not placed into a holistic view of food and health by those who release the information. Feeding, nutrition and metabolism are three distinct processes which depend on each other (in fact one is the consequence of the other). They interact in the human body differently than in other animals and also depend on the state of health, the use of medicine, individual characteristics and on the environment.

Research is not perfect and its path is often winding and contradictory and this should be considered before giving out information. Scientific studies should be screened by the community of researchers before being released to the consumer as "information": Results should be checked and approved by several studies in different laboratories. A scrupulous analysis of the ups and downs of eating behaviours can show that whoever proposes diets and offers advice, without a specific qualification in nutrition, often supports inaccurate or unbalanced information.

Experts in human nutrition are scientific researchers in this sector but it is very difficult for them to inform the consumer correctly because they do not have easy access to the media. Their information has a small echo and generally does not reach the consumer. Recent scientific research in this field suggests that, as a paradoxical result, despite all of the sophisticated types and techniques of communication in our society, the consumer is still hesitant and in doubt when confronted with food choices, and he is driven more by individual or social factors (like flavour, price, habits, traditions, oral suggestions) than by knowledge of the food. Many factors are involved in food selection which characterize eating behaviour and the closely related to numerous factors: individual, social-economic, cultural. Correct information helps to achieve psycho-physical well-being.

Consumers are demanding accurate and balanced information about nutrition and they show more interest in health topics than in sports or local events. However they find contradictory information in the plethora of nutritional magazines and TV programs because nutritional studies are not always reported clearly. Media reports about nutrition-related studies should always indicate the characteristics of the study, address the relevance of the investigators in the field, understand what the study results really mean and correlate them with previous related ones.

Media reports differ from scientific reports. In many cases, media reports are based on the latest studies published in scientific journals, and they ignore that

research reports are meant to reach an audience of other scientists in the field and not the general public. The scientific presentation of research results aims to advance intellectual discussion on a particular topic or area, and to add elements to the body of scientific evidence. Media reports should never be sensational news and should not suggest a change in health behaviour on the basis of only one study.

I have briefly described the contradictory context in which food information is presently located. But now to improve the situation, let's try to understand to what extent nutrition information can be an effective tool to help consumers making informed choices.

Labelling, introduced and regulated by European directives, has been an important achievement to promote knowledge about the nutritional value of food, but these regulations are not always well applied to guarantee effective information. Consumers are supposed to be helped in their choices by nutrition labelling, but so far, this is not happening, as indicated by two different investigations conducted in Italy: the first one by my Institute on 1,000 subjects (GRAZIANI *et al.*, 2004) and the second one by Doxa (Institute of Statistical Research and Analysis on Public Opinion) on 2,041 subjects, in 2004. The Doxa study showed that only 25% of consumers always read the nutrition label, 44% occasionally and 31% never.

Actually, food labels are not easy and quick to understand and they often include information that is not of interest to the consumer and/or present misleading data. As a general rule, additives should be listed not only with their E-numbers but also with their names and the indication of their functions; in multilingual labels, the information about ingredients should be the same in each language. Furthermore, information on the label should be presented in a simple style highlighting the main characteristics of the product. A font size suitable for people over 50 years old should be used.

Another example of ambiguous labelling comes from the fact that "nutrition facts" can be expressed as a percentage of volume or of weight. In the case of a seed oil, this option causes confusion because if the fat content is reported as percentage of volume the oil could seem "light"! When consumers read it, they could be induced to prefer this "light" product since many of them don't know the trick between weight and volume.

The label sometimes contains scientific words that the consumer does not even know, but evokes the idea of good food properties and hence, persuades them wrongly. Vitamin F is an old fashion way to name EFA, but it is still used in labelling because it is well known that consumers look at the list of vitamins, even if they do not know their meaning.

Taking into consideration customers' behaviour in buying food which emerged from the research that my Institute conducted as a multiple choice inquiry at different supermarkets, the interest in food information was focused preferentially on the list of additives (51.8%), even if they did not know their functions and wanted more information about them. Moreover "technology of production" (33.6%) and "country of origin" (25.3%) were of greater interest and were requested more often than "food energy value" (24.4%) and/or the "list of ingredients" (22.1). This behaviour has been influenced by the low confidence in the food industry, as a consequence of previous scandals, such as BSE, involving food products. The Doxa study is also in agreement with our results, confirming the consumers' interest in the technology of production (38%), country of origin (24%) and list of ingredients (19%).

From our research it also emerged that customers are interested in the label reporting the certification of the health characteristics of not yet regulated food-

stuffs. When they found a quality control from a supervisor system, they attributed a "health" value to the foodstuff. Our data show that customers appreciate particularly informative "leaflets on food" freely available at shopping centres (48.3%) or posted by mail (33.0%). Moreover, we observed less interest in other media of communication such as TV (15.3%), magazines (8.3%) and even school education programs (5.0%) or family doctors (3.6%). Similar results are reported by the Doxa study. Customers in supermarkets are looking mainly at the quality/price ratio (38.0%), safety (37.6%), trademark (19.8%), taste (17.3%), etc.

On the basis of our inquiry, we would like to propose that the effectiveness of information on foodstuffs be improved by adding the following to the label:

- "recommendation on how to store" the foodstuff to preserve it safely;
- a "suggested portion size" in relationship to the energetic value, in addition to the actual indication referred to 100 g.

These indications could be very helpful to advise consumers on the correct use of a foodstuff: in the first case, how to store it at home or how to care for the leftover. In the second case, how to evaluate the appropriate amount of food to be eaten (well-being amount). Since it is very difficult for the consumer to count exactly the right amount of nutrients and/or calories, it would be helpful to the consumer to find the number of portions present in a package directly on the label.

So for example, on a 500 g package of pasta, it should be reported that it contains 6 portions because one portion is about 80 g (in fact consumers, except may be an Italian, do not know this). A 1 litre bottle of oil should be labelled as 100 portions because one portion is about a tablespoon or 10 mL. One litre carton of milk is 8 portions because one glass of milk is 125 mL... and so on...

Overweightness and obesity are emerging problems in our society and the correct way to prevent them is to educate the consumer on the portion size of foodstuffs. Producers should collaborate by making it possible to have a standard unit weight package for each foodstuff that corresponds to a portion size or its multiples as has already been achieved for example for a single size portion package of jam (25 g), butter (10 g) or sugar (5 g). So, 1 mozzarella package should be a 1 portion size (100 g) or its multiples, a standard unit weight for chocolate candy should be established (perhaps 15 g), and so on... for baked products, processed cheese, etc.

This additional nutrition information was endorsed a few months ago by the Italian Ministry of Health in the "Final Report" presented by an "ad hoc" committee to which I belong (ITALIAN MINISTRY OF HEALTH, 2003).

As a conclusion, we can say that there is a lot of work to be done in the future by producers and scientists together in order to produce more effective nutrition information with the common purpose of educating consumers to achieve well-being through a correct use of foodstuffs.

The consumer's nutrition awareness is now based on a poor scientific basis, while the desire for well-being and pleasure is very high. In this context, consumers are sometimes seduced by many persuasive pseudo-scientific messages transferring medicinal properties to foods.

The actual style of the label is trying to gain "neutral" credibility, rather than to be an effective tool to help consumers make informed choices. If labelling would be made more easily understandable by the producer, it would also better succeed in informing and educating the consumer. Credibility is not linked to language complexity or to coloured images and food quality and safety will be better per-

ceived and achieved when the consumer considers the producer a reliable source of nutrition information.

CARLO CANNELLA
Istituto di Scienza dell'Alimentazione, Università di Roma "La Sapienza", Piazzale A. Moro, 5 - 00185 Roma (Italy) - E-mail: carlo.cannella@uniroma1.it

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ABBREVIATIONS

EFA: essential fatty acids.

PUFA: polyunsaturated fatty acids.

PAPER

SHELF-LIFE OF EXTRA VIRGIN OLIVE OIL STORED IN PACKAGES WITH DIFFERENT OXYGEN BARRIER PROPERTIES

STUDIO DELLA SHELF-LIFE DI OLIO EXTRA VERGINE DI OLIVA CONDIZIONATO IN CONTENITORI CARATTERIZZATI DA DIFFERENTE PROPRIETÀ BARRIERA ALL'OSSIGENO

G. GAMBACORTA*, M.A. DEL NOBILE, P. TAMAGNONE[1], M. LEONARDI[1], M. FACCIA[2] and E. LA NOTTE
Dipartimento di Scienze degli Alimenti, Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy
[1]Sipa S.p.A., Via Caduti del Lavoro 3, 31029 Vittorio Veneto (TV), Italy
[2]Dipartimento di Produzione Animale, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy
*Corresponding author: Tel. +39 0881 589249, Fax +39 0881 740211, e-mail: giusgambacorta@unifg.it

ABSTRACT

A study is reported on shelf-life of extra virgin olive oil packaged in bottles having different oxygen barrier properties and stored in the dark at room temperature (RT) and at 37°C for 12 months. Five different materials were tested: polyethyleneterephthalate (PET), PET including 1% "oxygen scavenger" (PETA), PET including 3% "oxygen scavenger" (PETB), PET coated with high barrier resin (PETC) and PET coated with high barrier resin including "oxygen scavenger" (PETD); glass

RIASSUNTO

È stato condotto uno studio sulla shelf-life di un olio extra vergine di oliva confezionato in materiali aventi differenti proprietà barriera all'ossigeno e conservato per 12 mesi al buio a temperatura ambiente e a 37°C. In particolare sono state prese in esame 5 bottiglie realizzate con differenti materiali plastici [polietileneterftalato (PET), PET con l'1% di "oxygen scavenger" inglobato nel film (PETA), PET con il 3% di "oxygen scavenger" inglobato nel film (PETB), PET rivestito con resina ad alta

- Key words: extra virgin olive oil, oxygen scavenger, plastic packaging, quality, shelf-life, storage -

was used as the control. The following quality indices were monitored during storage: acidity, peroxide value, K_{232}, K_{270}, organoleptic assessment and the (E)-2-hexenal/hexanal ratio. A significant influence of the package on the quality decay kinetics of oil was found. Acidity did not change during storage, except for oil packaged in glass stored at 37°C, where about a 50% increase was observed. K_{232} and peroxide values of the samples packaged in glass, PETC and PETD were lower than those of oil packaged in PET, PETA and PETB. K_{270} increased in all samples, whereas the sensory score decreased from 7.69 (initial value) to about 6.5, for oils stored at RT, and to about 6.0, for those stored at 37°C. The value of the (E)-2-hexenal/hexanal ratio remained above 1, indicating the absence of off-flavours. The results indicate that the materials having higher oxygen barrier properties slow down the quality decay kinetics of extra virgin olive oil, suggesting that PETC and PETD could replace the traditional glass bottles compatible with the mechanical performance, which needs to be carefully tested.

barriera all'ossigeno (PETC) e PET con l'1% di "oxygen scavenger" inglobato nel film e rivestito con resina ad alta barriera all'ossigeno (PETD)] ed una di vetro, usata come controllo. Per la valutazione della shelf-life degli oli sono state effettuate le seguenti analisi qualitative: acidità, numero di perossidi, K_{232}, K_{270}, panel test e rapporto t-2-esenale/esanale. I risultati hanno evidenziato l'esistenza di una significativa influenza del tipo di materiale sulla cinetica di decadimento della qualità dell'olio. L'acidità è rimasta costante nel tempo ad eccezione di quello condizionato in vetro a 37°C il quale ha mostrato, dopo 1 anno di conservazione, un incremento del 50%. Il numero di perossidi ed il K_{232} sono risultati più bassi per gli oli conservati in vetro, PETC e PETD rispetto a quelli conservati in PET, PETA e PETB. Il K_{270} è aumentato per tutti i campioni, mentre il punteggio al panel test è sceso dal valore iniziale di 7,69 a circa 6,5 per gli oli conservati a temperatura ambiente e a circa 6,0 per quelli conservati a 37°C. Infine, il rapporto t-2-esenale/esanale è risultato sempre superiore ad 1, indicando l'assenza di composti secondari di ossidazione ("off-flavours"). I risultati indicano che i contenitori aventi maggiore proprietà barriera all'ossigeno rallentano la cinetica di decadimento dell'olio extra vergine d'oliva. Pertanto, i contenitori PETC e PETD potrebbero sostituire vantaggiosamente il vetro nel confezionamento degli oli extra vergini di oliva, compatibilmente con le loro prestazioni meccaniche, che dovranno essere opportunamente valutate.

INTRODUCTION

Virgin olive oil is the fat substance at the basis of the Mediterranean diet. It is valued for its particular organoleptic and nutritional characteristics. This kind of vegetable oil is considered to be resistant to oxidation because of the low content of polyunsaturated fatty acids and the presence of natural antioxidants such as phenols, tocopherols and carotenoids. Nevertheless, if it is subjected to strong oxidative stress, secondary products such as aldehydes, ketones and al-

cohols (off-flavours) are formed, which are responsible for the "rancid" defect. Consequently, during storage the package must ensure an adequate oxygen barrier to protect the oil from oxidation and preserve its particular organoleptic characteristics.

The most widely used materials for packaging of vegetable oils are glass, sheet metal and plastic films. To date, the influence of these materials on oil quality has not been sufficiently studied. DE LEONARDIS and MACCIOLA (1998) compared the shelf-life of clear glass-bottled virgin olive oil stored in the dark in a cold room versus ambient temperature with diffuse light. They concluded that after 18 months only the oils stored in the dark still maintained good quality characteristics. GUTIÉRREZ and FERNÁNDEZ (2002) studied the changes of the quality indices of two glass-bottled Spanish extra virgin olive oils stored in the dark at 2°C and with illumination (800 lx; 12 h/day) at 30°C, during 6 months of storage. They found k_{270} and sensory evaluation to be the only quality indices useful to indicate the loss of the extra quality of the product. KIRITSAKIS (1984) reported that glass ensures better protection from oxidation than polyethylene (PE).

SHARMA *et al.* (1990) studied the effect of plastic film contact, including PE, polypropylene (PP) and butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) incorporated in PP on the storage stability at 37°C of refined sunflower and peanut oils. They found that the changes in the peroxide value were significantly less in the presence of plastic films than in the control samples. Moreover, they observed a migration of both BHT and BHA from plastic films into vegetable oils during storage. NPKA *et al.* (1990, 1992) observed that crude palm oil, packaged in clear plastic bottles, sealed PE film and clear glass bottles, showed higher total oxidation values than oils packaged in either lacquered metal or amber and green glass bottles. TAWFIK and HUYGHEBAERT (1999) evaluated the effect of the type of packaging material, oxygen permeability, storage time and temperature and antioxidants, on the stability and quality of olive, sunflower and palm oils. KAYA *et al.* (1993) studied the effect of permeability and transparency of polyethyleneterephthalate (PET) and glass bottles on the quality of sunflower and olive oils, evaluating their oxidative stability by peroxide value. They concluded that stability during storage increased in the following order with respect to packaging materials: coloured glass >clear glass> PET.

The aim of this work was to evaluate the possibility of substituting the glass bottles, traditionally used for packaging of extra virgin olive oil with innovative materials. The innovative packages tested were: passive (PET, PET coated with high barrier resin), active (PET including "oxygen scavenger") and mixed (PET coated with high barrier resin including "oxygen scavenger").

MATERIALS AND METHODS

Materials

Filtered oil of the "Cima di Bitonto" cultivar, which is used as the base for producing the Protected Designation of Origin (PDO) extra virgin olive oil "Terra di Bari", was used. It was extracted in industrial mills using a three-phase continuous system and Table 1 gives its initial characteristics.

Plastic bottles (1.0 L, 5 dm^2 and 0.21±0.01 mm thickness) with screw caps were obtained from SIPA (SIPA S.p.A., Vittorio Veneto, TV, Italy). They were made of materials with different oxygen barrier properties: polyethyleneterephthalate (PET), PET including 1% "oxygen scavenger" (PETA), PET including 3% "oxygen scavenger" (PETB), PET coated with high barrier resin (PETC)

Table 1 - Initial characteristics of oil (average value ± standard deviation, n = 3).

Acidity (g/100 g)	0.32±0.01
Peroxide value (meqO_2/kg)	8.72±0.10
K_{232}	1.77±0.00
K_{270}	0.095±0.001
Sensory evaluation	7.69±0.36
(E)-2-hexenal/hexanal	30.35±1.63

and PET including 1% "oxygen scavenger" and coated with high barrier resin (PETD). The PET including "oxygen scavenger" was a PET copolymer with an organic macromolecular oxygen scavenger covered by patents (BP CHEMICALS, Italy); the coating resin was epoxyammine applied with a spray system, thickness 6 µm. Table 2 shows the Oxygen Transmission Rate (OTR) of each type of package. The measurements were performed by the manufacturer (SIPA) on bottles put in air in a lab at an oxygen pressure rate of 0.21 bar and under controlled temperature and humidity (23°C and 50% UR) using an Ox-tran 2/20 permeabilimeter (Mocon, Minneapolis, MN, USA). Green glass bottles (1.0 L and 5 dm^2) with screw caps having a total oxygen barrier were used as the control.

Experimental design

Oil (252 L) was put into the glass bottles and the plastic packages leaving 2% head-space. For each material, 18 bottles were stored at room temperature (RT) and 24 bottles were stored at 37°C, both in the dark. The bottles stored at RT were sampled every month for 1 year, whereas those stored at 37°C were sampled every 15 days during the first 7 months and subsequently every month until 1 year of storage.

Analytical determinations

Acidity (in terms of oleic acid, g per 100 g), peroxide value (PV, meqO_2/kg), specific extinction coefficients K_{232} and K_{270} and organoleptic assessment were carried out according to the European Official Method of Analysis (EOMA), in force at the beginning of this experimental study (EEC, 1991). In particular, sensory analysis was performed by a panel consisting of a panel head, who was a soundly trained expert in the various types of oil, and 8 qualified virgin oil tasters using the profile sheet indicated by EOMA (EEC, 1991). The descriptive analysis used a six-point intensity scale, ranging from 0 (no perception) to 5 (extreme). Overall grading used a nine-point scale, ranging from 1 (the lowest quality) to 9 (maximum quality). Using this scale, the virgin oils are classified according to their scores: "extra virgin" ≥ 6.5; "virgin" ≥ 5.5; "ordinary virgin" ≥ 3.5; and "virgin lampante" < 3.5. The (E)-2-hexenal/hexanal ratio was assessed by analyzing the volatile fraction according

Table 2 - Initial values of oxygen transmission rate of the investigated packages (average value ± standard deviation, n = 10).

Material	Oxygen Transmission Rate (cm^3 pkg^{-1} day^{-1})
PET	0.083±0.002
PET + 1% "Oxygen Scavenger" (PETA)	0.063±0.005
PET + 3% "Oxygen Scavenger" (PETB)	0.029±0.002
PET + "Coating" (PETC)	0.024±0.000
PET + 1% "Oxygen Scavenger" + "Coating" (PETD)	0.013±0.001

to the method reported by ANGEROSA *et al.* (1997).

Statistical analysis

All chemical analyses were made in triplicate. Mean values and standard deviations were calculated and analyzed with the JMP package program (SAS Ins., CARY, NC, USA) for analysis of variance at one, two and three ways. Statistical discrimination of the mean values was performed using the method of contrasts.

RESULTS AND DISCUSSION

Evolution in acidity

Fig. 1a and 1b show the trend of acidity of the samples stored at RT and at 37°C, respectively. According to this quality index, all the samples preserved their extra virgin quality (acidity less than 1.0%) after 1 year; the initial values did not change except for the samples at 37°C in glass, which exhibited a significant increase ($p<0.001$) from the 195th day to the end of the storage period (from 0.38 to 0.49 g/100 g), according to the following exponential curve: $y = 0.2782e^{0.0219x}$. This finding, which agrees with the results reported by PAGANUZZI *et al.* (1997) in oils stored in similar conditions and by GUTIERREZ and FERNANDEZ (2002) in oils kept at 30°C with illumination, can only be explained by triglyceride hydrolysis with the formation of free fatty acids.

Evolution in peroxide value

The evolution of this parameter is reported in the Fig. 2a and 2b. The PV of oils packed in PET, PETA and PETB at RT slightly increased from the 6th month on, reaching about 11 meq O_2/kg at the end of storage, whereas the PV of oils packed in glass, PETC and PETD did not change. Concerning the oils kept at 37°C, at the end of the storage period those packed in glass, PETC and PETD had lower values with respect to the initial one (6.5-8.0 meqO_2/kg), whereas those packed in PET, PETA and PETB had higher values (10-11 meqO_2/kg). Glass always exhibited the lowest values during the storage time. On the whole, PV did not change or slightly decreased for oils in glass, PETC and PETD, stored in the dark both at RT and 37°C. These results agree with those reported by DE LEONARDIS and MACCIOLA (1998) and by GUTIÉRREZ and FERNÁNDEZ (2002) for oils stored in similar conditions. In the present study, the slight decrease in PV of the oil stored in packages having lower oxygen permeability could be explained by the fact that, due to the low oxygen concentration, the hydroperoxide formation rate is lower than its decomposition rate. In fact, due to the higher oxygen barrier of these materials, the oxygen consumed during storage was almost limited to the oxygen already contained (head-space and dissolved in the oil). According to this quality index, all samples maintained their extra virgin quality (<20 meqO_2/kg) after 1 year of storage.

Evolution in specific extinction coefficients K_{232} and K_{270}

Fig. 3a and 3b show the evolution of K_{232} of the samples stored at RT and at 37°C. The K_{232} coefficient increased in oils stored at RT in PET, PETA and PETB, according to a linear correlation (R^2=0.9393, 0.9495 and 0.8339, respectively; $p<0.001$), ranging from 1.77 (initial value) to about 2.00 (final value), whereas in oils stored in glass, PETC and PETD, the values remained almost constant. This trend was more marked for the oils stored at 37°C, in fact, K_{232} of PET, PETA and PETB bottled oils reached values higher than 2.1 after 1 year, according to linear correla-

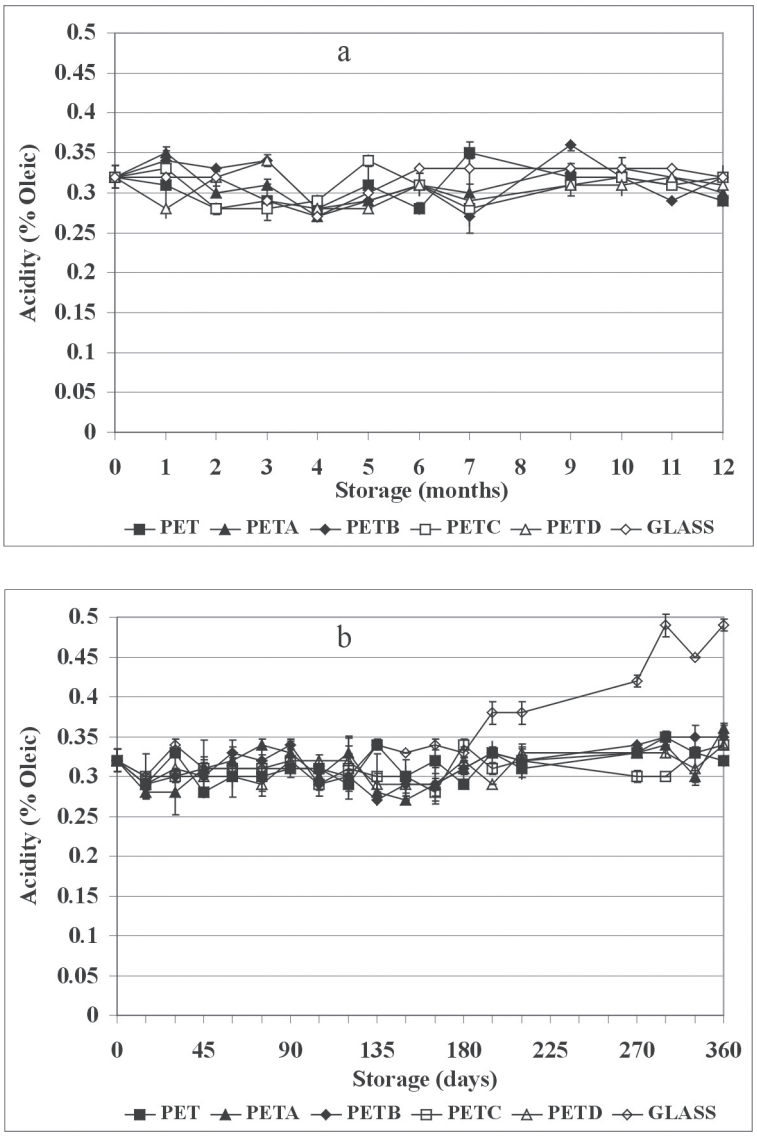


Fig. 1 - Trend of free acidity in the oils stored at RT (a) and at 37°C (b) at different storage times.

tion (R^2=0.9776, 0.969 and 0.9779, respectively; p<0.001), whereas for glass, PETC and PETD, no significant change was observed. It is clear that the low permeability to oxygen of these latter materials allowed the K_{232} and PV values to be kept constant.

As far as the K_{270} trend of the oils stored at RT is concerned, a slight increase was detected for all packages (Fig. 4a); the oil stored in glass had the lowest values. For the samples at 37°C the increases showed linear correlations (R^2>0.822 for all oils; p<0.001) and were higher than those observed for the oils at RT, ranging from 0.095 (initial val-

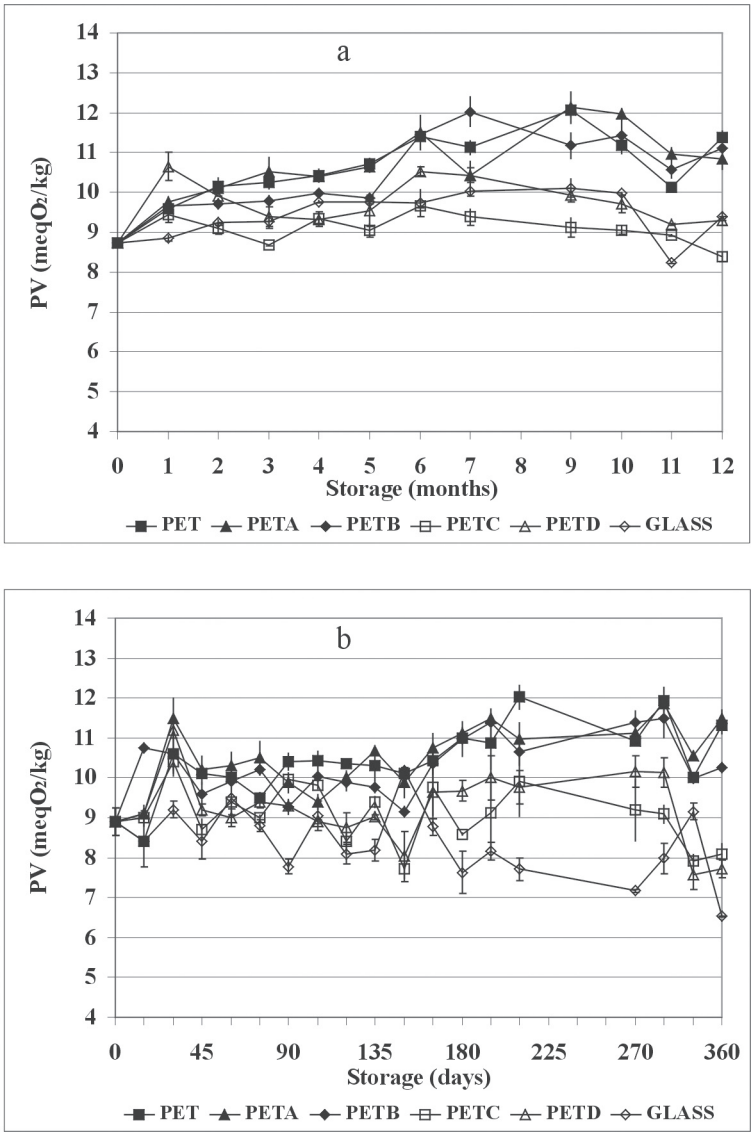


Fig. 2 - Trend of peroxide value in the oils stored at RT (a) and at 37°C (b) at different storage times.

ue) to 0.148 (oil in glass) or 0.171 (oil in PET) (Fig. 4b). Similar results have been reported by OLIAS and GUTIÉRREZ (1981) and by GUTIÉRREZ *et al.* (1988) for oils stored in different packages at 30°C. Regarding the influence of the material, for the oils stored in glass, PETC and PETD, the K_{270} increase was found to be lower than the other materials. Also in this case, such behaviour has to be attributed to the lower amount of oxygen penetrated into the package because of the high barrier properties. Nevertheless, for all packages tested and for both of the storage temperatures, the extra virgin quality was maintained after

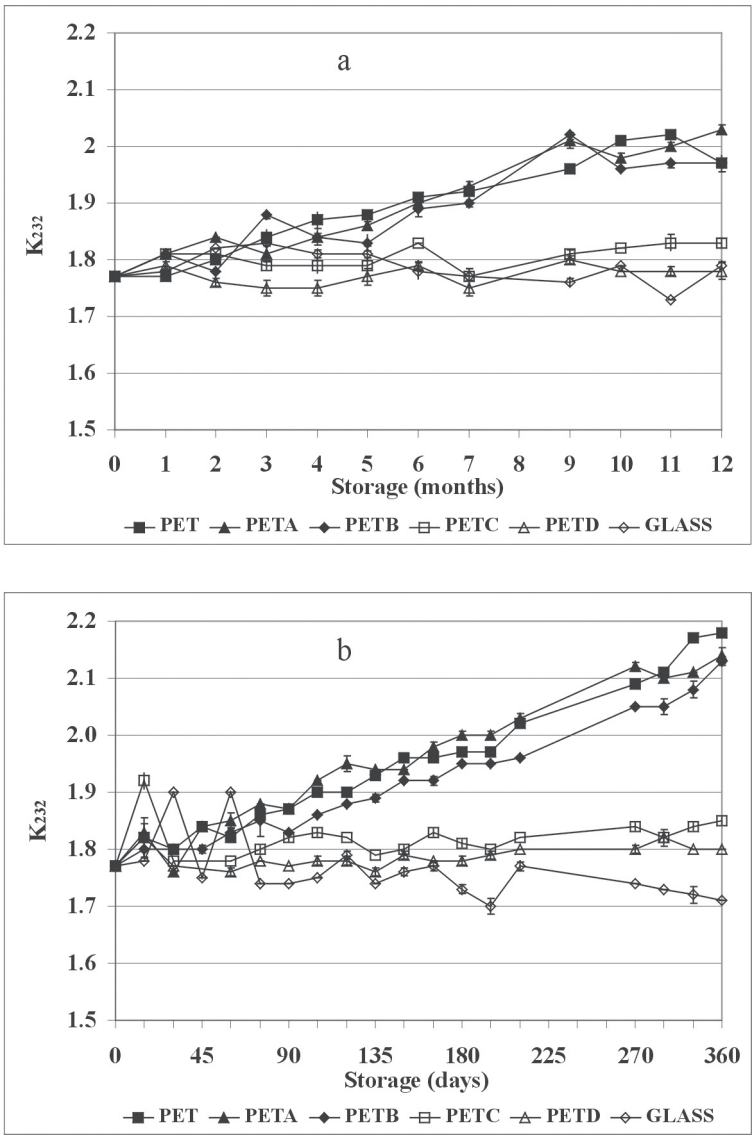


Fig. 3 - Trend of the K_{232} coefficient in the oils stored at RT (a) and at 37°C (b) at different storage times.

1 year for these parameters ($K_{232} < 2.50$, $K_{270} < 0.20$).

Evolution in sensory score

Fig. 5a and 5b show the evolution of the sensory score. All oils stored at RT had similar sensorial characteristics which tended to decrease with time, with PETC showing slightly lower scores starting from the 5th month of storage; for this latter oil the majority of tasters found a slight "winey" defect. For all samples the decrease followed logarithmic type equations ($R^2>0.828$; $p<0.001$), reaching scores higher than 6.0 after 1 year. As for

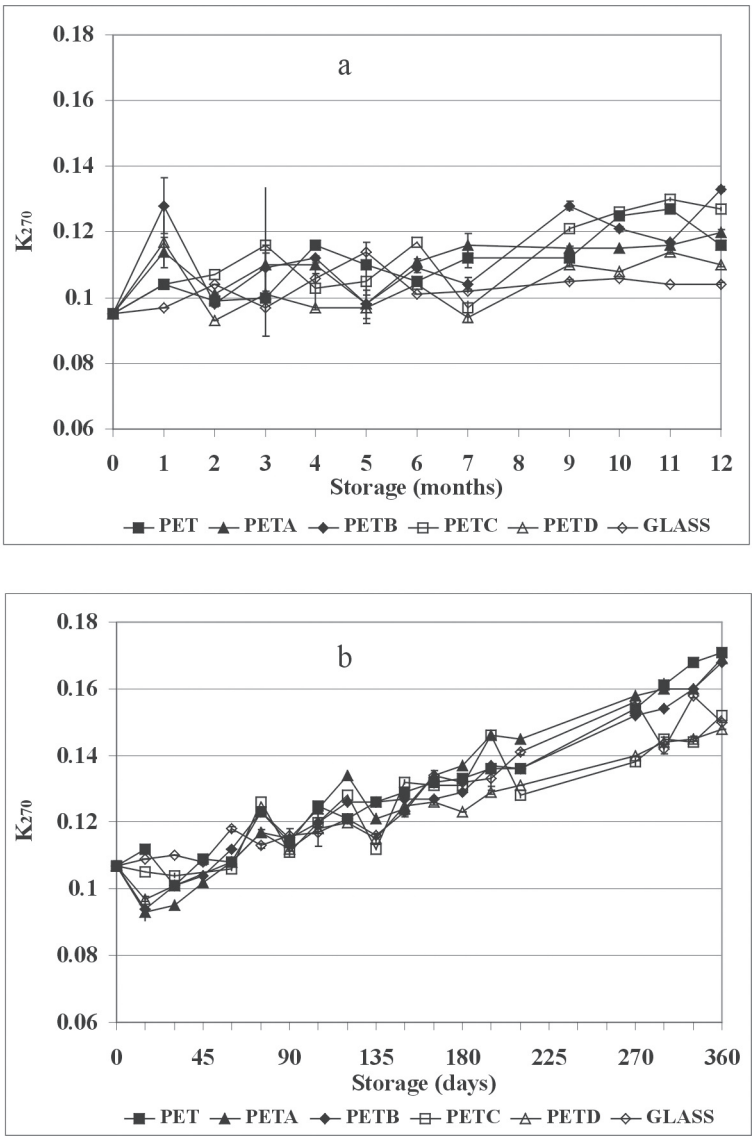
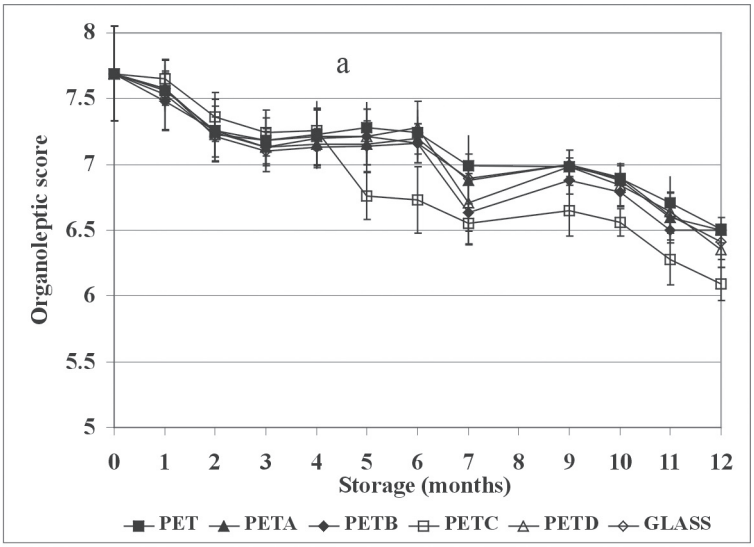


Fig. 4 - Trend of the K_{270} coefficient in the oils stored at RT (a) and at 37°C (b) at different storage times.

oils stored at 37°C, already after 2 months the scores decreased by about 1 (6.08-6.48) with respect to the initial values. In the successive stages of storage the sensory score did not significantly change, showing only a slight decrease. The variation observed at 2 months was mainly due to the loss of the fruity characteristics. A similar trend was observed starting from the 40th day of storage by GUTIÉRREZ and FERNÁNDEZ (2002) for Spanish oils stored at 30°C with illumination. On the whole, after 1 year all oils stored at RT obtained the minimum score for the "extra virgin" category, except the products in PETD and in PETC (6.35 and 6.1,



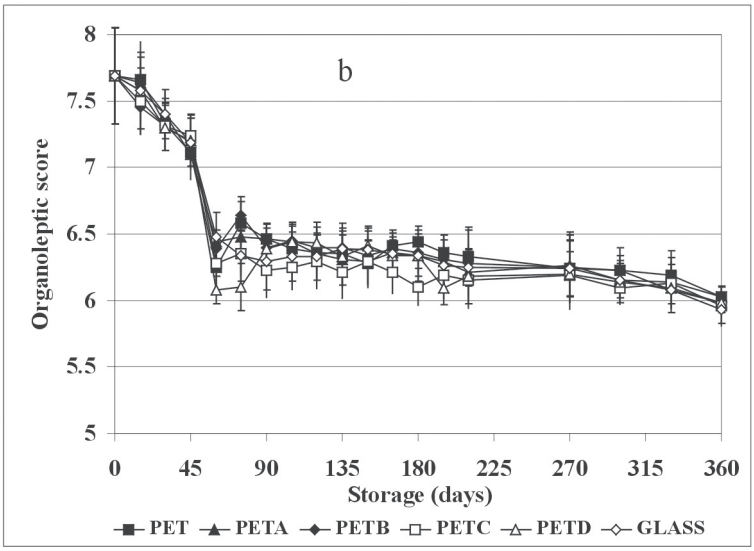


Fig. 5 - Trend of the sensory score in the oils stored at RT (a) and at 37°C (b) at different storage times.

"virgin" category, respectively). The samples stored at 37°C were all classified as "virgin olive oil" (scores about 6.0).

Evolution of (E)-2-hexenal/hexanal

The (E)-2-hexenal/hexanal ratio showed a similar trend among the different packages (Fig. 6a and 6b). This index remained almost constant in the oils stored at RT until 5 months of storage (> 20), then slightly decreased, reaching a value of about 15 at the end of the storage. The oils kept at 37°C showed a linear decrease following exponential curves (R^2>0.786; p<0.001), ranging from 30 (in-

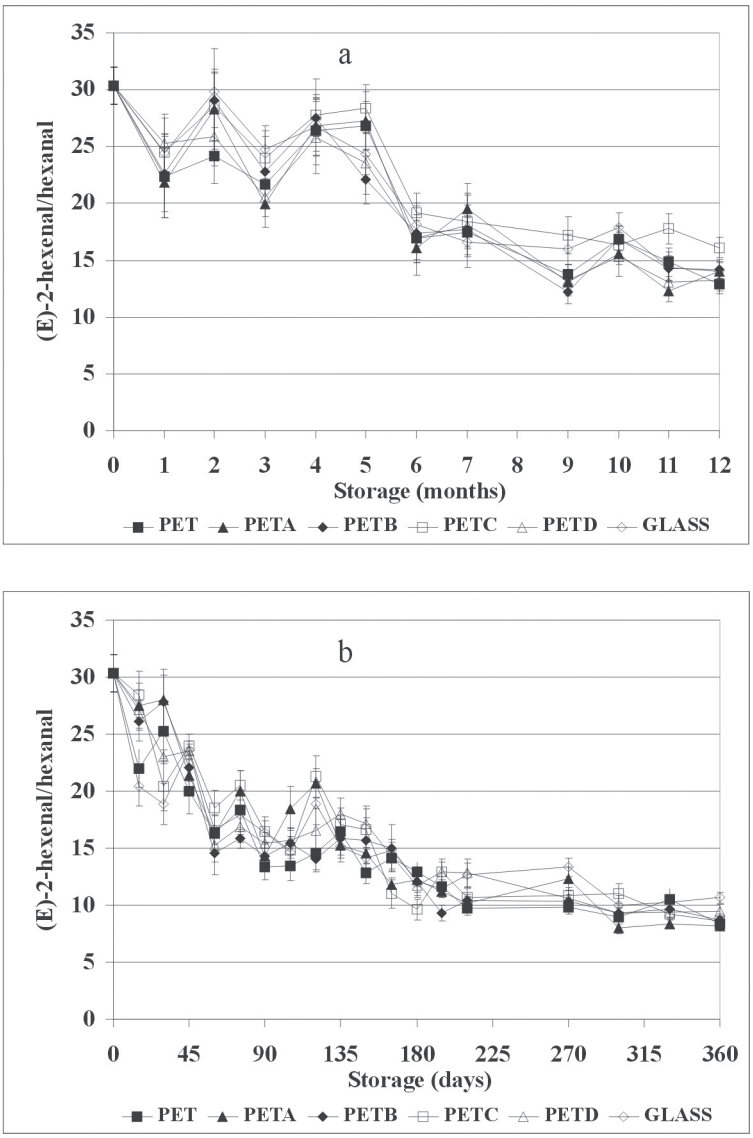


Fig. 6 - Trend of the (E)-2-hexenal/exanal ratio in the oils stored at RT (a) and at 37°C (b) at different storage times.

itial value) to less than 10 after 1 year. The decrease in (E)-2-hexenal/hexanal ratio indicates the loss of volatile molecules with odorous impact of herbaceous and the formation of hydroperoxide decomposition compounds responsible for the rancid defect. The trend of this parameter matches with the results of the sensory evaluation (decrease of the scores) and with the increase in K_{270}.

CONCLUSIONS

The results of the investigation indicate that among the packages tested,

those having high oxygen barrier properties (glass, PETC and PETD) maintained the values of the primary oxidation compounds (PV and K_{232}) of the oils, both at RT and 37°C almost unchanged. Moreover, these packages retarded the formation of the secondary oxidation compounds (K_{270}). Regarding the organoleptic properties, only very slight variations were observed among the different packages and only at RT. Finally, the value of the (E)-2-hexenal/hexanal ratio remained above 1, indicating the absence of off-flavour compounds. In conclusion, considering the negligible loss of the organoleptic characteristics but the higher efficiency in protecting the product from oxidation, PETC and PETD could replace glass in the packaging of extra virgin olive oils. This, compatible with the mechanical performance, needs to be carefully tested.

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PAPER

URACIL AS AN INDEX OF HYGIENIC QUALITY IN EGG PRODUCTS

L'URACILE COME INDICE
DELLA QUALITÀ IGIENICA DEGLI OVOPRODOTTI

A. HIDALGO*, M. ROSSI, C. POMPEI and E. CASIRAGHI
Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche
(DISTAM), Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy
*Corresponding author: Tel. +39 02 50316639, Fax +39 02 50316632,
e-mail: alyssa.hidalgovidal@unimi.it

ABSTRACT

A preliminary study was carried out to test the possibility of using uracil as a hygienic quality index for egg products, by comparing it with the legal European indices. Whole egg products prepared at the laboratory scale from eggs with different hygienic quality, as well as industrial egg samples were analyzed. Pasteurization caused a reduction of succinic acid levels, while uracil, lactic and 3-hydroxybutyric acids were not affected. Egg products obtained from sound shell eggs and stored at 4°C for

RIASSUNTO

Scopo della ricerca è stata la verifica dell'efficacia dell'uracile quale indice della qualità igienica degli ovoprodotti, in confronto con gli indici di legge europei. Sono stati analizzati campioni di misto d'uovo sia prodotti industrialmente sia a livello di laboratorio, a partire da uova di differenti qualità igieniche. Il trattamento di pastorizzazione ha ridotto i livelli di acido succinico, mentre non ha avuto effetto sull'uracile e sugli acidi lattico e 3OH-butirrico. Durante la conservazione a 4°C

- Key words: egg product, hygienic quality, lactic acid, 3OH-butyric acid, succinic acid, uracil -

up to 28 days had satisfactory hygienic quality and uracil was not detectable; uracil was detectable in samples made with contaminated raw material. Almost 50% of the industrial samples had detectable uracil values, while only 16% of them exceeded the legal limits using lactic and succinic acids. Preliminary studies proved that uracil, outstanding for its thermostability and high sensitivity, could be a reliable index for evaluating the hygienic quality of the raw material used in manufacturing egg products

fino a 28 giorni, gli ovoprodotti ottenuti da uova integre hanno mostrato una qualità igienica nei limiti di legge e un livello non rilevabile di uracile; gli ovoprodotti ottenuti da materia prima contaminata hanno invece evidenziato livelli quantificabili di uracile. Quasi il 50% dei campioni industriali ha presentato livelli di uracile quantificabili, mentre solo il 16% di essi ha superato i limiti di legge per gli acidi lattico e succinico. Studi preliminari hanno provato che l'uracile, parametro sensibile e termostabile, potrebbe essere un indice affidabile per la valutazione della qualità igienica della materia prima utilizzata per l'ottenimento di ovoprodotti.

INTRODUCTION

Pasteurized whole eggs are widely used in the food industry for the production of pasta, baked products, pastries, mayonnaise, ice cream, etc. The European regulation (EEC, 1989) for preparing egg products requires the use of eggs with intact shells or at least with intact shell membranes with no leaking contents, and prohibits the use of incubator reject eggs. Any direct contact between the internal egg contents and the egg shell should be avoided during egg breaking; thus, centrifuging and pressing are unacceptable practices for obtaining edible egg products.

If a hen is healthy, the egg contains no viable organisms. The level of shell contamination may range from 10^3-10^5 cfu per egg in clean laying conditions and from 10^7-10^8 cfu under dirty conditions (BAXTER-JONES, 1991). The European regulation (EEC, 1989) establishes limits for microbial (e.g. mesophilic aerobic bacteria $<10^5$ cfu/g) and chemical parameters (3OH-butyric acid ≤10 mg/kg d.m., lactic acid ≤1,000 mg/kg d.m., succinic acid ≤25 mg/kg d.m.) in pasteurized egg products. To guarantee good hygienic handling conditions before pasteurization treatment, 3OH-butyric and lactic acids are used as embryonic development indices, while succinic acid and lactic acid are microbial spoilage indicators (STIJVE and DISERENS, 1987).

Organic acids (LITTMAN *et al.*, 1982) and uracil have been studied to evaluate egg product deterioration. MORRIS *et al.* (1989) proposed uracil, a thermal stable and non volatile base, as a potentially useful indicator of spoilage in egg products and hypothesized the nucleoside, uridine, as one of its precursors. In fact, a wide range of bacteria convert exogenous uridine into uracil by the action of uridine-phosphorylase, an enzyme belonging to the nucleoside phosphorylases involved in bacterial nucleoside synthesis (UTAGAWA *et al.*, 1985; WANG *et al.*, 1983). MORRIS *et al.* (1989) investigated the relationship between egg product odor acceptability and their uracil content, but no further studies were performed to relate uracil level to bacterial

count or other spoilage indices. Uracil has also been found in incubator reject eggs together with 3OH-butyric and lactic acids, as a consequence of embryonic development (ROSSI *et al.*, 1999).

The aim of the present study was to test the possibility of using uracil as a hygienic quality index of egg products. Lactic and succinic acids were also evaluated for comparison. To this end, whole egg products prepared at laboratory scale from eggs of known origin and commercial samples of whole eggs for industrial food use were analyzed.

MATERIALS AND METHODS

Experimental whole egg products

Six pasteurized whole egg products were produced at laboratory scale using different egg lots: grade A-extra eggs (EC, 1994), from a commercial channel (lot N. 1); newly-laid eggs, obtained directly from the producer (lot N. 2); grade B eggs (EC, 1994), obtained from shell eggs stored at 4°C for six months (lots N. 3 and 4); discarded broken eggs collected during one day of production from an industrial shell egg-packing center (lot N. 5); and raw whole egg, obtained by industrial centrifugation of shell eggs stored at 4°C for six months (lot N. 6).

For lots N. 1, 2, 3, and 4, eighty shell eggs per lot were manually broken and, after removing the *chalazae*, were manually mixed to break the yolks. For lots N. 5 and 6, five kg of each of the crude whole egg mixes were directly filtered through a 0.8 mm sieve. All the lots were then homogenized using a Niro Soavi homogenizer (model Panda, Parma, Italy).

Pasteurization (63.5°C for 3.5 min) of the six lots of raw whole egg was carried out in a purposely built lab scale tubular heat exchanger, equipped with an aseptic stainless steel bottling system.

In order to verify the effect of egg pasteurization on the level of succinic acid, two spoiled liquid whole egg samples (S1 and S2) were prepared by breaking dirty eggs and leaving them in direct contact with their shells for 1 day at ca. 25°C. Less contaminated whole egg samples (DS1 and DS2) were also prepared by diluting S1 and S2, respectively, with sound liquid whole egg. The succinic acid values were determined in the four samples before and after batch pasteurization at 63.5°C for 3.5 min.

Whole egg product storage

Experimental whole egg products N. 1, 3, 5, 6 and a commercial whole egg product (packed in 1 L tetra brick®, declared shelf life: 28 days) freshly produced using grade A eggs and provided directly by the manufacturer (lot N. 7), were stored at 4°C for up to 21-32 days

Industrial whole egg products

Seventeen samples of commercial liquid whole egg products from eight different manufacturers (A, B, C, D, E, F, G, and H) furnished by different food industries and two pasteurized whole egg samples (I) provided directly by the manufacturer and obtained by industrial centrifugation of broken shell eggs, were analyzed before the “use by date”.

Analyses

The total mesophilic aerobic bacteria count, 3OH-butyric, lactic and succinic acids, and uracil levels of the lab-prepared samples were determined before and after pasteurization. These chemical analyses were also performed on the industrial whole egg products, while microbial conformity of commercial products was checked by the food industries.

The total mesophilic aerobic bacteria count was determined following AOAC method 966.23 (AOAC, 1995b). Succinic and 3OH-butyric acids were determined using enzymatic analysis kits

from Boehringer (Mannheim, Germany). Lactic acid and uracil were quantified by HPLC following the procedure of MORRIS (1987) modified as described by ROSSI and POMPEI (1995). Lactic acid was detected using a refractive index detector (model 1037A, Hewlett Packard Co., Palo Alto, CA), while uracil was detected at 260 nm using a photodiode array detector (model 996, Waters Corp., Milford, MA). The HPLC calibration curves were prepared using 10 different standard concentrations for each compound: from 10 to 400 mg/L for lactic acid (Sigma Chem. Co., St Louis, MO) and from 0.2 to 10 mg/L for uracil (Merck, Darmstadt, Germany). Based on the calibration curves, the detection limits for lactic acid and uracil were calculated as the intercept value of the regression line plus 3 times the standard error of the estimate (MILLER and MILLER, 1988). The calibration curves were linear in the concentration ranges considered (r^2=1, $p \leq 0.001$), giving detection limits of 17.5 and 0.2 mg/L in the standard solutions, corresponding to 281.9 and 2.9, respectively, on an average dry matter (d.m.) of 23.7 g/100 g of sample. For 3OH-butyric and succinic acids, the detection limits reported on the enzymatic kit instructions (0.2 and 0.6 mg/L in the standard solution, respectively) were used, corresponding to an average of 3.1 and 12.1 mg/kg d.m. in the sample. The results, expressed as mg/kg d.m., were the average of triplicate analyses. Dry matter was assessed as the average of two measurements following AOAC method 925.30 (AOAC, 1995a).

RESULTS AND CONCLUSIONS

Effect of pasteurization on experimental samples

The microbiological analysis run before pasteurization showed low levels of contamination (about 10^4 cfu/g) in the whole egg samples from sound, fresh (lots N. 1 and 2) and stored (lots N. 3 and 4) shell eggs, while high contamination levels (about 10^9 cfu/g) were found in samples from illicit practices (lots N. 5 and 6). Heat treatment reduced the total bacterial count by ca. 3-4 log cycles, producing pasteurized egg products with total bacterial counts below the legal limit, except for lot N. 5 which slightly exceeded 10^5 cfu/g.

Table 1 shows the effect of the pasteurization treatment on the levels of organic acids and uracil in the six experimental whole-egg samples. The severe contamination of samples N. 5 and 6 is also revealed by the high lactic and succinic acid levels and by the presence of uracil. All the samples had levels of 3OH-butyric acid, that were below the legal limit, thus confirming that this index is not dependent on microbial count and shell egg age, as previously reported by SALWIN *et al.* (1972) and ROBINSON *et al.* (1975).

No significant differences between raw and pasteurized samples were observed with respect to 3OH-butyric acid, lactic acid, and uracil levels. On the contrary, the very high succinic acid levels in lots N. 5 and 6 significantly decreased after pasteurization. A decrease in the level of succinic acid as a consequence of pasteurization could give misleading evaluations of the hygienic quality of egg products if the initial succinic acid content was slightly above the legal limit in the raw product. This hypothesis is confirmed by the results reported in Table 2. In fact, thermal treatment reduced the succinic acid level to below the detection limit in the DS1 and DS2 samples, whose levels approached the legal limit before pasteurization. The decrease in succinic acid as a consequence of whole egg pasteurization has not been reported before and the explanation for this phenomenon is not known.

Table 1 - Effect of pasteurization on organic acid and uracil contents (mg/kg d.m.) in experimental whole egg products. Values in parentheses indicate standard deviation.

Lot	3OH-butyric acid		Lactic acid		Succinic acid		Uracil	
N.*	raw	pasteurized	raw	pasteurized	raw	pasteurized	raw	pasteurized
1	nd	nd	nd	nd	nd	nd	nd	nd
2	nd	nd	nd	nd	nd	nd	nd	nd
3	2.7(1.3)^{a}	2.1(0.4)^{a}	401.7(85.8)^{a}	420.4(14.2)^{a}	nd	nd	nd	nd
4	3.9(0.9)^{a}	3.4(1.4)^{a}	nd	nd	nd	nd	nd	nd
5	3.9(1.2)^{a}	3.8(1.1)^{a}	11,350 (740.5)^{a}	12,740 (903.6)^{a}	526 (1.3)^{a*}	391 (33.5)^{b*}	48.2(2.8)^{a}	44.0(3.0)^{a}
6	nd	nd	4,225 (249.8)^{a}	4,164 (244.4)^{a}	804 (24.1)^{a**}	406 (10.4)^{b**}	229 (7.1)^{a}	252 (31.7)^{a}

*: see materials and methods for detailed description; nd: non-detectable; a, b: values in the same row, per each variable, bearing different superscripts are significantly different at $p \leq 0.05$ (*) and $p \leq 0.01$ (**).

Table 2 - Effect of pasteurization on succinic acid levels (mg/kg d.m.) in spoiled (S1, S2) and diluted spoiled (DS1, DS2) whole egg samples. Values in parentheses indicate standard deviation.

Treatment	S1	S2	DS1	DS2
Raw	649.2 (15.6)	546.7 (18.9)	29.6 (5.2)	25.6 (5.3)
Pasteurized	368.6 (7.0)	266.8 (9.2)	nd	nd

nd: non-detectable; limit of detection: 12.1 mg/kg d.m.

Hygienic quality indices during egg product storage

Fig. 1 presents the evolution of the hygienic quality indices during storage at 4°C of samples N. 1, 3, 5, 6, and 7. For total bacterial count and lactic and succinic acid levels, experimental egg products N. 1 and 3, and industrial sample N. 7 remained below the legal limits up to 21-28 days and uracil remained below the limit of detection.

After the expiration date (28 days), sample N. 7 showed hygienic quality decay exceeding the legal limits for total bacterial count and succinic acid levels at 32 day-storage. It is noteworthy that there was a simultaneous formation of uracil (6 mg/kg d.m.). Lactic acid, instead, did not show significant changes, remaining below the legal limit, suggesting low sensitivity as a hygienic quality index.

The experimental heat treatment conditions were not sufficient to guarantee to the samples obtained with illegal practices (N. 5 and 6) the same shelf life of the other samples: at time 0, sample N. 5 was already above the microbial threshold, while sample N. 6 crossed this legal limit between days 5 and 12. However, these samples should already be considered inedible because of the high lactic and succinic acids levels, above legal limits from the beginning. In sample N. 6, obtained from centrifugation of 6 month-stored eggs, the very high uracil content remained constant, as a consequence of the complete transformation of uridine in uracil before pasteurization. In sample N. 5, prepared using losses from an industrial fresh shell egg packing line, a simultaneous increase of uracil and uridine throughout storage was observed. Uracil increased as a consequence of

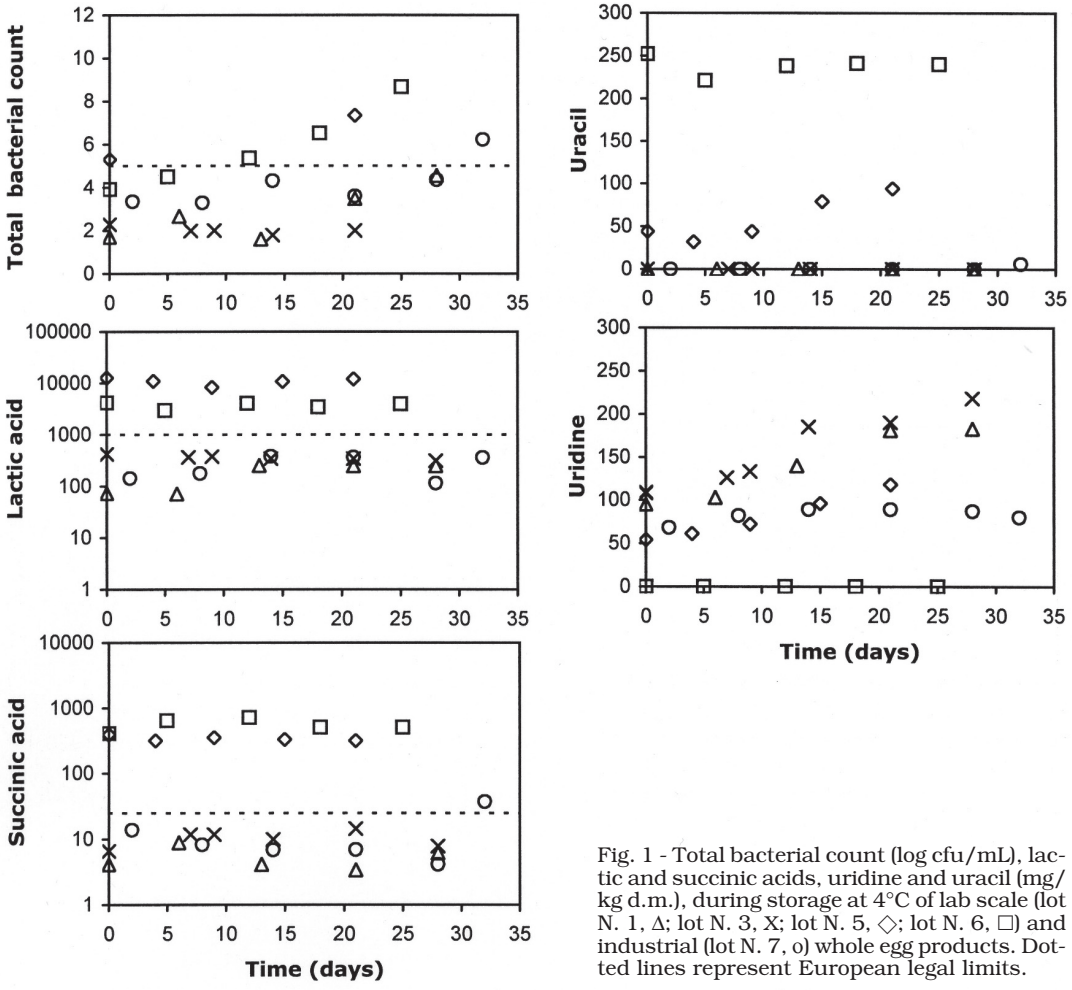


Fig. 1 - Total bacterial count (log cfu/mL), lactic and succinic acids, uridine and uracil (mg/kg d.m.), during storage at 4°C of lab scale (lot N. 1, Δ; lot N. 3, X; lot N. 5, ◇; lot N. 6, □) and industrial (lot N. 7, o) whole egg products. Dotted lines represent European legal limits.

the growing microbial count, while the increase in uridine, similar to what was observed during aging of shell eggs (ROSSI *et al.*, 1995), may be the result of residual native enzyme activity releasing this nucleoside from RNA. The same phenomenon induced uridine increase in samples N. 1 and 3, while the almost constant content in industrial sample N. 7 might be due to the relatively severe pasteurization conditions applied by the manufacturer (67°C x 200 s), that inactivated the enzymes responsible for uridine formation.

Industrial samples

Fig. 2 reports the analytical values of the 19 industrial egg product samples. Only sample H1 had a 3OH-butyric acid level higher than the legal limit of 10 mg/kg d.m., which indicates the presence of incubator rejects, as confirmed by the high levels of lactic acid and uracil (ROSSI *et al.*, 1999). Furthermore, the high level of succinic acid, together with the lactic acid and uracil levels, indicates very poor hygienic quality of the raw material. Sample H2, notwithstanding its accept-

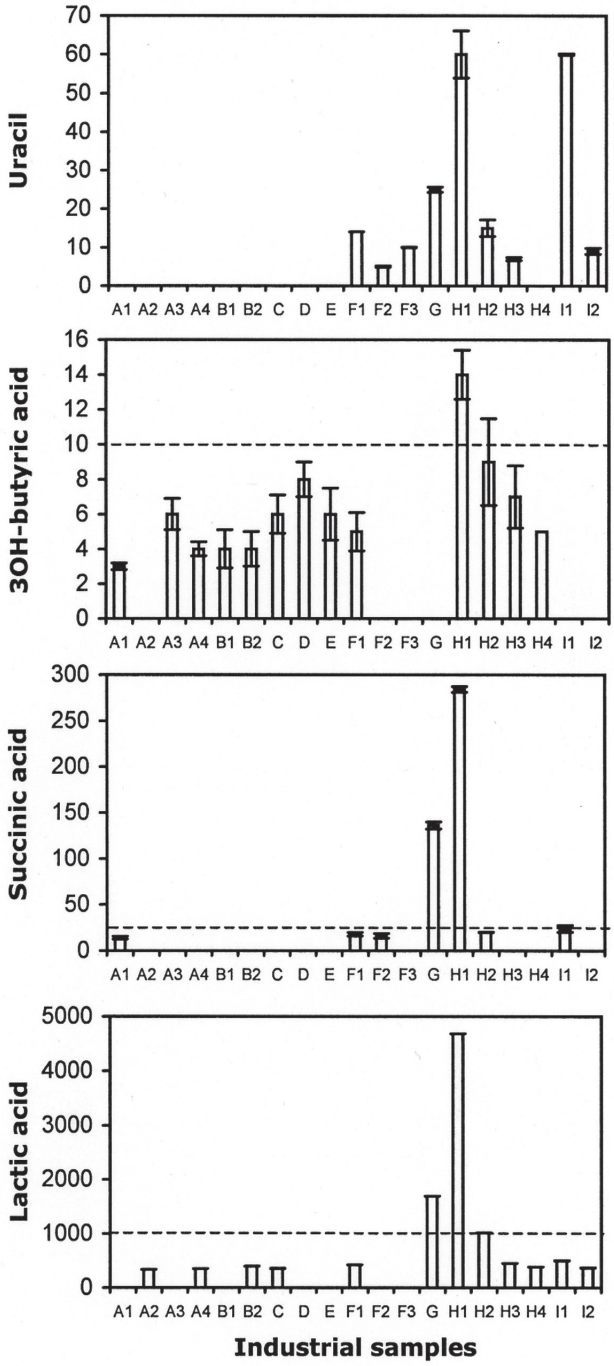


Fig. 2 - Uracil and organic acid concentrations (mg/kg d.m.) of industrial liquid egg products. Dotted lines represent European legal limits.

able succinic acid level, probably had reject eggs added because of the detectable uracil, and 3OH-butyric and lactic acid levels that were near the legal limits.

In sample G, the use of spoiled eggs and/or poor hygienic processing practices was indicated by the high levels of lactic and succinic acids, and uracil. Samples F1, F2, F3, H3, I1, and I2 had detectable levels of uracil, even though the succinic and lactic acid values were acceptable (with the exception of succinic acid in sample I1, which approached the legal limit). Almost 50% of the industrial samples (9 out of 19) had detectable uracil values, indicating the poor hygienic quality of the raw material, while lactic and succinic acid levels only indicated low quality in 16% (3 out of 19) of the samples. The deficiency of the legal chemical indices to evaluate hygienic quality is particularly evident in samples I1 and I2, obtained using an illegal practice (centrifugation). Moreover, an unacceptable odor was perceived by the analyst in most of the samples that had detectable uracil levels. MORRIS *et al.* (1989) detected spoilage odors in egg products containing ≥1.7 µg/g uracil, corresponding to about 7 mg/kg d.m., with an average dry matter of 23.7 g/100 g.

Even though the number of samples analyzed for each manufacturer was low, it can be affirmed that manufacturer A followed high quality standards, while manufacturers F and H produced poor quality egg products.

CONCLUSIONS

Thermostable microbial metabolites are useful indicators of the

hygienic quality of eggs used to manufacture egg products. Microbial count tests are not reliable since pasteurization treatments reduce microbial contamination even in highly spoiled raw materials. Thus, microbial tests performed on egg products do not give any information about the hygienic quality of the raw material used. The results of the present study clearly point out the deficiency of the legal European chemical parameters, i.e. succinic and lactic acids. The former seems to be influenced by heat treatment and can hardly be related to the hygienic quality of the raw materials, while the latter, even if thermostable, has a low sensitivity. In fact, succinic and lactic acids only identified a few of the samples that were prepared with spoiled raw material; in contrast, uracil identified many more poor quality samples, including those obtained using illegal practices. Therefore the use of uracil, outstanding for its thermostability and high sensitivity, could be a more reliable index for evaluating the hygienic quality of the raw materials used to manufacture egg products. Experiments are in progress to investigate the level of spoilage needed to produce detectable uracil levels in egg products.

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PAPER

INTERNAL MOULD – RIPENED CHEESES: CHARACTERISTICS, COMPOSITION AND PROTEOLYSIS OF THE MAIN EUROPEAN BLUE VEIN VARIETIES

FORMAGGI MATURATI CON MUFFE INTERNE: CARATTERISTICHE, COMPOSIZIONE E PROTEOLISI DELLE PIÙ IMPORTANTI VARIETÀ EUROPEE A VENATURA BLU

J. FERNÁNDEZ-SALGUERO
Departamento de Bromatología y Tecnología de los Alimentos
Universidad de Córdoba, Campus de Rabanales,
Edificio C-1, E-14071 Córdoba, Spain
Tel. +34 957 212010, Fax +34 957 212000, e-mail: ao1fecaj@uco.es

ABSTRACT

The general characteristics of manufacturing, gross chemical composition, metabolizable energy, sodium chloride, pH, water activity, major soluble nitrogen compounds, soluble tyrosine and tryptophan and degree of casein hydrolysis of the main commercially available European blue vein cheeses: Bleu de Bresse, Cabrales, Danablu, Edelpilzkäse, Gorgonzola, Roquefort and Stilton are given. The strong proteolysis undergone by blue vein cheeses is reflected in their high content of the dif-

RIASSUNTO

Sono indicate le caratteristiche generali di fabbricazione, la composizione chimica, l'energia metabolizzabile, il contenuto in cloruro di sodio, il pH, l'attività dell'acqua, i più importanti componenti azotati solubili, la tirosina e il triptofano solubili e il grado di idrolisi delle caseine dei seguenti formaggi europei a venatura blu di larga commercializzazione: De di Blue che Bresse, Cabrales, Danablu, Edelpilzkäse, Gorgonzola, Roquefort e Stilton. La forte proteolisi subita dai formaggi a vena-

- Key words: Bleu de Bresse, Blue veined cheeses, Cabrales, Danablu, Edelpilzkäse, Gorgonzola, mould ripened cheeses, Roquefort, Stilton -

ferent nitrogen fractions analysed. The Urea-PAGE technique revealed the occurrence of α_{s1}-I-CN in all cheese varieties except Roquefort, indicating the presence of cow milk.

tura blu si riflette nell'elevato contenuto delle frazioni azotate analizzate. La tecnica dell'Urea-PAGE ha rilevato la presenza di α_{s1}-I-CN in tutte le varietà di formaggio, ad eccezione del Roquefort, indicando così la presenza di latte vaccino.

INTRODUCTION

This category of cheese is really classified as "internal mould-ripened semi-soft cheese" but it is also possible to see them included within different groups of cheese, such as "ripened soft cheese" or "pâtes persillées", "mould ripened cheese" and, mainly, "blue-veined cheese". These cheeses utilize the species, *Penicillium roqueforti* and *Penicillium glaucum*, either growing naturally or by introducing mould spores into the milk or curds before pressing. Milk for blue cheese is either raw or pasteurised, and may even be homogenized with the aim of increasing more free fatty acids, leading to more flavour.

Cheese produced with raw milk has a very complex microflora. At the beginning of ripening, lactococci are always the main group of microorganisms inside the cheese but yeast, lactobacilli, leuconostocs, micrococci, staphylococci and enterobacteria are also present. However, in blue-veined cheeses, *Penicillium* spp. is also one of the main groups of the flora and the ripened cheeses are marked by their characteristics. The secondary flora plays an essential complementary role in the development of its organoleptic properties.

Although some years ago a significant amount of blue-veined cheese was made with raw milk and contaminating flora, the industrialization of all main European blue cheese varieties is carried out, in many cases, with the use of pasteurised milk and the addition of starter cultures. The purpose of using starters is justified because it ensures the consistent development of acidity at a controllable rate which aids rennet action. The acidity also improves draining of moisture from the curd, governs the flavour, body and texture of the cheese and controls undesirable bacteria. In blue-veined cheeses, the organisms selected as starters are different depending on varieties. To produce a cheese with some openness in texture, such as Stilton, strains of *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar. *diacetylactis* (also "citrate-positive *lactococci*") are used for gas and flavour production. Other lactic acid bacteria such as *Leuconostoc* spp. can also be used.

In the past, when the production of some varieties was carried out on a small scale, such as in the Spanish blue cheese "Cabrales", the cheese was not pierced with needles and very often some of the cheese had the defect of the lack of proliferation of the *Penicillium* spp. inside the cheese. *P. roqueforti* and *P. glaucum* are naturally present in some cheese ripening areas or caves, but now they are available as pure strain cultures from commercial suppliers. Nevertheless, the selection of *P. roqueforti* for manufacturing of some blue-veined cheese is frequently based on experience rather than on rigorous studies of their enzymatic characteristics. Investigations have shown differences in proteolytic as well as lipolytic activity between strains of *P. roquerforti* and thereby also differences

in the formation of free fatty acids and aroma compounds.

The manufacturing process of some semi-soft blue cheese is characterised by the homogenisation step of the milk. The milk is normally pasteurised and lactic starter cultures are added (0.2-0.5%). *P. roqueforti* is either added to the milk, or at a later stage, such as to the curd at salting. The curd is not pressed, but knits together under its own weight in open-ended moulds, which are turned frequently. In the ripening stage, the cheese, obtained in a round-wheel form, must be pierced to allow the escape of carbon dioxide and the entry of oxygen, which promotes mould growth. In general, the texture of blue cheese is moist, showing slight stickiness with a tendency to crumble.

Some characteristics of the seven main European blue-veined varieties have been reported (CARR, 1981; ENSRUD, 1981; SHAW, 1986; LAW, 1997; KOSIKOWSKI and MISTRY, 1997).

Bleu de Bresse

Manufacturing of this cheese started in France during World War II due to the lack of Gorgonzola cheese. It is made with cow's milk in the Eastern part of the country and is creamy, milder than most blues but has a savoury flavour and a soft and spreadable texture. Bleu de Bresse is one of the most popular blue-veined cheeses.

Cabrales

It is the most genuine and representative of Spanish blue-veined cheeses. It is manufactured in the Cabrales area (Picos de Europa, Asturias), and was officially awarded the Denomination of Origin in 1990. It is made with cows' milk, but also with binary and ternary mixtures of cow, sheep and/or goat milk. Its paste is compact, but spreadable, without openings, white with blue-green veins and strong flavour and piquant taste. The rind is thin, sticky and grey with yellow-reddish patches. Ripening takes a minimum of 2 months in mountain caves under air currents (at about 10°C and 90-95% relative humidity), with periodic turning and cleaning. Aged cheese may be sold wrapped in leaves of *Acer pseudoplatanus.*

Danablu

Also called Danish Blue was developed just before World War I in Denmark from cow's milk. It has a light, uniform, yellow background and a mellow flavour. It is modelled after Roquefort, but is not as piquant or salty. It has a semi-soft texture and slices and spreads easily, but it also crumbles nicely for use in salads. Ripening takes 6-8 months. In the USA blue-veined cow's milk cheese is called Blue. In the USA legally the cheese must be held for at least two months before consumption and normally it is not ready for distribution until about four months. The technique of ultrafiltration has been used for the manufacture of many soft cheeses such as Danish Blue.

Edelpilzkäse

Cheese made with cow's milk in the Bavarian region (Germany), also called German Blue. It is a fine blue cheese with a crumbly texture, a rich taste and is creamy and has an assertive piquant flavour. Edelpilzkäse is a semi-soft cheese in the shape of a bar or cylinder and weighs 2-5 kg.

Gorgonzola

It is one of most famous cheeses from Italy and one of the world's most distinguished blues. Gorgonzola ranks with Roquefort and Stilton, but is creamier than either of them. Gorgonzola was originally named "Stracchino" and was first made in a town with the same name, near Milan in the Lombardy region. It

was originally made from sheep's milk, but now, although some goat's milk may be used, it is made from cow's milk. Its veins, formed by *P. glaucum*, first develop when the cheese is ripened for many months in caves and they are more greenish than blue in colour. Aged Gorgonzola can be one of the most pungent and strong-flavoured of the blues and has very heavy mould and is quite strong and sharp; it is also drier and more crumbly than younger versions. From the point of view of economy and palate, the ripening time has been reduced to around six months and sometimes even less, so that the cheese is milder.

Roquefort

Possibly the world's most celebrated cheese and one of the oldest cheeses in existence, being known in Roman times. Roquefort cheese is made only in the Causses, in Southern France, from whole sheep's milk clotted with lamb rennet rather than with calf rennet and is protected by Appellation d'Origine status. Roquefort is aged in natural limestone caverns of Mount Combalou, ventilated with air currents called "fleurines" that are optimal for the growth of *Penicillium roqueforti*. The curd is inoculated with mould spores and then drained. The cheese is dry salted by rubbing salt on the surface and then taken to the caves, spiked or needled for air passage and left to ripen at 8°C and about 95% relative humidity for about three months. The lack of carotene in sheep's milk produces cheese, unless artificially coloured, that is white with a creamy paste rather than crumbly, not overly salty and with veins slightly greener that blue. The red sheep emblem on the foil wrapper is assurance of authenticity.

Stilton

Made with whole cow's milk in the region of Leicester, Derbyshire and Nottinghamshire in England. Milk for Stilton is not homogenized and the starter inoculation is very low (0.01%). There is no cooking stage and the cheese is pierced fairly late in ripening. It has a white to pale amber interior appearance with evenly spread blue veins in the form of tall cylinders with a crusty rind and a rich, piquant taste with Cheddar undertones. The Stilton cheese combines the most distinctive virtues of blue and Cheddar cheese; the mellow richness of Cheddar is appealingly accented with a mouldy tang that is neither overly pungent nor salty. Stilton is ripened from four to twelve months in cool ripening rooms; after, the rind is brushed or wiped to keep it clean as the cheese matures. Stilton displays a very delicate, mild, carbonyl flavour and the paste is highly marbled with mould.

In addition to the above main European blue-veined cheeses, there are many other blue-veined varieties in different countries than can be found in specialized shop, such as:

- In Denmark: "Mycella" is a Danish blue cheese with a milder flavour than Danablu because it is made with non-homogenized milk. "Blue Castello" and "Saga Blue" are also from Denmark.
- In France: "Belle Bressane", "Bleu d'Auvergue", "Bleu des Causses", "Bleu du Haut-Jura" (Bleus from Gez, Septmoncel and elsewhere in the region), "Fourme d'Ambert" and "Pipo Crem'".
- In Germany: "Bavarian Blue".
- In Italy: "Castelmagno" and "Montecenisio".
- In Norway: "Gammelost".
- In Spain: "Picón Bejes-Tresviso" protected by Denomination of Origin and "Gamonedo".
- In the United Kingdom: "Blue Cheshire", "Blue Wensleydale" and "Dorset Blue".

The Controlled Denomination of Origin (DOC) or Appellation d'Origine Controllée is a concept of international legislation established by the Commission of the European Communities to recog-

nise the specific heritage of the products of particular regions, while at the same time providing consumers with a guarantee of authenticity (BERTOZZI and PANARI, 1993). In the cheese sector, various European countries such as France, Italy, Portugal and Spain, have recognised the DOC concept in their respective legislation, following common principles based on their own judicial traditions and international provisions. The manufacturing and final features of cheese with Controlled Denomination of Origin are controlled by their respective Regulatory Councils.

During ripening numerous changes of microbiological, biochemical and chemical events occur as a result of which the main constituents of cheese are degraded to primary, and later to secondary compounds. With the addition of starter culture to milk in standard amounts, depending on the cheese varieties, the fermentation process starts; this involves the metabolic conversion of lactose by lactic acid bacteria (LAB) to produce lactic acid. The degree and rate of acidification achieved influences the size of casein micellar aggregates that precipitate due to the action of chymosin and the rate and intensity of syneresis during the curd formation. Also the acidification in combination with the decrease in pH and redox potential prevents the subsequent growth of spoilage and pathogenic microorganisms.

The degree of ripening and type of flavour developed in each variety of blue cheese depends on a number of factors, which include: the moisture, salt and fat content of the cheese, the action of proteases and lipases derived from the coagulant, the species of the starter bacteria and non-starter bacteria, the surface and mainly internal microflora of the cheese and the ripening humidity, temperature and time of ripening. The extent of proteolysis in blue-mould cheeses is very extensive, producing modifications in the texture. In blue vein cheeses the enzymes released from secondary starters (*P. roqueforti* and *P. glaucum*) are of major importance, besides the enzymes from rennet, milk proteinases (mainly, plasmin), enzymes from the starter bacteria and from non-starter bacteria. *Penicillium roqueforti* secretes potent aspartyl-proteinases (also called acid proteinases) with pH optima at 5.5 and acid and neutral metallo-proteinases (their pH optima are at 5.5-6.0) and several aminopeptidases and carboxypeptidases. Alkaline aminopeptidase is a metalloenzyme with an optimum pH of 8.0. It releases apolar amino acids but glycine in the penultimate or N-terminal position causes low activity. Acid carboxypeptidase is a serine enzyme with an optimum pH of 3.5. It has broad specificity and releases acidic, basic or hydrophobic amino acids. The mycelia also contain intracellular proteinases and peptidases that are released when the moulds die and lyse.

As with proteolysis, lipolysis in blue-veined cheeses is higher than in other varieties of cheese included in surface mould-ripened cheeses. Although data in the literature vary greatly, probably depending on the degree of ripening, the levels of free fatty acids in blue-veined cheeses reach about 10-25% of the total fatty acids. This major lipolysis does not lead to any rancid taste, probably due to neutralization of fatty acids on elevation of the pH (GRIPON, 1993). Native milk lipase contributes to degradation of triacylglycerides, especially during the early stages of ripening. Also microorganisms other than *Penicillium* spp. have very low lipolytic activity in mould-ripened cheese, however the surface microflora such as yeast, which can be present with counts higher than 7.0 log cfu/g, could have a positive effect on lipolysis in cheeses such as Gorgonzola. *P. roqueforti* has been reported to produce two lipases extracellulary, one with an optimum at acid pH values (6.0-6.5), the other with an optimum in the alkaline pH range (pH about

7.5-9.5), but this enzyme still has 20% of its activity at pH 6.0 (GRIPON, 1993). The free acids themselves contribute to the flavour characteristics of blue-veined cheeses. Nevertheless, *P. roqueforti* produces methyl ketones from fatty acids via the β-oxidation pathway. In blue-veined cheeses the methyl ketones play a key role in the typical flavour of the cheese, 2-heptanone and 2-nonanone being the most important and 2-pentanone, 2-propanone, 2-undecanone and 2-tridecanone being present in lower amounts. Besides methyl ketones, a complex volatile fraction composed of secondary alcohols via reduction of methyl ketones, other alcohols, many esters and aldehydes, phenols, γ-lactones, sulphur compounds, anisoles, volatile amines, ammonia and short branched-chain fatty acids can also be included.

The aim of this study is to give information on the seven main European blue-veined cheese varieties and their characteristics of production as well as to compare the degree of proteolysis and some chemical components.

MATERIALS AND METHODS

Samples

Thirty four different cheese samples of the main European cheese varieties: Blue de Bresse (four), Cabrales (six), Danablu (three), Edelpilzkäse (four), Gorgonzola (five), Roquefort (seven) and Stilton (five), were purchased from retail markets in several areas of Spain. The rinds of the cheese were removed when necessary and the internal portions were ground and stored at -24°C in airtight containers until their analysis.

Determination of chemical and nitrogen fractions

Determinations of the chemical composition of the samples including moisture, protein, fat, ash, and sodium chloride, were conducted in duplicate as described by AOAC (1980). The pH was measured in the aqueous extract (1:1 w/w) by using a Beckman 3500 digital pH-meter and the water activity was determined at 20°C by means of a CX-1 dewpoint hygrometer from Decagon Devices (Pullman, Washington, DC, USA).

Nitrogen fractions, viz. soluble nitrogen (SN) at pH 4.6, non-protein nitrogen (NPN soluble in 12% trichloroacetic acid, TCA), ammonia nitrogen (NH_3-N), and soluble tyrosine (Tyr) and tryptophan (Trp), were determined as described elsewhere (FERNÁNDEZ-SALGUERO *et al.*, 1989; CARMONA *et al.*, 1999; VIOQUE *et al.*, 2000). Amino acid nitrogen (AAN) was quantified in the SN extract as described by FOLKERTSMA and FOX (1992). All samples were analyzed in duplicate.

Samples for electrophoretic preparations of caseins were prepared from 0.2 g of cheese, 0.8 mL of distilled water, 2 mL of 7 M urea and 0.2 mL of 2-mercaptoethanol (LEDFORD *et al.*, 1966). Electrophoretic separation was carried out, gels were scanned and bands were densitometrically quantified at 600 nm as described by MARCOS *et al.* (1979).

RESULTS AND DISCUSSION

Chemical composition, caloric values, pH and water activity (a_w) of the seven main European blue-veined cheeses are shown in Table 1. Most of them had (in g/100 g cheese) about 45 g of water, 30 g of fat and 20 g of protein. The pH of most of the cheeses was higher than 6.0. The mean values for water activity ranged from 0.90 for Cabrales to 0.95 for the Bleu de Bresse and Gorgonzola varieties. In ripened cheese, the decrease in a_w is mainly influenced by the presence of salt, moisture loss and the gradual hydrolysis of proteins to soluble low molecular weight compounds.

Table 1 - Average values and standard deviations for moisture, fat, protein, and ash (g/100 g cheese), caloric value (kJ/100 g cheese), NaCl (g/100 g dry matter), water activity and pH of the main European blue-veined cheeses.

Cheese	n*	Moisture	Fat	Protein	Ash	Caloric value	NaCl	a_w	pH
Bleu Bresse	4	49.5±0.6	28.0±0.2	19.7±0.2	3.8±0.4	1,410±12	4.4±0.9	0.95±0.01	6.9±0.2
Cabrales	6	36.4±4.1	32.7±2.6	26.6±1.8	5.0±0.5	1,715±56	5.1±1.0	0.90±0.02	5.7±0.3
Danablu	3	46.0±0.5	28.3±0.6	20.2±0.4	4.7±0.2	1,412±18	7.0±0.8	0.93±0.01	5.3±0.1
Edelpilkäse	4	47.0±1.3	27.0±2.2	20.9±1.1	5.0±0.8	1,374±92	6.5±1.4	0.93±0.01	6.1±0.2
Gorgonzola	5	46.8±0.6	30.0±1.6	19.7±0.9	3.6±0.4	1,465±35	4.0±1.0	0.95±0.01	6.5±0.4
Roquefort	7	42.7±2.4	32.1±4.3	19.6±2.4	5.5±1.8	1,543±72	6.5±1.8	0.92±0.01	6.2±0.5
Stilton	5	42.0±1.6	31.5±2.7	22.0±1.2	4.9±0.4	1,575±34	3.6±0.6	0.93±0.01	6.0±0.3

*n = number of samples of each cheese variety.

Table 2 - Average values and standard deviations of different nitrogen components* (g/100 g TN) and soluble tyrosine and tryptophan (g/100 g cheese) of the main European blue-veined cheeses.

Cheese	SN	NPN	AAN	NH_3N	Tyr	Trp
Bleu de Bresse	58.6±12.6	49.8±3.4	23.3±6.5	14.7±1.2	0.24±0.03	0.41±0.03
Cabrales	55.9± 4.8	38.8±7.0	11.0±1.5	8.3±1.5	0.33±0.17	0.29±0.05
Danablu	61.3± 7.4	52.5±8.9	20.5±0.6	5.7±0.5	0.31±0.07	0.35±0.02
Edelpilkäse	59.8± 9.7	45.0±2.1	24.6±4.9	9.2±0.9	0.31±0.04	0.43±0.07
Gorgonzola	59.5± 9.9	50.0±8.9	25.0±9.3	9.9±6.9	0.24±0.09	0.41±0.08
Roquefort	72.8± 8.5	51.3±7.0	29.1±5.3	10.5±6.6	0.36±0.11	0.39±0.04
Stilton	56.0± 6.8	41.6±6.7	16.5±4.1	7.4±3.7	0.27±0.04	0.32±0.03

*SN = soluble nitrogen; NPN = Non-protein nitrogen; AAN = Amino acid nitrogen; NH_3N = Ammonia nitrogen; Tyr = Tyrosine; Trp = Tryptophan.

Table 2 shows the average values and standard deviation for different soluble fractions for the main European blue-veined cheeses. In all seven varieties, more than 55% of the total nitrogen (TN) was soluble, with Roquefort and Cabrales having the highest and lowest amounts respectively. NPN in Bleu de Bresse, Danablu, Gorgonzola and Roquefort was about 50% TN. Free amino acids and ammonia were also abundant, with the production of NH_3N in Bleu de Bresse being very high. In another blue-veined cheese such as "Picón Bejes-Tresviso", a similar proteolysis was reported by PRIETO *et al.* (2000). Strong proteolysis in blue-veined cheese is also reflected in their high contents of two soluble aromatic amino acids, tyrosine (Tyr) and tryptophan (Trp) (Table 2). The contents of Tyr and Trp are used as rapid methods for monitoring cheese ripening.

Table 3 lists the relative percentage of the electrophoretic compounds of the cheeses. The strong proteolysis in Blue de Bresse and Roquefort varieties caused almost complete disappearance of the α_s-casein fraction. The other four varieties yielded different electrophoretic bands in the α_s-casein region. The β-casein is also hydrolysed to a great extent and the major products show low mobility in the region of γ-CN probably corresponded to γ_1, γ_2, and γ_3-caseins, resulting from the degradation of β-casein by plasmin.

Initial proteolysis is, however, due

Table 3 - Relative percentage of the electrophoretic components of different blue-veined cheeses.

Cheese	Para-κ-CN	γ-CN	β-CN	α_{s2}-CN	α_{s1}-CN	α_{s1}-I-CN
Bleu de Bresse	9.4	49.2	13.8	-	7.6	13.7
Cabrales	5.4	40.6	22.1	13.8	5.9	11.7
Danablu	7.9	47.0	18.4	9.9	9.0	6.9
Edelpilkäse	6.0	55.9	13.1	8.2	6.6	10.2
Gorgonzola	7.9	50.8	7.4	11.2	10.6	12.3
Roquefort	12.7	46.3	41.6	-	-	-
Stilton	8.6	42.7	20.5	11.2	7.1	9.7

mainly to hydrolysis of α_{s1}-CN by chymosin and hydrolysis of β-CN by plasmin, α_{s1}-CN f24-199 (α_{s1}-I CN) being the principal large peptide formed initially, but following sporulation of the mould at about 15 days, its extracellular proteinases become dominant. Most of the water-soluble peptides identified are released from the N-terminal halves of α_{s1}- and β-caseins; so the primary chymosin cleavage sites in α_{s1}-CN are Phe_{23}-Phe_{24}, Leu_{101}-Lys_{102} and to a lesser extent Phe_{28}-Pro_{29} and Leu_{40}-Ser_{41} and the primary plasmin cleavage sites in β-CN are Lys_{28}-Lys_{29}, Lys_{105}-His_{106} and Lys_{107}-Glu_{108}. On the other hand, the large C-terminal fragments, α_{s1}-CN f24-199 (α_{s1}-I CN), α_{s1}-CN f102-199, β-CN f29-209 (γ_1-CN), β-CN f106-209 (γ_2-CN) and β-CN f108-209 (γ_3-CN) are accumulated (FOX and MCSWEENEY, 1997). Oligopeptides are hydrolysed by peptidases from non-starter bacteria and secondary starters. Regarding the products with the highest electrophoretic mobility, all the varieties except Roquefort contained α_{s1}-I-casein (Table 3), the major products in the degradation [hydrolysis of the 23-24 peptide bond (FOX and GUINEY, 1973)] of α_{s1}-casein by chymosin (LARSEN *et al.*, 1998). The presence of this C-end polypeptide of α_{s1}-CN in all cheeses except Roquefort indicates that they were made from cows' milk. Conversely, Roquefort cheese does not contain the α_{s1}-I-CN component as it is made from ewes' milk, where α_{s1}-CN is a minor component.

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PAPER

A RAPID γ-CASEIN ISOELECTROFOCUSING METHOD FOR DETECTING AND QUANTIFYING BOVINE MILK USED IN CHEESE MAKING: APPLICATION TO SHEEP CHEESE

METODO RAPIDO DI ISOELETTROFOCALIZZAZIONE DELLE γ-CASEINE PER LA DETERMINAZIONE E QUANTIFICAZIONE DEL LATTE BOVINO IMPIEGATO NELLA PREPARAZIONE DI FORMAGGI: APPLICAZIONE AI FORMAGGI DI PECORA

O. CERQUAGLIA and P. AVELLINI[1]
Ministero delle Politiche Agricole e Forestali, Ispettorato Centrale Repressione Frodi, Via Omicini 161, 06124 Perugia, Italy
[1]Università degli Studi di Perugia, Dipartimento di Scienze degli Alimenti, Sezione Sicurezza e Qualità degli Alimenti di Origine Animale, Via San Costanzo, 06126 Perugia, Italy

ABSTRACT

An analytical method useful to detect the presence of bovine caseins and/or milk in "pecorino" sheep cheese is described. Results are compared with those obtained by the official procedure reported in the EC Regulation n. 1081/96. The proposed method has more rapid sample preparation, uses ready-to-use polyacrylamide gel plates and the "Phast System" to reduce the time necessary for focalisation, fixing

RIASSUNTO

Viene proposto un protocollo analitico in grado di rivelare la presenza di latte o caseine bovine fraudolentemente impiegati nella preparazione di formaggio pecorino, confrontando i risultati ottenuti con quelli forniti dalla procedura ufficiale riportata nel Regolamento CE/1081/96. Il metodo proposto prevede una fase di preparazione dei campioni più rapida, l'impiego di lastre in gel di poliacrilamide già pronte e del

- Key words: bovine milk detection, casein isoelectrofocusing, sheep cheese -

and staining. The fast preparation of the sample simplifies the official procedure but does not allow small quantities of bovine caseins to be revealed (<4%); therefore this method can only be used for non PDO cheeses. The use of ready-to-use polyacrylamide gel plates offers various advantages such as higher sensitivity, higher repeatability as well as speed of execution.

"Phast-System" e l'utilizzo di standard a varie concentrazioni di latte vaccino. La preparazione rapida dei campioni semplifica la fase preliminare dell'indagine, ma non consente di evidenziare piccole quantità di caseine bovine (<4%), quindi può essere utile nell'analisi di routine di formaggi non DOP. L'impiego di lastre pronte di gel offre numerosi vantaggi, tra i quali una maggiore sensibilità, ripetibilità e velocità di esecuzione dell'analisi.

INTRODUCTION

Illegal use of bovine milk in the production of pecorino cheese allows producers to obtain unfair gains by marketing high-quality products at a lower cost. This is often made possible by the difficulty and laboriousness of analytical control methods. The PDO (Protected Designation of Origin) status of various Italian sheep cheeses (such as "Fiore Sardo", "Casciotta d'Urbino", "Pecorino Romano" and others) is a reason that control laboratories need to have valid analytical methods to protect these products from possible unfair competition of national and international origin.

Various analytical methods based on different principles and methodologies have been proposed to detect bovine milk or caseins used in the production of pecorino cheese. There are extensive references on immunochemical methods based on the use of specific antiserums against soluble milk proteins (AVELLINI, 1986; SIMONETTI *et al.*, 1991), those that employ electrophoretic techniques to detect different casein fractions (AVELLINI, 1986; ADDEO *et al.*, 1990, MOIO *et al.*, 1990; REA *et al.*, 1991; IANNIBELLI *et al.*, 1999), those that use RP-HPLC and cation-exchange HPLC (PELLEGRINO *et al.*, 1992; MAYER *et al.*, 1997) and recently, those based on molecular methods to identify the mitochondrial DNA present in the somatic cells of the milk (BRANCIARI *et al.*, 1999, 2000, 2001; REA *et al.*, 2001). The validity of the different methods is strictly linked to the specific problem to be faced and each time should be evaluated with respect to the cost/benefit obtained.

In the official control laboratories the use of a reference method is absolutely necessary. The one presently in force (O. J., 1996) is based on the isolation of caseins from the cheese followed by their solubilization and hydrolysis with plasmin to convert β-caseins to γ-caseins. The protein fractions obtained are then submitted to isoelectric focalisation in the presence of urea and the profiles are then compared with those obtained from standard products of known composition. Because this method is complicated and only a few laboratories in Italy are accredited for carrying it out, it appears that modifications should be made to facilitate its use and to obtain results faster, while still ensuring reliability of the method.

Furthermore it is necessary that the method can also quantify bovine milk. The reference method, in fact, allows qualitative evaluation only. Quantitative evaluation is therefore very important for some PDO cheeses (e.g. "Casciotta d'Urbino") in which precise quantities of bovine milk (20-30%) have been

specified. For these types of products it is therefore necessary to verify the percentages of milk used. In this paper a rapid screening method useful to detect and quantitatively determine bovine milk in sheep cheese is described.

MATERIAL AND METHODS

Materials

Certified reference material CRM 599, consisting of sheep/goat rennet with 0% and 1% bovine milk, was obtained from Lab Service Analytica (Anzola Emilia, Bologna, Italy). Standards of sheep caseins with a known amount of casein from bovine milk (containing, respectively, 1, 5, 10, 30 and 50%) were prepared in the laboratory in accordance with the reference method (O.J., 1996). Pecorino cheese samples were obtained from the market. Plasmin was obtained from Roche Diagnostics (Milano, Italy). Phast System, densitometric apparatus and software were purchased from Pharmacia (Uppsala, Sweden). Ready-to-use gel plates (Phast-Gel 4-6.5) and Ampholine pH 3.5-9.5 were obtained from Amersham Bioscience (Uppsala, Sweden). Biolyte pH 5-7 and pH 6-8 were obtained from Bio-Rad Laboratories (Segrate, Milano, Italy). All other chemicals, furnished by different dealers, were reagent grade.

Sample preparation and plasminolysis

The extraction of caseins from pecorino cheese samples and plasminolysis of these caseins, of the certified reference material and of the standard were conducted according to the procedures for the reference method (Mu), Regulation EC 1081/96 (O.J., 1996). They were also tested using the rapid method (Mr) described here: about 8 g of dry cheese or 10 g of fresh cheese were suspended in 25 mL of sodium tetraborate buffer solution (0.05 M pH 9) and homogenized for about 1 min. After being maintained at room temperature for 15 min, the suspension was centrifuged at 3000 rpm for 10 min at 10°C and the fat layer was discarded. The supernatant (0.5 mL) was placed in a screw-tap test tube containing 12 µL of bovine plasmin solution and the suspension was incubated at 37°C for 1 h with shaking. Plasminolysis was quenched by adding 0.5 mL of 24% trichloroacetic acid and the product was centrifuged at 2,000 rpm for 5 min at room temperature. After discarding the supernatant the precipitate was recovered by dissolving it in 0.5 mL 9 M urea containing 3% β-mercaptoethanol.

Electrophoretic analysis

The electrophoretic analysis of the solutions was performed on a Phast System using both gel plates (Lu) prepared according to the reference method, cut to be used in this type of apparatus, and ready-to-use gel plates (Lp) (Phast-Gel IEF pH 4-5.6) dipped for 90 min in an ampholine-urea solution prepared as follows: to 48 g of urea weighed in a 100 mL flask, 1 mL of Triton-X 100, 8 mL of Ampholine pH 3.5-9.5, 4 mL of Biolyte pH 5-7 and 4 mL of Biolyte pH 6-8 were added and brought to mark with water.

After eliminating excess liquid, the gel plate was held on a separation desk and used for electrophoresis. The separation procedure for both types of gel plates was carried out with the Phast System program described at point 6.3.2 of the Reference method (Mr).

Development and quantitative evaluation of electrophoretic patterns

Fixing and staining of the electrophoretic patterns was performed automatically in the development section of the Phast System using the procedure described at point 6.4 of the Reference method (Mr).

After separation and development of the electrophoretic patterns, for each type of γ-casein, up to three different bands (γ1, γ2, γ3), related to the casein concentrations, could be observed on the plate.

Visual evaluation of the patterns was used to verify the presence of bovine γ-casein bands, while the quantitative determination of bovine caseins was performed by densitometric scanning of the patterns, calculating the ratio (Ra) between the relative area of bovine γ-caseins and the sum of all γ-caseins.

$$Ra = \frac{\Sigma\gamma_{bovine}}{\Sigma\gamma_{bovine} + \Sigma\gamma_{sheep}}$$

To calculate the percentage of bovine milk in the cheese samples, the Ra value was interpolated on the straight calibration line, obtained from the analysis of the standards, by plotting the known amount of bovine milk against the relative (Ra) ratio.

RESULTS AND DISCUSSION

Evaluation of standards on different gel-plates

Electrophoretic separation using ready-to-use gel plates (Lp) as well as gel plates prepared according to the reference procedure (Lu) was performed on standards with a known amount of bovine milk, extracted and submitted to plasminolysis with the reference procedure.

Examples of patterns of the two gels are reported in Figs. 1 and 2, respectively.

Figs. 3 and 4 show the densitometric profiles of the electrophoretic separation of standards on Lp and Lu reported in Figs. 1 and 2.

Table 1 reports the theoretical bovine milk content of the standards, the calculated ratio (Ra) and the bovine casein amounts evaluated on the straight calibration lines (Y1 and Y2) both after separation on ready-to-use gel plates

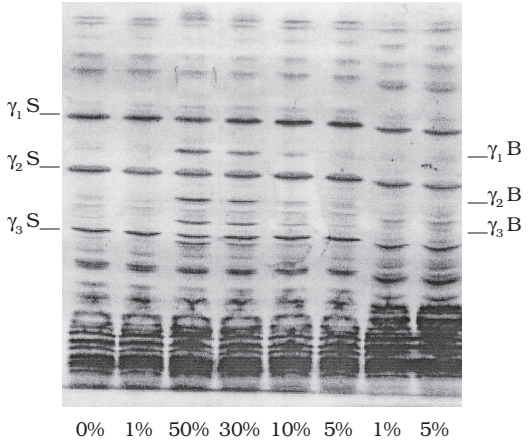


Fig. 1 - Electrophoretic separation of standards on ready-to-use gel plate (Lp).

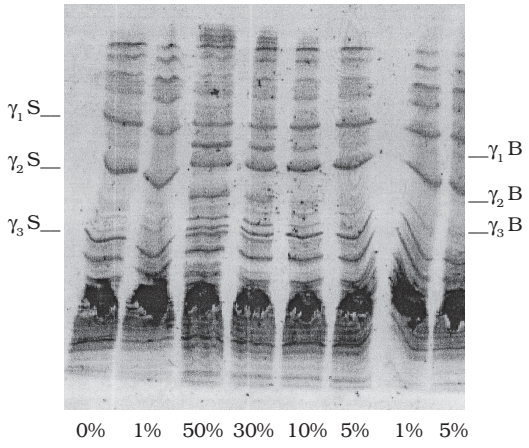


Fig. 2 - Electrophoretic separation of standards on gel plate prepared according to the reference procedure (Lu).

In both Figs. 1 and 2, for each pattern, the percentages of bovine milk of the standard sheep caseins used are indicated; the positions of sheep ($\gamma_{1,2}$ and γ_3 S) and bovine ($\gamma_{1,2}$ and γ_3 B) caseins are also shown.

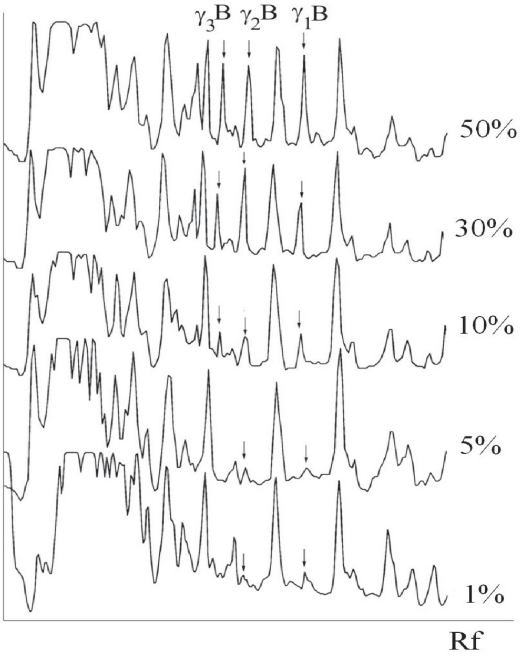


Fig. 3 - Densitometric profiles of standards obtained using ready-to-use gel plate (Lp).

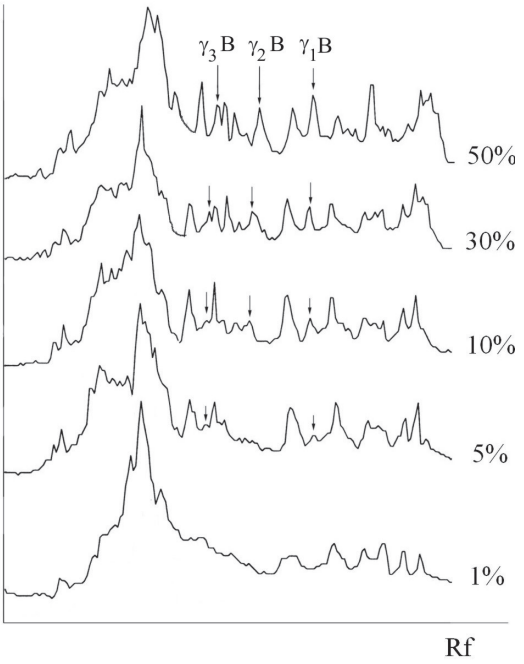


Fig. 4 - Densitometric profiles of standards obtained using gel plate prepared according the reference procedure (Lu).

(Lp) and after separation on plates prepared according to the reference procedure (Lu).

The data show how the 1% standard could be clearly seen using the ready-to-use gel plates (Lp). The presence of 1% bovine milk in pecorino cheese could not be seen using plates prepared according to the reference procedure (Lu). It is possible that gels prepared according to the reference procedure contain air bubbles or impurities and do not allow the thinnest bands to be seen. In this case the entire procedure should be repeated.

Table 1 - Results obtained for standards using ready-to-use gel plates (Lp) and gel plates prepared according to the reference procedure (Lu).

Percent of bovine milk (theoretical)	Area ratio (Ra) on Lp	Area ratio (Ra) on Lu	Percent bovine milk Determined on Lp (Y1)*	Percent bovine milk Determined on Lu (Y2)**
0	-	-	-	-
1.0	0.127	-	1.0	-
5.0	0.143	0.054	5.8	4.1
10.0	0.159	0.135	10.6	12.2
30.0	0.235	0.216	33.4	26.3
50.0	0.283	0.400	47.8	51.5

* Y1 straight calibration line on Lp (r=0.9940; r^2=0.9880); ** Y2 straight calibration line on Lu (r=0.9848; r^2=0.9698).

The use of a standard with 1% bovine milk is essential because it has the dual purpose of evaluating if the method reaches the required sensitivity and of establishing the lowest limit for which samples are considered negative.

False positives were never obtained, even in the presence of a thin band corresponding to the γ-2 fraction of bovine casein, as observed in Fig. 1, because the calculation made using the above mentioned formula (Ra) never gave percentages of bovine milk over 0.5%. It is possible to state that the use of ready-to-use gel plates, which are always homogeneous, improved the visibility of the bands and thus the performance of the entire analytical procedure.

Analysis of pecorino cheese samples using different preparation methods

Electrophoretic separation using plates prepared according to reference procedure (Lu) was performed on 4 pecorino cheese samples (a, b, c, d) extracted and submitted to plasminolysis with the rapid method (Mr) and the reference procedure (Mu).

Examples of patterns of a sample obtained by the two extraction procedures are reported in Figs. 5 and 6, respectively.

The preparation of a sample with the reference procedure (Mu) gave the same electrophoretic profiles as that obtained with the rapid method, although the rapid method (Mr) uses variable quantities of casein, in relation to the water content of the sample (e.g. in relation to the ripening of the cheese), and the reference method (Mu) works with constant and exact casein concentrations (25 mg).

Therefore the reference method (Mu) could be considered more reliable, since the use of exact quantities of caseins should result in better repeatability. Nevertheless, considering that the evaluation of the amount of milk used in cheese making is based on the ratio between the intensity of bovine γ-caseins and that of total γ-caseins, the quantity of caseins an-

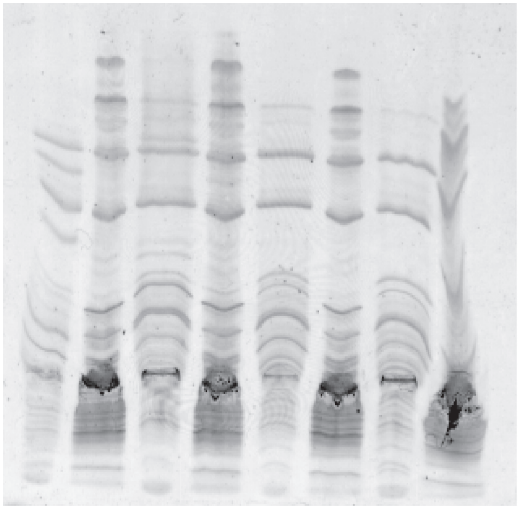


Fig. 5 - Electrophoretic separation of four samples extracted by the rapid method (Mr) and analysed on Lu.

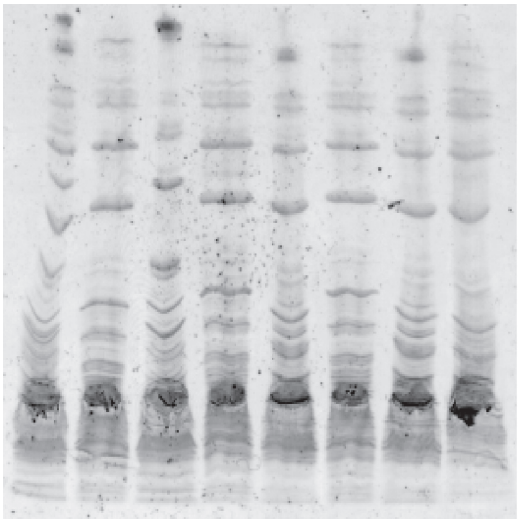


Fig. 6 - Electrophoretic separation of four samples extracted by the reference procedure (Mu) and analysed on Lu.

alysed cannot influence the results and does not represent a restrictive condition for applying the rapid method (Mr). Low quantities of caseins extracted from products with a high water content could also affect the intensity of the electrophoretic bands, thus limiting the sensitivity of the analytical procedure. However the characteristics of the pecorino cheese commonly marketed (normally ripened for almost two months) guarantees the validity for the proposed application

The percentage of bovine milk was calculated on 4 other cheese samples, obtained from markets labelled A, B, C and D, and extracted and submitted to plasminolysis with both methods (Table 2).

The data reported in Table 2 show that (Mr) did not provide "false positive" results; its sensitivity was slightly lower than that of (Mu) even though the percent variation coefficient was low; finally the limit of detection of bovine milk using (Mr) (about 5%) was low enough to allow it to be used to indicate the absence of fraud. In fact small quantities of proteins of bovine origin in PDO pecorino cheeses could be due to the use of lacto graft and so possible frauds lose their significance if the substitution of the worthier sheep milk with bovine milk is performed in a very low percentage.

Analysis of pecorino cheese samples using different gel plates

Analysis of pecorino cheese using ready-to-use gel plates (Lp) as well as gel plates prepared according to the reference procedure (Lu) was performed on 4 cheese samples (Eu, Fu, Gu, Hu) extracted and submitted to plasminolysis with the reference procedure (Mu). Examples of patterns of the two gels are reported in Figs. 7 and 8, respectively.

Six other pecorino cheeses (1-6) were extracted and submitted to plasminolysis with the official method (Mu). The extracts were placed together with the standard 10% bovine milk on both electrophoretic plates. In this way both types of gels gave the same results with small variability, despite the fact that the samples contained rather small amounts of bovine milk. This was confirmed by the comparison between the percentage obtained with the two procedures. The values of the percent variation coefficient (CV%) of the bovine milk content of various samples (Table 3) also confirm that the use of different types of gel slides offers comparable results.

It can see that ready-to-use gel plates (Lp) allowed the best qualitative and quantitative results with respect to those obtained from gel plates prepared according to the reference method (Lu). In fact on Lp the electrophoretic traces were always clearer and more homogeneous and the bands were more intense. This allows precise densitometric evaluations to be obtained and lower percentages of bovine milk to be determined.

Table 2 - Results of cheese analysis using the rapid method (Mr) and the reference procedure (Mu) for preparation and plasminolysis of samples.

Cheese sample	% Bovine milk determined using (Mu)	% Bovine milk determined using (Mr)	Percent variation coefficient
A	30	26	7
B	23	18	12
C	35	31	6
D	5	4	11

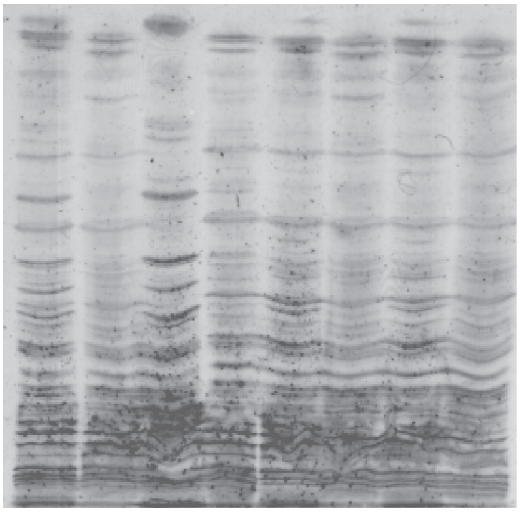


Fig. 7 - Isoelectrofocusing of four samples extracted by the reference procedure (Mu) and analysed on Lp.

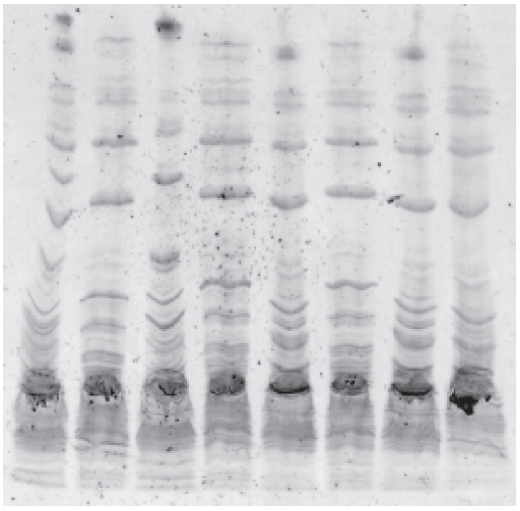


Fig. 8 - Isoelectrofocusing of four samples extracted by the reference procedure (Mu) and analysed on Lu.

Table 3 - Comparison between the percentages of bovine milk estimated through electrophoretic analysis conducted on ready-to-use gel plates (Lp) and gel plates prepared according to the reference procedure (Lu).

Sample	% Bovine milk (Lp)	% Bovine milk (Lu)	CV%
1	7.1	5.7	11
2	7.0	5.6	11
3	5.0	3.9	12
4	6.0	4.7	12
5	5.6	6.3	6
6	6.6	6.9	2

CV% = percent variation coefficient between results obtained for the same sample.

CONCLUSIONS

The rapid method of sample preparation and plasminolysis (Mr) did not give false negatives, but resulted in a slightly lower sensitivity in comparison to the reference procedure (Mu). In fact, using (Mr) there may be more interference in the electrophoretic gel plate due to poorer purification of the matrix. This did not allow very small quantities of bovine casein (<4%) in sheep cheese to be detected. Regulations for PDO pecorino cheese production absolutely forbid the use of proteins of bovine origin (e.g. the use of whey inoculation or that of bovine curds), thus the use of (Mr) is not advisable to guarantee the genuineness of PDO sheep cheeses. The proposed rapid method of extraction and plasminoly-

sis (Mr), due to its simplicity and rapidity, could be used as a screening method in routine laboratory analysis for common cheeses.

The use of ready-to-use gel plates offer various advantages:

- greater sensitivity as the electrophoretic patterns are well-defined with intense and precise bands;
- more repeatable results as the bands are more easily quantified by densitometry;
- faster results because the gel plates are already prepared;
- avoids laboratory manipulation of acrylamide and NN'-methylenebisacrylamide, the use of which is correlated with health risks for the operators;
- avoids the use of gels containing air bubbles and/or impurities which can be present in laboratory prepared plates;
- provides gel plates with a defined composition and precise thickness.

Thus we suggest the use of ready-to-use gel plates (Lp) to carry out the analytical method, with the aim of simplifying and improving the performance of the methodology. Furthermore, the method described is very useful for quantitative evaluations that can be performed on Lp and with the aid of standards with various concentrations of bovine milk.

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PAPER

STUDY ON pH LOWERING IN PRESERVED ASPARAGUS CREAM

STUDIO SULLA RIDUZIONE DEL VALORE DI pH IN CREMA DI ASPARAGI

A. DEROSSI*, L. PALMIERI[1], A. DE GIORGI[1], R. LOIUDICE[1] and C. SEVERINI
Dipartimento di Scienze degli Alimenti, Facoltà di Agraria, University of Foggia, Via Napoli 25, 71100 Foggia, Italy
[1] Stazione Sperimentale per l'Industria delle Conserve Alimentari (SSICA), Via Nazionale 121, 84012 Angri (SA), Italy
*Corresponding author: antonioderossi@yahoo.it

ABSTRACT

The acidification process is an important operation to stabilize canned vegetables. Many variables (temperature, time, etc.) can influence the final results. Two different treatments were compared to evaluate their acidification capacity: a) blanching in an acidified solution and b) addition of acids to blanched and homogenized asparagus. The effect of the combination of acids on the pH of the products was studied by Central Composite Design. In both treatments, lactic acid was more effective in lowering the

RIASSUNTO

Per le conserve vegetali l'acidificazione è un importante trattamento stabilizzante, il cui esito dipende da numerose variabili. In questo lavoro sono stati confrontati due metodi di acidificazione: a) blanching in soluzione acquosa di una miscela di acidi; b) aggiunta diretta di acidi alla matrice vegetale (asparagi) scottata in acqua e triturata. Gli effetti delle combinazioni degli acidi sul pH finale del prodotto sono stati studiati applicando un Central Composite Design. L'acido lattico è stato l'acidificante più

- Key words: acids, asparagus, blanching, Central Composite Design, vegetable creams -

pH than citric acid and gluconic-δ-lactone (GDL). Good results were obtained by applying method b) to pasteurized products, because hydrolysis of GDL was favoured.

efficace. In particolare, i migliori risultati sono stati ottenuti con il metodo b) dopo pastorizzazione poiché le elevate temperature hanno favorito l'idrolisi del glucono-δ-lattone.

INTRODUCTION

In recent years there has been an increase in interest in *Asparagus officinalis* in the Mediterranean area, for both its organoleptic features and its nutritional value. For this reason, some studies have been conducted on genetic improvement of the cultivars, as well as the optimization and innovation of systems of cultivation and marketing. Furthermore, food industries have increased their interest in asparagus to obtain canned vegetables. In particular, second-rate products are normally used to produce vegetable creams.

There have been only a few studies on optimizing the industrial process (RODRIGO *et al.*, 1997, 1998; LAU *et al.*, 2000). Lowering of the pH is considered one of the most important technological operations to stabilize preserved vegetables. With acidifying blanching the vegetable pH can be reduced to 4.6, which is the upper limit recommended by the Food and Drug Administration (2002) to assure the safety of canned food. This pH value assures the prevention of germination, growth and toxin production by *Clostridium botulinum* in acidified food.

Another important treatment to assure the safety of canned vegetables is pasteurization or sterilization. Pathogenic or non pathogenic microorganisms that can grow even at low pH are inactivated by a correct heat treatment. The acid blanching routinely used in food processing industries may be affected by a number of parameters (time, temperature, surface contact between the product and the acid solution, acid concentration, acid solubility) (YANG and YEN, 1998, JACKSON *et al.*, 1996). Furthermore, the pH value of the blanched product can be affected by the release of organic compounds from the product to the solution, by acid transfer from the solution to the product and by a decrease in the solution-product ratio (ABDEL, 1992, GEKAS *et al.*, 1993, SAM *et al.*, 1994). Some researchers have studied (JACKSON *et al.*, 1996, YANG and YEN, 1998) the effect of process variables on blanching treatment by Central Composite Design.

It is well known that the organic acids, normally used to acidify vegetables for canned foods, have different abilities to lower the pH, mainly depending on the number of acid functions, the k_a of the acid and the pH of the medium. Likewise, the effect of the type of acid on the taste of the product needs to be taken into account. For these reasons optimization of the blanching process to obtain a standardized pH value of the final product is one of the most difficult steps in preserved vegetable manufacturing.

Recently gluconic-δ-lactone (GDL) has been studied as a new compound to acidify canned vegetables. This compound is commonly obtained from *Aspergillus niger* (FIEDUREK, 2001) and *Acetobacter suboxidans* and its slightly sweet taste produces a low impact on the organoleptic features of treated foods. Some studies carried out by PREVIDI and GOLA (1990) on vegetables (carrots, mushrooms and fennel) showed that products submitted to acidification with GDL and heat treated had good organoleptic characteristics. They obtained canned vegetables with good characteristics (fla-

vour, colour and firmness) when gluconic-δ-lactone was added to the final solution in the can. Fish (trout fillet), treated with gluconic-δ-lactone and stored at 4°C, had a prolonged shelf life under protective atmosphere (PREVIDI and GOLA, 1990). In addition, several studies have been carried out on the operating conditions of blanching and their effects on the quality of treated vegetables (LEDENEVA, 1990, SANCHEZ PINEDA *et al.*, 1996). MCGLYNN *et al.* (1993) studied the influence of gluconic acid on the texture and colour of canned asparagus. RODRIGO *et al.* (1999) evaluated the effect of gluconic-δ-lactone on the quality of canned acidified mushrooms and showed that it appeared to be more suitable than citric acid; in fact, it did not confer as acid a taste as citric acid or an extraneous flavour.

Instead, there are few papers in the literature concerning the direct addition of organic acids to vegetable creams. The great importance of this method of acidification is the reduction of the process variables that influence the pH in vegetable foods. The aim of this study was to compare the difference in pH lowering effectiveness between conventional acid blanching and non-conventional blanching using response surface methodology. In this way the composition of the acidifying solution could be optimized and this step of the process could be simplified.

MATERIALS AND METHODS

Thirty-four trials of acidification were performed on samples of *Asparagus officinalis* purchased on the local market. Three sets of operating conditions were considered:

(A) Blanching in acidified solution (conventional method). The solution was prepared with 1% (w/w) NaCl (Carlo Erba, Milan, Italy) and citric (Carlo Erba, Milan, Italy) [CITR], lactic (Carlo Erba, Milan, Italy) [LAT] and gluconic (Sigma Aldrich chemie, Milan, Italy) [GLC] acids added following the concentration called for by a three-factor five-level Central Composite Design (CCD 1);

(B) Addition of citric, lactic and gluconic-δ-lactone (Sigma Aldrich chemie, Milan, Italy) [GDL] acids to blanched (solution of 1% (w/w) NaCl), ground and homogenized asparagus (non-conventional method), according to the concentration called for by a three-factor five-level Central Composite Design (CCD 2);

(C) Application of the same operating conditions as B samples and successive pasteurization (100°C for 30 min) (CCD 3).

For planning the Central Composite Design, some preliminary tests were performed to determine the level of concentration of the acids (citric acid, lactic acid, gluconic acid and gluconic-δ-lactone) in order to obtain a pH value of the cream ranging from 4.0 to 4.3, by using a single acid (data not shown). The product/solution ratio was 1/6 and the time of treatment was 10 min. The grinding-homogenization was performed with an electric mixer (Tecator 1094, Hoganas, Sweden) and the pH measurements were carried out with a model micropH 2002 pH meter (Crison Instruments, Allen, Spain). At least three replications were performed per trial.

Experimental design

The values chosen for each independent variable (Table 1) were within the ranges usually considered in industrial canned vegetable production. The effects of each factor, considered singularly and in combination with the others, were investigated by modulating their levels according to a three-factor, five-level Central Composite Design. The 17 combinations obtained are reported in Table 2. The fractional experimental plan reduces the total number of possible combinations to a manageable size.

Table 1 - Levels of the 3 variables considered in the experimental design applied to the acidifying treatments (CCD).

Codified levels	Citric acid (%w/w)	Lactic acid (%w/w)	Gluconic acid or gluconic-δ-lactone (%w/w)
-2	0.10	0.10	0.10
-1	0.15	0.15	0.15
0	0.20	0.20	0.20
+1	0.25	0.25	0.25
+2	0.30	0.30	0.30

Table 2 - Codified levels for the 3-factor 5-level Central Composite Design (CCD).

Runs	Citric acid (% w/w)	Lactic acid (% w/w)	Gluconic acid or gluconic-δ-Lactone (% w/w)
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1
9	0	0	0
10	0	0	0
11	-2	0	0
12	+2	0	0
13	0	-2	0
14	0	+2	0
15	0	0	-2
16	0	0	+2
17	0	0	0

Statistical analysis

pH measurements performed on samples and acid solutions after each blanching treatment were submitted to statistical analysis by a stepwise regression to identify the relevant variables and a multiple regression (Standard Least Square Fitting) to fit a second order mathematical model using the following polynomial equation:

$$y = B_0 + \Sigma B_i x_i + \Sigma B_{ii} x^2 + \Sigma B_{ij} x_i x_j$$

where *y* is the modelled independent variable (pH value of the processed sample), B_i, B_{ii} and B_{ij} are the regression coefficients of the model. Variables showing a significance less than 99% ($p<0.01$) were not included in the equation. This model allowed the effects on the dependent variable (pH) of linear (i), quadratic (Σx_i^2) and synergistic terms ($\Sigma x_i x_j$) of the independent variables ([citric acid], [lactic acid] and [gluconic acid]) to be assessed. In order to describe the individual and synergistic effects of the independent variables on the positive outcome of the acidifying treatments (in terms of final sample pH), regression iso-response surfaces were developed imposing a constant value to one of the independent variables at the

central point of the CCD. Statsoft (ver. 5, Tulsa, USA) software was used.

RESULTS AND DISCUSSION

The pH values measured on *Asparagus officinalis* samples (raw material) showed a natural variability of 0.2 to 0.3 units (average between 7.03 and 7.30). For this reason the range of pH considered was between a maximum value of 4.3 and a minimum of 4.0 (below this value the taste of vegetables is generally considered too acid).

The partial equations with respect to individual and synergistic effects of the independent variables on the final pH of samples are shown in Table 3. The goodness of the used polynomial models was evaluated by the coefficients of correlation (r), p levels and standard error values (SE).

When the traditional method of acidification was used, the synergic effect of lactic and citric acids with GLC was balanced by the positive values of the quadratic terms of GLC, so its influence was the lowest (equations 1a and 2a). Lactic and citric acids showed a good effectiveness in pH lowering (equation 3a) in particular as individual terms. The best combinations of the three acids in the blanching solution are shown in Figs. 1, 2 and 3. In fact, if the target is to bring down and keep the pH between 4.0 and 4.3, both lactic (Fig. 1) and the citric (Fig. 2) acids had to be more than 0.12%, when used with GLC. Instead, using lactic and citric acids, when 0.10% (minimum value) lactic acid was added, a concentration of citric acid of about 0.26% was necessary to obtain a pH value of 4.3. The same pH value could be obtained by adding the minimum concentration of citric acid (0.10%) when 0.23% of lactic acid was added (Fig. 3).

The cooled addition of acids to the

Table 3 - Partial equations processed imposing a constant value to one of the independent variables at the central point of the CCD.

Partial equations		
r = 0.9677	p<0.000001	SE = 0.055
Partial equation 1a (GLC-LAT)	$y = 4.8032+7.5822[GLC]^2-2.0185[GLC]-13.4764[GLC][LAT]$	
Partial equation 2a (GLC-CITR)	$y = 4.8032+7.5822[GLC]^2-10.0925[GLC][CITR]-2.695[GLC]$	
Partial equation 3a (CITR-LAT)	$y = 5.1062-2.0185[CITR]-2.695[LAT]$	
r = 0.959	p<0.000002	SE = 0.13741
Partial equation 4b (GDL-LAT)	$y = 5.0202+5.2682[GDL]-13.3872[LAT]^2-2.1744[LAT]+29.316[LAT][GDL]$	
Partial equation 5b (GDL-CITR)	$y = 4.4847+0.595[GDL]-2.1744[CITR]$	
Partial equation 6b (CITR-LAT)	$y = 3.9665-13.3872[LAT]^2-10.8722[CITR][LAT]+5.8632[LAT]$	
r = 0.7407	p<0.004	SE = 0.11107
Partial equation 7c (GDL-LAT)	$y = 3.3557-3.1091[LAT]^2$	
Partial equation 8c (GDL-CITR)	$y = 3.6858-4.7505[CITR]^2$	
Partial equation 9c (CITR-LAT)	$y = 4.30766-4.7505[CITR]^2-3.1091[LAT]^2$	

a: acid blanching;
b: cooled addition of acids after grinding and mixing;
c: b samples after pasteurization.

ground product led to a decrease of the pH until the desired value only for high concentrations of GDL (0.20-0.30%) and medium concentrations of lactic acid (0.15-0.25%), when the citric acid content was constant (Fig. 4). Moreover, for high concentrations of lactic acid, an increase of GDL led to an increase of the pH. These results were confirmed by data (not shown) obtained in model systems. In fact, the increasing addition of GDL to an aqueous solution of citric and lactic acids induced the expected decrease of the pH, but the use of the same solution for blanching of the asparagus induced its increase, probably due to interactions with organic compounds of the product.

Following this non-conventional method of acidification, the influence of lactic acid, either as individual or quadratic term, was the highest (equations 4b, 5b and 6b). This effect was likely because lactic and citric acids have a similar pk_a (3.07 for lactic acid and 3.13 for the first acid function of citric acid, respectively), but the molecular weight is lower for lactic acid (90 vs 178); so for the same concentration (w/w), lactic acid was more present in comparison to citric acid. The influence on pH of second and third acid functions of citric acid is negligible because they are not dissociated at the pH level of the samples.

In this method, too, when 0.10% citric acid was added (with a constant concentration of 0.20% lactic acid), the GDL was not able to bring down the pH value

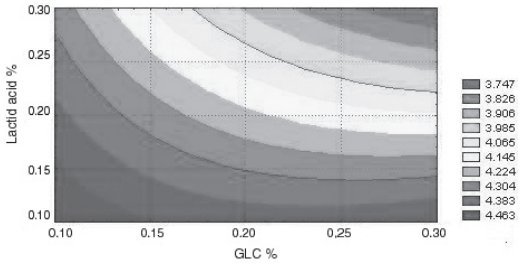


Fig. 1 - Plot of lines and levels for the effects of lactic acid and gluconic acid (GLC) interactions on pH values of *Asparagus officinalis* samples produced according to the A operating conditions.

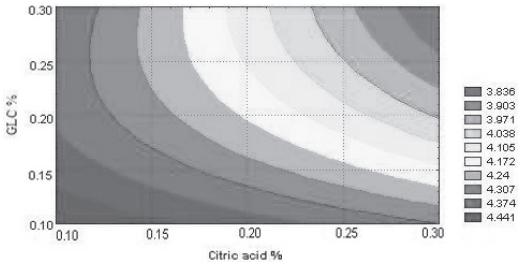


Fig. 2 - Plot of lines and levels for the effects of citric acid and gluconic acid (GLC) interactions on pH values of *Asparagus officinalis* samples produced according to the A operating conditions.

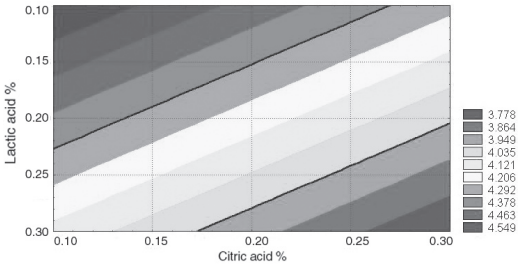


Fig. 3 - Plot of lines and levels for the effects of lactic acid and citric acid interactions on pH values of *Asparagus officinalis* samples produced according to the A operating conditions.

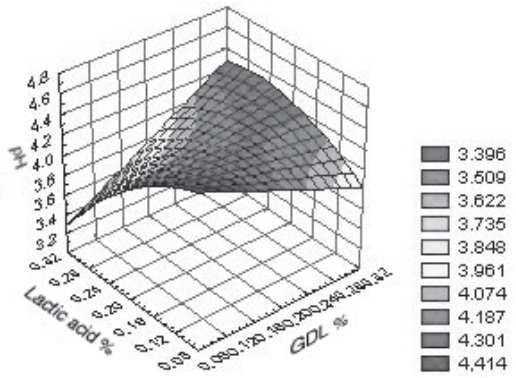


Fig. 4 - Surface plot for the effects of lactic acid and gluconic-δ-lactone (GDL) interactions on pH values of *Asparagus officinalis* samples produced according to the B operating conditions.

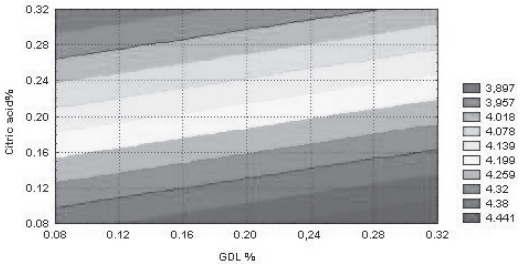


Fig. 5 - Plot of lines and levels for the effects of citric acid and gluconic-δ-lactone (GDL) interactions on pH values of *Asparagus officinalis* samples produced according to the B operating conditions.

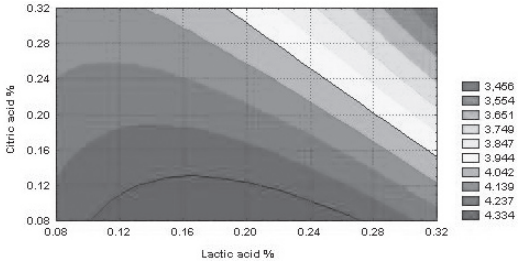


Fig. 6 - Plot of lines and levels for the effects of citric acid and lactic acid interactions on pH values of *Asparagus officinalis* samples produced according to the B operating conditions.

to 4.3 (Fig. 5). With a constant concentration of GDL (Fig. 6), lactic and citric acids can be variously combined, even if it was not advisable to use high concentrations of lactic acid that may lead to an excessive decrease of the pH with high concentrations of citric acid.

It is well known, the GDL exhibits its acidifying effect only after heating, in the GLC form. So, the effect of GDL on pH lowering needs to be taken into account when the products have to be pasteurized.

After heat treatment, the results show that the influence of the increasing addition of GDL on the product pH did not seem to be significant. In fact, this variable is not present in the partial equation (Table 3) because it is not significant ($p>0.05$). The increase of GDL concentration in a range from 0.10 to 0.30% did not influence the pH lowering (data not shown).

As observed in the partial equations and figures, after pasteurization, as expected, all the pH values were lower than those of the non pasteurized products (Fig. 7). In addition, after pasteurization, several samples showed a pH value less than 4.0, considered too low for a good taste of the product.

CONCLUSION

Results showed that the balance of different acids was favourable to obtain pH lowering until the correct range of values.

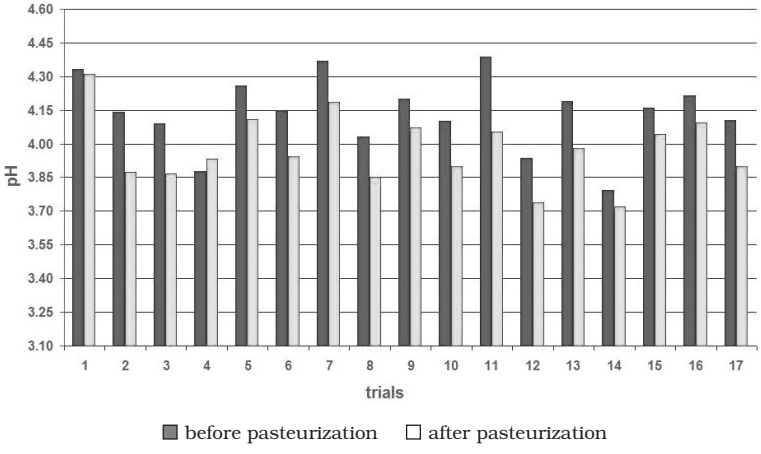


Fig. 7 - pH values of samples following B (CCD 2) and C (CCD 3) operating conditions.

In this way, the use of citric acid that has a strong organoleptic impact on acidified products, could be reduced.

The cooled mixing of acids with the ground product allowed the same results to be obtained as with traditional acid blanching in terms of safety, using a lower quantity of lactic and citric acids and counting on a greater number of combinations.

The choice of acids proved to be important. Lactic acid was more effective than citric acid at the same concentration. Gluconic-δ-lactone, chosen for its mild taste, was partially effective in traditional blanching and it lowered the pH only after heat treatment, in the proposed non conventional method. Its use has to be particularly regulated if applied to pasteurized products, so good results can be produced enforcing the effect of the other acids.

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PAPER

MICROBIAL ANTAGONISM OF *LACTOBACILLUS CASEI* ADDED TO FRESH VEGETABLES

ANTAGONISMO MICROBICO DI *LACTOBACILLUS CASEI* AGGIUNTO A PRODOTTI DI QUARTA GAMMA

G. SCOLARI* and M. VESCOVO
Istituto di Microbiologia, Università Cattolica del Sacro Cuore,
Via Emilia Parmense, 84, 29100 Piacenza, Italy
* Corresponding author: e-mail: gianluigi.scolari@unicatt.it

ABSTRACT

The ecological aspects of Scarola salad leaves, mechanically damaged and singly inoculated with *Lactobacillus casei*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Escherichia coli* and *Listeria monocytogenes*, were studied. SEM observations and bacterial counts of the damaged tissues or intact leaf surface demonstrated that the added cells of each species mainly colonized the cut-edges, trichomes and stomata of the salad leaves. Challenge experiments performed on salads inoculated

RIASSUNTO

Sono stati studiati gli aspetti ecologici delle foglie di insalata Scarola, meccanicamente danneggiate e singolarmente inoculate con *Lactobacillus casei*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Escherichia coli* e *Listeria monocytogenes*. Le osservazioni in microscopia SEM e le conte microbiche sulla superficie fogliare integra o lungo le sezioni di taglio, hanno dimostrato che le cellule di ciascuna specie aggiunta colonizzano prevalentemente le parti danneggiate, i tricomi e gli stomi. Gli

- Key words: foodborne pathogens, food safety, *Lactobacillus casei*, produce -

simultaneously with *Lactobacillus casei* and each individual pathogen showed an inhibitory effect by the lactic culture towards all the pathogenic strains. The protective effect of the antimicrobical-producing *L. casei* strain in relation to bacterial cell location is discussed.

esperimenti di competizione condotti su foglie di insalata, inoculata simultaneamente con *Lactobacillus casei* e ciascun singolo patogeno, hanno evidenziato un effetto inibitorio da parte della coltura lattica nei confronti di tutti i patogeni esaminati. La tendenza delle cellule aggiunte ad accumularsi nelle stesse nicchie ecologiche può spiegare l'effetto protettivo dimostrato dai batteri lattici produttori di sostanze antimicrobiche.

INTRODUCTION

In recent years, increasing consumer demand for convenience food items has led to a wide diffusion of new categories of ready-to-eat vegetable products. Freshness, economic handling and attractive presentation are essential characteristics for their progressive success, and there is no need for further washing, slicing or chopping (NGUYEN-THE and CARLIN, 1994; VESCOVO *et al.*, 1997).

Pathogens can readily contaminate fresh produce via contact with the decaying vegetation, soil, polluted water, manure or sewage sludge (BEUCHAT, 1998). In addition, contamination with pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* may also occur during post-harvest handling, processing, distribution and marketing, as demonstrated by several researchers (BERRANG *et al.*,1989; BEUCHAT and BRACKETT, 1990; ODUMERU *et al.*, 1997).

To preserve the typical fresh character only mild stabilizing treatments are used on such products, combining chemical and physical barriers (chlorine, low temperatures, modified atmosphere packaging) (VESCOVO *et al.*, 1997). However, controversial opinions exist among researchers about the effectiveness of classical sanitizers like chlorine, as it does not assure the complete elimination of the contaminating microorganisms and can reduce the image of freshness of the product. In fact, it is well known that the difference of total microbial counts between vegetables either water washed or treated with 200 ppm chlorine, is usually not more than 1 log CFU/g (BEUCHAT, 2002), probably due to the presence of protective hydrophobic pockets and crevices on the leaf surface (ADAMS *et al.*, 1989) or an internalizaton process within the growing plants (WARRINER *et al.*, 2003). Furthermore, the presence of stomata, trichomes and cut edges can provide ecological niches suitable for pathogen survival and reproduction (BEUCHAT, 1999; SEO and FRANK, 1999; TAKEUCHI and FRANK, 2000, 2001). These reasons stimulated food microbiologists to try new biological approaches to preserve minimally processed fruits and vegetables (VESCOVO *et al.*, 1995, 1996; CAY *et al.*, 1997; SCHUENZEL and HARRISON, 2002).

Lactic acid bacteria (LAB) may fulfil this purpose, due to their protective effects widely demonstrated in a large variety of foods. Their ability to produce organic acids, H_2O_2 (HARP and GILLILAND, 2003) and particularly antimicrobial compounds such as bacteriocins (STILES, 1996; O'SULLIVAN *et al.*, 2002) or bacteriocin-like substances (SCOLARI *et al.*, 1999, GANZLE *et al.*, 2000) explain their competitive action towards undesirable microorganisms.

However, a better understanding of the microbial ecosystem of raw vegetable surfaces would be extremely useful when developing strategies to minimize contamination and to prevent pathogen growth at various stages of processing, marketing and preparation for consumption.

The objective of this study was to investigate the microbial ecosystems of Scarola salad leaves, contaminated with single pathogenic strains or inoculated with an antimicrobial-producing *Lactobacillus casei* strain. Moreover challenge experiments were carried out between the lactic culture and each pathogen, to evaluate the inhibition potential of *L. casei* in fresh vegetables during storage at 8°C (maximum temperature allowed for commercial storage).

MATERIALS AND METHODS

Salad

Endive Scarola salad (*Cichorium endivia Latifolium*, cultivar Congo) was purchased at a local supermarket and immediately utilized after transporting to the lab. Outer leaves were discarded, while the inner pale-coloured leaves were subjected to extensive washing in sterile milli-Q water containing 200 ppm chlorine at 25°C for 15 min, using a mild ultrasound water-bath. Treated samples were rinsed four times in sterile milli-Q water until the sanitizer was completely removed. They were then cut into 3x3 cm pieces with a sterile surgical knife and used for the experiments. Cuts were made on the leaf surface to simulate post-harvest mechanical damage.

Strain and culture conditions

L. casei LC34GF strain was selected among the various mesophilic LAB strains isolated from fresh vegetables and characterized for their peculiar inhibitory activity against pathogenic and spoilage microorganisms (VESCOVO *et al.*, 1996). Other characteristic traits of this strain, such as pectinolytic activity and growth at refrigeration temperatures (4°C), were absent.

E. coli Ct_1 and Ct_2 were isolated from raw milk, while *E. coli* EcV8 was from fresh mixed salads; *S. aureus* Sa4 and Sa5 strains were isolated from lettuce and basil, respectively, while *L. monocytogenes* LGB and Sp2 were isolated from cauliflower and smoked salmon, respectively. *Aeromonas hydrophila* 2 was kindly provided by Prof. A. Galli, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università di Milano, Italy. All the strains were stored in the collection of the Istituto di Microbiologia under liquid nitrogen until use.

L. casei LC34GF was cultured for 15 h at 30°C in MRS broth (Oxoid) under anaerobiosis. Each pathogenic strain was activated by three successive 24 h transfers in Tryptic Soy Broth (Difco) at 37°C in aerobiosis, except for *A. hydrophila* which was incubated at 30°C.

Inoculum procedure

Bacterial cells were collected from active cultures by centrifugation at 3,000 x g, 4°C, 15 min; pellets were washed twice and resuspended in sterile milli-Q water in order to obtain a level of approximately 6-7 log $CFUg^{-1}$ for the *L. casei* strain and 4-5 log $CFUg^{-1}$ for each pathogen on the salad leaves. The inoculum of each strain was sprayed singly on the whole leaf surface (30 $\mu L\ g^{-1}$) which was left 18 h at 4°C in a sterile cabinet to allow the complete absorption of the added cells; following two gentle rinses in sterile water to remove the non absorbed cells, and spinning in a manual centrifuge, samples were incubated 6 days at 8°C in sealed 90 µm thick nylon/polyethylene bags with permeability to oxygen and carbon dioxide of 40 and 150 cc m^{-2}

$24h^{-1}$, 23°C, respectively. Control samples were prepared using sterile water as inoculum.

Challenge experiments

Challenge experiments were carried out in two steps using the inocula of LAB and the pathogens described above. At first, salad leaves were sprinkled with the strain mixture of each pathogenic species and left 2 h for absorption in a sterile cabinet; then, LAB inoculum was added and the leaves were maintained for 18 h at 4°C to ensure complete absorption. Samples were gently rinsed two times and centrifuged as previously described, before incubation at 8°C for 6 days in nylon/polyethylene pouches. The same procedure was used on control samples except for the LAB inoculum which was substituted by sterile water.

Labelling of *L. casei* LC34GF strain

To evaluate the ability of the added LAB cells to colonize the Scarola salad leaves, *L. casei* LC34GF strain was transformed by electroporation with pPSC22 plasmid vector (COCCONCELLI *et al.*, 1996) carrying resistance to erythromycin and chloramphenicol. The transformation procedure was performed employing the apparatus and the protocol described by VESCOVO *et al. (*1991) with some modifications: the *L. casei* strain was grown up to 0.630 OD_{560}; pulse time was 3.8 ms and 1 h was required for gene expression. Transformed cells were selected on MRS containing 50 µg mL^{-1} erythromycin.

Bacterial counts

Strip samples (CE) from each experiment, for a total of 1 g, were removed from salad leaves 0.3 cm from each cut edge using a sterile surgical knife; 1 g of intact samples was taken from the remaining intact surfaces. All the samples were homogenized in 10 mL of sterile Ringer solution with a Stomacher Lab-Blender 400 for 2 min at maximum speed. Decimal dilutions were plated in MRS agar to which 50 µgmL^{-1} erythromycin was added for *L. casei* LC34GF strain.

L. monocytogenes populations were estimated plating the dilutions on PALCAM (polymixin acriflavine LiCl ceftazidime aesculin mannitol) agar (DIFCO) and incubating the plates at 30°C for 48 h; presumptive *L. monocytogenes* colonies were transferred to Tryptic Soy Agar (BBL) for confirmation by *L. monocytogenes* latex test (OXOID). Uninoculated Scarola samples were analysed for the presence of *L. monocytogenes* in Listeria Enrichment Broth (OXOID) using the above incubation conditions.

E. coli counts were performed, plating dilutions on VRBA containing 100 mgL^{-1} MUG supplement (OXOID); plates were incubated at 37°C for 24 h.

Baird Parker agar medium, with the addition of 5% egg yolk tellurite (OXOID), was used for *S. aureus* evaluation, after 48h incubation at 37°C; randomly picked colonies were subjected to confirmation by *S. aureus* latex test (OXOID).

A. hydrophila was enumerated plating dilutions on starch-ampicillin agar medium (PALUMBO *et al.*, 1985) and aerobically incubating plates at 30°C for 24h.

SEM observations

Salad samples, inoculated with each single strain and stored in the previous conditions, were dehydrated by increasing ethanol concentrations up to absolute alcohol with CO_2 to the critical point as described by SCOLARI and VESCOVO (1996), then coated with gold under vacuum. Observations were made with a Philips XL30 ESEM electron microscope. Samples for SEM observations were also taken from the challenge experiments between *L. casei* LC34GF and *S. aureus*.

pH measurement

pH was measured during storage with a Crison pH-meter (CRISON) fitted with a type HI1883B Mettler microelectrode (METTLER); the microelectrode was plunged into the samples of leaf homogenate (100 mg) obtained by grinding 10 g of salad leaves in a mortar.

Statistical analysis

Experiments on samples singly inoculated with strain mixtures of each species and challenge experiments were replicated four and three times, respectively. The data were analysed using the general linear models procedure of the Statistical Analysis Software (SAS Institute, Cary, N.C.). Significant differences were determined using Duncan's multiple range test; the level of significance employed for all tests was $\alpha = 0.05$.

RESULTS AND DISCUSSION

The pH measured in control sample homogenates ranged from 5.8 to 5.6 at day 0 and after 6 days of storage at the experimental conditions, while in the samples treated with the *L. casei* strain it was between 5.6 and 5.4 at the end of storage. No difference in pH was detected in salads singly inoculated with the mixture of each pathogenic strain compared to the control samples. Moreover, no growth of *S. aureus*, *E. coli*, *L. monocytogenes*, and *A. hydrophila* was detectable on the uninoculated leaves during storage.

The bacterial growth and death in challenge experiments carried out on salad leaves inoculated with both the *L. casei* LC34GF strain and the strain mixture of each pathogenic species, are summarized in Table 1; the growth kinetics of the control samples singly inoculated with each pathogen mix are also reported.

During the storage period, the inoculated LAB progressively colonized the substrate with increases between 1 and 2 log $CFUg^{-1}$. *S. aureus* was the most sensitive to the antagonistic effect of *L. casei* cells, as it was not detectable after 6 days at 8°C; the inhibition was already evident since the second day (2 log reduction).

A. hydrophila and *E. coli* followed a similar inhibition kinetics with a count decrease ranging from approximately 1 log $CFUg^{-1}$ on the second day to more than 5 log $CFUg^{-1}$ at the end of leaf sample incubation. On the contrary, *L. monocytogenes* was the most resistant among the examined species, although more than 1 log $CFUg^{-1}$ reduction was observed on the fourth day, and 2.4 log $CFUg^{-1}$ at the end of incubation time. The results are comparable to those reported by VESCOVO *et al.* (1996) using a *L. casei* strain isolated from vegetable products, thus suggesting that *L. casei* species could be a good candidate for studies on fresh vegetable preservation.

As the inhibitory potential of the *L. casei* LC34GF strain was more evident when used on fresh vegetables compared to other food matrixes (unpublished results), study of the ecological location of the microflora in the abiotic components of this ecosystem is of great interest and may offer new perspectives to explain the observed phenomena.

Some indications on these aspects are provided by the attachment of the different bacterial species to Scarola salad leaves after the artificial contamination. Bacterial counts related to the intact leaf surface and to cut edges, respectively, are shown in Table 2; the counts on the cut edge samples were significantly higher than those of the intact surface, showing differences of about 1.5; 0.9; 1.1; 0.6 and 0.6 log $CFUg^{-1}$ for *L. casei, S. aureus, A. hydrophila*, *E. coli* and *L. moncytogenes,* respectively. Similar results were obtained by SEO and FRANK (1999) and by TAKEUCHI and FRANK

Table 1 - Bacterial count* on Scarola leaves stored 6 days at 8°C after inoculation with single pathogenic strain mixes, with or without the addition of *Lactobacillus casei* LC34GF.

Strains added	Incubation time (days) 0	2	4	6
Staphylococcus aureus Sa4 – Sa5	5.0±0.2[b]	4.8±0.4[a]	5.2±0.3[a]	5.8±0.2[a]
Staphylococcus aureus Sa4 – Sa5 *Lactobacillus casei* LC34GF	5.0±0.2[b] 6.2±0.2	2.3±0.2[b] 6.5±0.3	<1[b] 7.2±0.6	<1[b] 8.5±0.5
Aeromonas hydrophila 2	5.0± 0.3[b]	5.7±0.1[b]	5.9±0.4[b]	6.9±0.3[b]
Aeromonas hydrophila 2 *Lactobacillus casei* LC34GF	5.0±0.3[b] 7.2±0.3	4.3±0.2[c] 8.4±0.3	2.2±0.2[c] 8.6±0.1	<1[c] 9.1±0.3
Escherichia coli Ct1-Ct2-EcV8	4.8±0.3[b]	5.1±0.2[b]	5.2±0.3[b]	5.9±0.3[b]
Escherichia coli Ct1-Ct2-EcV8 *Lactobacillus casei* LC34GF	4.8±0.3[b] 6.9±0.2	4.2±0.2[c] 7.5±0.2	2.5±0.2[c] 7.9±0.4	<1[c] 8.1±0.4
Listeria monocytogenes LGB-Sp2	4.9±0.3[b]	4.2±0.1[b]	4.6±0.3[b]	4.8±0.2[b]
Listeria monocytogenes LGB-Sp2 *Lactobacillus casei* LC34GF	4.9±0.3[b] 6.6±0.3	4.0±0.2[b] 7.1±0.2	3.2±0.1[c] 8.0±0.5	2.4±0.2[c] 8.0±0.7

* each value is the mean of three separate experiments and is expressed as log $CFUg^{-1}$ ± SD; a,b: different letters within the same column of each challenge experiment, indicate significant differences (P>0.05).

Table 2 - Inoculum size* and bacterial counts* at different sites of Scarola leaves after single strain inoculation and storage at 8°C for 6 days. All the mean differences between intact leaf surface and cut edge counts were statistically significant. Variance analysis of the population differences was performed separately for each site.

Bacterial strains	Inoculum size	Intact leaf surface	Cut edge sample
Lactobacillus casei LC34	6.1±0.2	6.6±0.3[a]	8.2±0.2[b]
	7.0±0.4	6.9±0.3[d]	8.5±1.0[b]
Staphylococcus aureus Sa3 – Sa4	4.1±0.1	4.0±0.1[a]	5.0±0.3[b]
	5.2±0.2	4.8±0.3[d]	5.6±0.4[b]
Aeromonas hydrophila 2	4.3±0.1	4.5±0.2[a]	5.8±0.4[b]
	5.1±0.2	5.2±0.2[a]	6.1±0.3[b]
Escherichia coli Ct1-Ct2-EcV8	4.2±0.1	4.0±0.1[a]	4.5±0.1[b]
	5.0±0.3	5.2±0.3[a]	5.8±0.3[b]
Listeria monocytogenes LGB-Sp2	4.0±0.2	4.2±0.3[a]	4.7±0.4[b]
	4.8±0.3	4.5±0.2[a]	5.1±0.2[b]

* each value is the mean of four separate experiments and is expressed as log $CFUg^{-1}$ ± SD; a,b: different letters in the same row indicate significant differences (P>0.05).

(2000) studying the location of *E. coli* O157:H7 cells on iceberg lettuce leaves. TAKEUCHI and FRANK (2000) observed that a high inoculum size of this species caused fewer differences in cell population between the surface and the cut edges, whereas in our experiments significant differences were not observed also starting with different bacterial loads (Table 2). This is probably due to the low initial levels of pathogens used in our experiments (4-5 log $CFUg^{-1}$). It is interesting to note that the maximum difference in cell location was observed for the *L. casei* LC34GF strain, showing its ability to grow on the damaged leaf

tissues, which is necessary for antagonistic action.

The results from bacterial counts were also supported by SEM observations, as shown in some significant microphotos. Microcolonies of *S. aureus* Sa5 were visible as a biofilm structure adhering to the cut edge tissues of a salad leaf (Fig. 1a) as well as for *S. aureus* Sa4 (Fig. 1b). It was observed that cells of this species were usually concentrated near the damaged lymphatic vessels on cut edges of leaves probably for a greater availability of nutrients. The same reason may explain the higher concentration of *E. coli* cells on cut edges, as illustrated in Fig. 2, in which the difference in cellular density between the surface and cut edges is evident; moreover, cells of *E. coli* strains frequently accumulated deep by within the leaf stomata (Fig. 3). When sprayed on salad leaves, *A. hydrophila* cells also occupied the same preferential ecological niches as the previous pathogens (Fig. 4a and b). The behaviour of *L. monocytogenes* was the same as the other pathogens.

It is worth noting that the colonizing ability of the *L. casei* strain was maximum on damaged lymphatic vessels (cut edges), stomata and trichomes, as shown in Figs. 5a, 5b and 6, respectively.

All of the data suggest that the contamination sites of pathogens in fresh vegetable leaves are mainly localized on the cut edges and damaged tissues; moreover a deep penetration into the stomata is also possible. Although the attachment of bacteria to the surfaces of plant tissues involves numerous complex mechanisms, including cell entrapment or specific electrostatic and hydrophobic/hydrophilic interactions, the location of contamination sites could be attributable to the inability of pathogenic bacteria to degrade the hydrophobic cuticle layer present on the leaf surface in order to reach the soluble nutrients (FRANK, 2001).

These results agree with the observations reported by SEO and FRANK (1999), TAKEUCHI and FRANK (2000) and TAKEUCHI *et al.* (2000), who also showed that cellular viability of *E. coli* O157: H7 in such niches is poorly affected by sanitizing treatments.

In the present study it was shown that LAB colonization occurs in the same ecological niches occupied by pathogenic strains. This ecological aspect assumes particular importance as it could explain the protective effect of LAB against pathogens in ready-to-eat vegetables observed in the present study and also

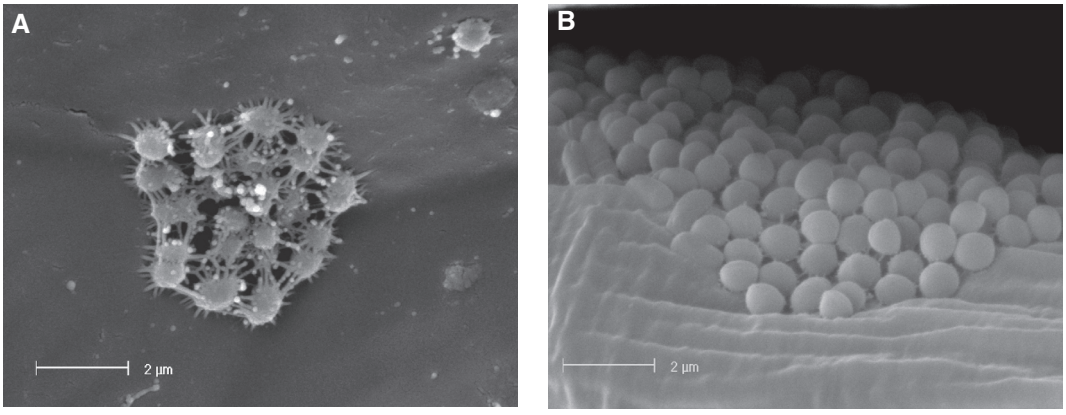


Fig. 1 - Biofilm of a cut edge of a Scarola leaf inoculated with *Staphylococcus aureus* Sa5 (a) and *Staphylococcus aureus* Sa4 (b) after incubation at 8°C for 6 days.

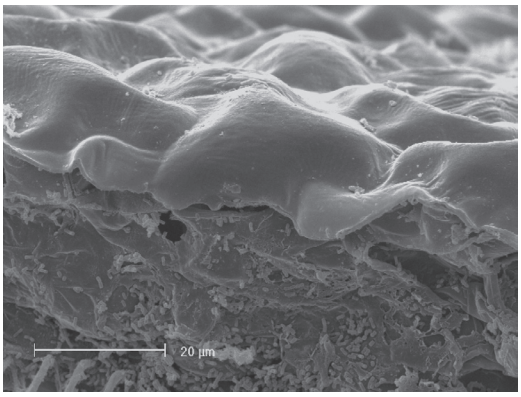


Fig. 2 - Microflora density of the surface and cut edges on a salad leaf inoculated with *Escherichia* coli EcV8 and incubated at 8°C for 6 days.

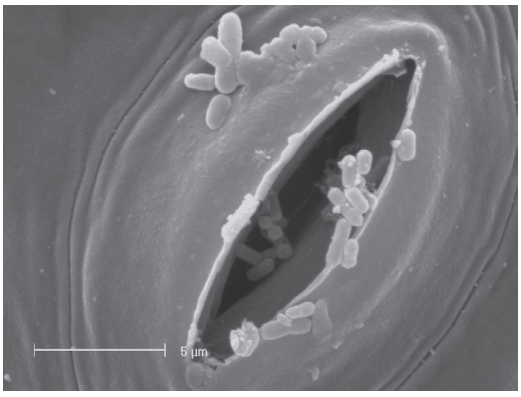
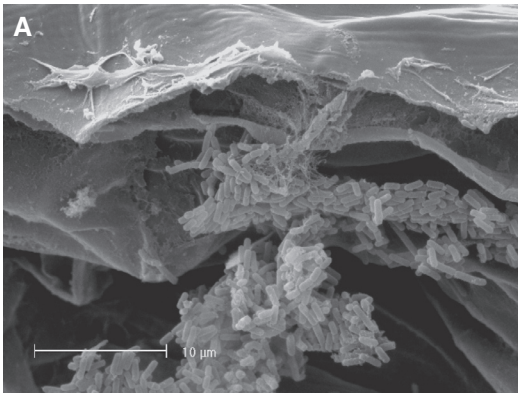


Fig. 3 - Deep internalization of inoculated *Escherichia coli* Ct1 cells in Scarola leaf stomata at the sixth day of storage at 8°C.



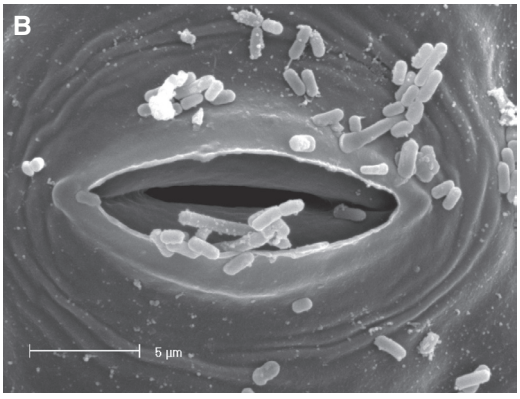
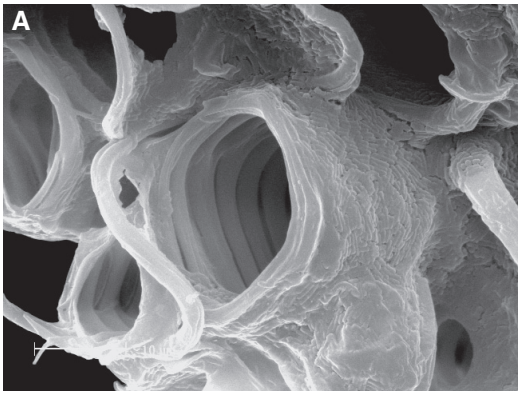


Fig. 4 - Microflora density on the surface and cut edges of a salad leaf inoculated with *Aeromonas hydrophila* 2 and incubated at 8°C, 6 days (a). Colonization of leaf stomata by *Aeromonas hydrophila* inoculated cells (b).



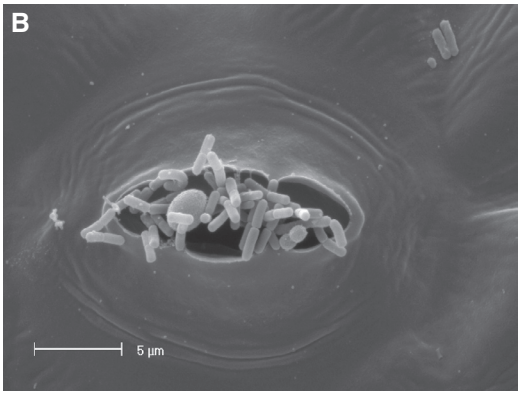


Fig. 5 - Preferential localization of *Lactobacillus casei* LC34GF strain on damaged lymphatic vessels in cut-edge surfaces (a) and penetration of cells in Scarola leaf stomata (b), 6 days after its inoculation at 8°C.

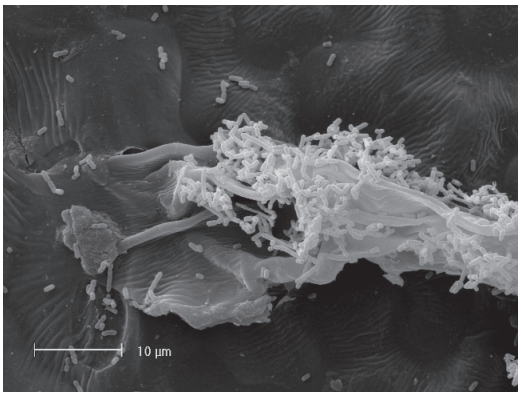


Fig. 6 - Intense growth of *Lactobacillus casei* LC34GF strain on damaged trichomes of Scarola leaf.

by VESCOVO *et al.* (1996). In fact the localized multiplication of inoculated LAB cells in the observed microenvironments, allows the inhibiting metabolites to be maintained in a concentrated form, thus assuring effective antagonism toward the competing pathogenic microflora.

SEM observations of cut edges, carried out on samples taken during challenge experiments between *L. casei* LC34GF and *S. aureus*, showed an increasing prevalence of LAB compared to pathogens at the first, second and fourth day of leaf storage at 8°C (Fig. 7a, b, c). It is noteworthy to underline that the inhibitory effect of LAB occurs even if the pathogenic strain is organized in a protective biofilm structure (Fig. 7b).

These data could explain the conflicting results in the literature about the possible protective role of the indigenous microflora against pathogenic species (CARLIN *et al.*, 1996; DEL-CAMPO *et al.*, 2001; FRANCIS and O'BEIRNE, 2002). In fact, it is well known that such microflora usually colonize undamaged surface areas not available to pathogens (TAKEUCHI *et al.*, 2000); therefore a close inhibiting interaction with pathogens is not always possible. The last considerations could also justify the need of a high inoculum of *L. casei* (6-7 log CFUg^{-1}) to get successful control of the total aerobic microflora mainly represented by pseudomonads (SCOLARI and VESCOVO, 1997).

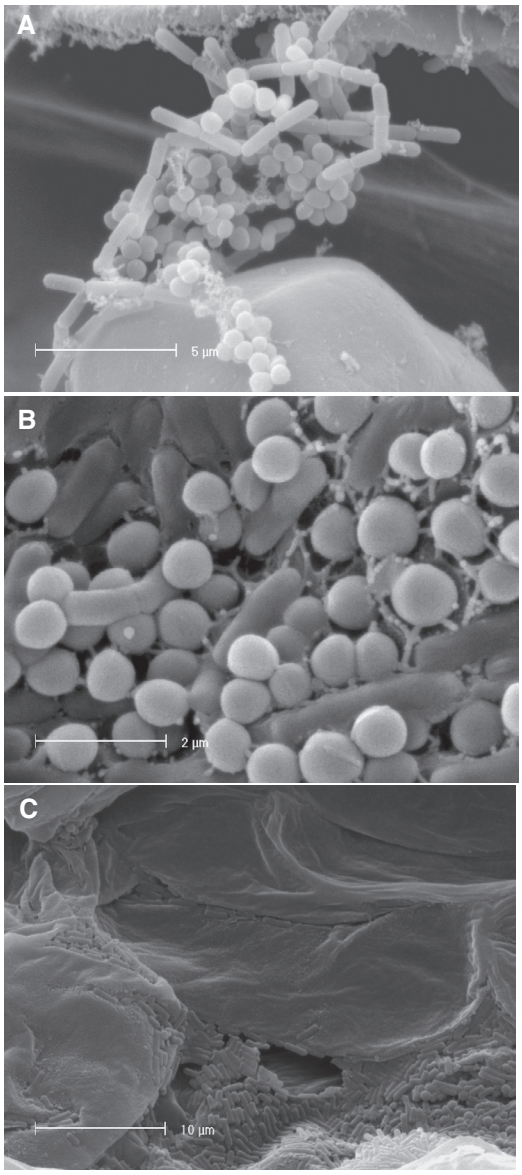


Fig. 7 - Visualization of a cut-edge on Scarola leaf inoculated with *Staphylococcus aureus* Sa4 (5 log CFU g^{-1}) and subsequently with *Lactobacillus casei* LC34GF (6 log CFU g^{-1}) on the first (a), second (b) and fourth (c) day of incubation at 8°C.

CONCLUSIONS

The *L. casei* strain mainly grows in the same ecological niches where pathogenic cells adhere; the effectiveness of added LAB cells extends during the entire storage period contrary to the activity of sanitizing agents which decline suddenly after application. Moreover the protective effect is probably enhanced by abuse temperatures, making LAB addition to fresh salads particularly interesting for food safety in the goal of preventing risks from cold-chain failure.

Further studies are in progress to better understand the interactions occurring in the leaf niches between LAB and pathogens through cell viability assessment. It is advisable to conduct studies in order to improve the knowledge about the mechanism of bacteria attachment to the plant tissues, as well as the washing process by the use of proper physical methods to remove the epiphytic spoilage microflora, especially when it is organized as a biofilm.

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SHORT COMMUNICATION

VOLATILE COMPOUNDS IN DIFFERENT CULTIVARS OF *APIUM GRAVEOLENS* L.

COMPOSTI VOLATILI IN DIFFERENTI CULTIVARS DI *APIUM GRAVEOLENS* L.

B. TIRILLINI[*], R. PELLEGRINO[1], R. PAGIOTTI[2], N. POCCESCHI[2] and L. MENGHINI[3]

Istituto di Botanica, Università di Urbino, Via Bramante 28, 61029 Urbino, Italy

[1]Laboratorio di Chimica Organica, Dipartimento di Chimica, Università di Perugia, Via Elce di Sotto 8, 06123 Perugia, Italy

[2]Dipartimento di Biologia Vegetale, Università di Perugia, Borgo XX Giugno, 06100 Perugia, Italy

[3]Dip. di Scienze del Farmaco, Università di Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy

*Corresponding author: Tel. +39 0722 2428, Fax +39 0722 4092, e-mail: tirillini@uniurb.it

ABSTRACT

The volatile compounds (VC) of four cultivars of *Apium graveolens* L. (cv. Black Celery of Trevi, cv. D'Elne, cv. Dorato d'Asti, and cv. Verde Pascal) were determined with HS-SPME and GC/MS. Eleven compounds were identified in cv. Black Celery of Trevi, seventeen compounds in cv. D'Elne and twenty-one compounds in cv. Verde Pascal. The main constituent of these cultivars was limonene. Twenty-one compounds were identified in cv. Dorato d'Asti. The main constituent was γ-ter-

RIASSUNTO

Le frazioni volatili (VC) di quattro cultivars di *Apium graveolens* L. (cv. Sedano Nero di Trevi, cv. D'Elne, cv. Dorato d'Asti and cv. Verde Pascal) sono state determinate usando la HS-SPME e la GC/MS. Undici composti sono stati identificati nella cv. Sedano Nero di Trevi. Diciassette composti sono stati identificati nella cv. D'Elne. Ventuno composti sono stati identificati nella cv. Verde Pascal. Il limonene è il composto principale di queste tre cultivars. Ventuno composti sono stati identificati nella cv.

- Key words: *Apium graveolens*, GC-MS, HS-SPME, volatiles -

pinene. The cv. Black Celery of Trevi is different with respect to the other cultivars, particularly due to the low amount of γ-terpinene.

Dorato d'Asti. Il γ-terpinene è il composto principale. La cv. Sedano Nero di Trevi si distingue nettamente dalle altre cultivar studiate, in particolare per il basso contenuto di γ-terpinene.

INTRODUCTION

Apium graveolens L. (Umbelliferae) is a biennal herbal plant that can grow to a height of 100 cm. It has strong, characteristic volatile compounds. The varieties *rapaceum* (Miller) DC and *dulce* (Miller) Pers. are widely cultivated for their edible petioles (TUTIN *et al.*, 1968). Of the eighty cultivars of *Apium graveolens* cultivated as vegetables in Europe (COMMISSIONE-EUROPEA, 2003), those most commonly cultivated in central Italy are Black Celery of Trevi, D'Elne, Dorato d'Asti and Verde Pascal. The cv. Black Celery of Trevi is one of the most ancient ecotypes in central Italy. A one hundred year-old document reports that Black Celery of Trevi was one of the vegetables taken to the "new world", because it could be preserved for a long time (REGIONE-UMBRIA, 1999).

Apium graveolens spontaneously growing in Germany contains more than 10% each of myrcene and limonene (FEHR, 1974). Native celery from Estonia contains limonene, (Z)-β-ocimene and phthalide isomers (ORAV *et al.*, 2003). *A. graveolens* spontaneously growing in Cuba contains 3-butyl-4,5-dihydrophthalide, limonene and β-caryophyllene as major constituents (PINO *et al.*, 1997). *Apium graveolens* L. var. *dulce* from India contains senkyunolides, limonene, β-selinene and butylphthalides as major constituents (CHOUDHARY and KAUL, 1992).

Headspace solid-phase microextraction (HS-SPME), a technique introduced by ZHANG and PAWLISZYN (1993), may replace the large and expensive equipment used in traditional headspace techniques. It has the further advantage that injection of relatively large amounts of water into the GC and loss of analytes by adsorption onto large surfaces is avoided. The most important physicochemical parameters for the sensitivity in HS-SPME analyses have been reported to be moderate volatility and high lipophilicity of the analyte. HS-SPME combined with GC-MS detection is a powerful technique for analysis of volatile compounds, but since HS-SPME strongly discriminates more polar and very volatile compounds, it is not suited for quantitative analysis (PELUSO *et al.*, 1995).

The aim of this study was to characterize the volatile compounds of some of the most common horticultural celery cultivars grown in Central Italy using HS-SPME and GC-MS analysis. To the best of our knowledge, this is the first report on the application of this technique on celery and the first report on the determination of volatile compounds of the cultivars Black Celery of Trevi, D'Elne, Dorato d'Asti, and Verde Pascal.

MATERIALS AND METHODS

Plant material

Fresh materials (*Apium graveolens* L. var. *dulce* (Miller) Pers. cv. Black Celery of Trevi, cv. D'Elne, cv. Dorato d'Asti and cv. Verde Pascal) were collected in Trevi, (PG) central Italy in July 2003. The plants were grown from commercial

seeds obtained in the Trevi area. Randomly chosen plants (6 per cultivar) were cut with a plastic knife and approximately 200 g (wet weight) of the same plant was placed in a 750 mL glass vial closed with a Teflon-lined membrane cap. Six glass vials were prepared for each cultivar. After 12 h at 20°C the samples were analysed.

Headspace Solid-Phase Microextraction

The Solid-Phase Microextraction (SPME) device (Fiber holder 57330-U with Fiber 57300-U, Supelco, Bellefonte, USA) uses a fine (d=100 μm) fused silica fiber coated with a short (10 mm), thin (95 μm) layer of polydimethylsiloxane (PDMS). The fiber was exposed to the headspace above the celery sample for 30 min at 20°C. The fiber was then pulled into the housing and the SPME device was removed from the sample vial and inserted into the injection port of the gas chromatograph.

GC and GC-MS analyses

The GC analysis was carried out using a Varian 3300 instrument (Varian, Inc., Palo Alto, USA) equipped with a flame ionization detector and an HP-InnoWax capillary column (30 m x 0.25 mm, film thickness 0.17 μm), operating from 60°C (3 min) to 210°C (15 min) at 4°C/min or an HP-5 capillary column (30 m x 0.25 mm, film thickness 0.25 μm) operating from 60°C (3 min) to 300°C (15 min) at 4°C/min; injector and detector temperatures, 250°C; carrier gas, helium (1 mL/min); split ratio, 1: 10.

The GC-MS analysis was carried out using an HP 5890 GC-MS system (Hewlett-Packard, Palo Alto, USA) operating in the EI mode at 70 eV, using the two above-mentioned columns. The operating conditions were analogous to those reported in the GC analysis section. Injector and transfer line temperatures were 220°C and 280°C, respectively. Helium was used as the carrier gas at a flow rate 1 mL/min. Split ratio 1: 10.

Compound identification

The compounds were identified using both columns, by comparing their retention indices relative to the C6-C22 *n*-alkanes and the mass spectra with corresponding data standards (ADAMS, 2001, DAVIES, 1990, HELLER and MILNE, 1983, JENNINGS and SHIBAMOTO, 1980, MCLAFFERTY and STAUFER, 1989, STENHAGEN *et al.*, 1974). Reference compounds were also used to validate identification of the compounds.

The percentage composition of the volatile compounds was obtained by the normalization method from the GC peak areas, without using correction factors.

Statistical analysis

The percentages of the compounds recorded for each sample were submitted to numerical cluster analysis (centroid clustering of mean squared Euclidean distances) (SADOCCHI, 1981) from which the dendrogram was derived.

RESULTS AND DISCUSSION

The composition of the volatile compounds of the various celery cultivars is given in Table 1; the compounds are listed in the order of their elution from the HP-5 column.

Eleven compounds were identified in the cv. Black Celery of Trevi, representing 99.7% of the total volatile compounds. The monoterpene hydrocarbon fraction accounted for the highest mean contribution (98.4%). The sesquiterpene hydrocarbon content was low (1.1%), in which β-selinene was the major one (0.7%). Seventeen compounds were identified in the cv. D'Elne, representing

Table 1 - Chemical composition (%) of the volatile compounds of some cultivars of *Apium graveolens**.

Compounds	RI	cv. Black Celery of Trevi	cv. D'Elne	cv. Dorato d'Asti	cv. Verde Pascal
n-heptane	700	n.d.	0.2±0.1	0.2±0.1	0.1±0.1
n-octane	800	n.d.	0.3±0.1	n.d.	0.1±0.1
n-nonane	900	0.2±0.1	1.6±0.2	2.7±0.3	0.5±0.1
α-thujene	929	n.d.	0.5±0.1	0.6±0.1	0.2±0.1
α-pinene	938	0.2±0.1	1.7±0.2	3.8±0.4	2.2±0.3
camphene	955	n.d.	0.3±0.1	0.8±0.1	0.4±0.1
sabinene	974	0.2±0.1	1.9±0.2	2.5±0.3	1.3±0.2
β-pinene	981	0.8±0.1	2.3±0.3	6.6±0.7	1.5±0.3
myrcene	992	32.2±3.3	27.5±2.8	12.9±1.3	21.3±2.2
α-phellandrene	1,004	n.d.	n.d.	0.6±0.1	0.1±0.1
α-terpinene	1,018	n.d.	0.5±0.1	0.2±0.1	0.3±0.1
p-cymene	1,025	n.d.	n.d.	0.4±0.1	n.d.
limonene	1,029	53.4±5.4	34.9±3.5	26.9±2.7	4.0±4.1
(Z)-β-ocimene	1,037	7.1±0.7	n.d.	n.d.	5.3±0.6
(E)-β-ocimene	1,050	0.2±0.1	1.4±0.2	2.7±0.3	2.5±0.3
γ-terpinene	1,060	4.3±0.4	20.2±2.1	29.8±3.0	14.0±1.7
terpinolene	1,089	n.d.	0.4±0.1	0.8±0.1	0.5±0.1
n-undecane	1,100	n.d.	0.1±0.1	0.1±0.1	0.1±0.1
allo-ocimene	1,132	n.d.	n.d.	2.5±0.3	2.4±0.3
β-caryophyllene	1,420	0.4±0.1	2.2±0.2	1.0±0.1	4.5±0.4
β-selinene	1,491	0.7±0.1	0.2±0.1	0.3±0.1	0.4±0.1
n-hexadecane	1,600	n.d.	n.d.	0.1±0.1	n.d.
n-heptadecane	1,700	n.d.	n.d.	0.1±0.1	0.1±0.1

* Each value is the mean (± standard deviation) of six samples.
Retention indexes, relative to n-alkane series on a DB-5 column (RI).
n.d. not detected (limit of detection 0.05%).

96.2% of the total volatile compounds. The monoterpene hydrocarbon fraction accounted for the highest mean contribution (91.6%). This fraction was dominated by limonene (34.9%). The sesquiterpene hydrocarbon and n-paraffin contents were relatively low (2.4 and 2.2%, respectively) and the major compounds were β-caryophyllene (2.2%) and n-nonane (1.6%), respectively. Twenty-one compounds were identified in the cv. Dorato d'Asti, representing 95.6% of the total volatile compounds. The monoterpene hydrocarbon fraction accounted for the highest mean contribution (91.1%). This fraction was dominated by γ-terpinene (29.8±3.0%). The n-paraffin and sesquiterpene hydrocarbon contents were relatively low (3.2 and 1.3%, respectively) and the major compounds were n-nonane (2.7%) and β-caryophyllene (1.0%), respectively.

Twenty-one compounds were identified in the cv. Verde Pascal representing 97.8% of the total volatile compounds. The monoterpene hydrocarbon fraction accounted for the highest mean contribution (92.0%). This fraction was dominated by limonene (40.0%). The sesquiterpene hydrocarbon content was relatively low (4.9%) and the major compounds were β-caryophyllene (4.5%).

The number of volatile compounds in the cultivars ranged from eleven to twenty-one; limonene and myrcene were among the main compounds in all the

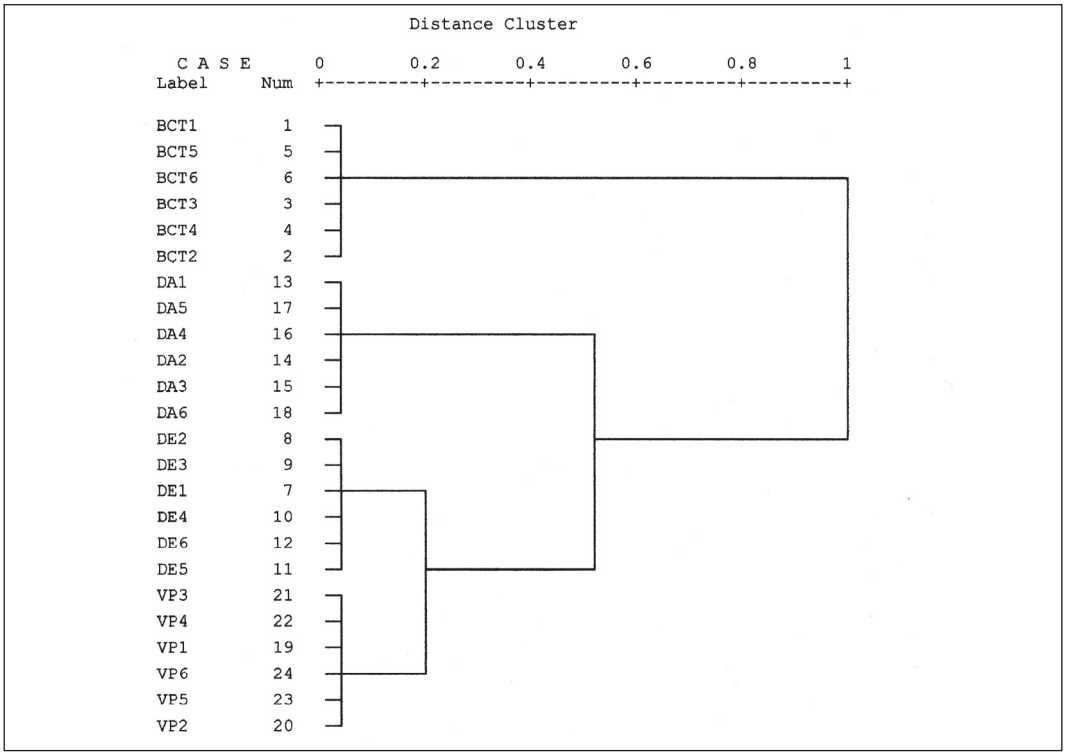


Fig. 1 - Dendrogram produced by the centroid clustering of the mean squared Euclidean distances computed from the percentages of the volatile composition of the cultivars. BCT=Black Celery of Trevi; DA=Dorato d'Asti; DE=D'Elne; VP=Verde Pascal.

cultivars; γ-terpinene was one of the principal compounds in the cultivars D'Elne, Dorato d'Asti and Verde Pascal and the low amounts of this compound in the cv. Black Celery of Trevi is one of the more important differences between this cultivar and the others. (Z)-β-ocimene was present only in the cultivars Black Celery of Trevi and Verde Pascal.

The hierarchical cluster analysis (Fig. 1) using average linkage showed that cv. D'Elne and cv. Verde Pascal join first at a distance cluster (ds) = 0.14, secondary cv. Dorato d'Asti joins at a ds = 0.48 and finally cv. Black Celery of Trevi joins at ds = 0.99. This indicates that the volatile compounds of cultivars D'Elne and Verde Pascal are quite similar and that Black Celery of Trevi cultivar is very unlike the others.

Limonene and (Z)-β-ocimene were present in high quantities in the celery oil of *Apium graveolens* from Estonia (ORAV, 2003), limonene and β-caryophyllene were the major components of the oil of *Apium graveolens* from Cuba (PINO, 1997) and myrcene was one of the main components of the essential oil of *Apium graveolens* var. *secalinum* from Egypt (SALEH *et al.*, 1986). The other compounds have been reported earlier as chemical constituents of the essential oil of leaves of *A. graveolens*.

Phthalide compounds such as sedanolide, 3-butylphthalide, 3-butyl-4,5-di-

hydrophthalide, ligustilide and 3-butylhexahydrophthalide which are relatively abundant in the leaf essential oil of *A. graveolens*, were not detected. The HS-SPME technique has not been reported to be able to capture phthalide isomers, even if in theory these compounds may be absorbed on PDMS. The presence of the phthalide isomers could be better confirmed with the traditional solvent extraction technique than with the headspace technique.

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SHORT COMMUNICATION

ALLERGY TO RED CHICORY (*CICHORIUM INTYBUS* VAR. *SYLVESTRE*)

ALLERGIA AL RADICCHIO (*CICHORIUM INTYBUS* VAR. *SYLVESTRE*)

P.L. MINCIULLO, A. SAIJA[1], D. TROMBETTA[1], D. BONANNO, B. FERLAZZO and S. GANGEMI*
Divisione e Scuola di Specializzazione in Allergia e Immunologia Clinica, Dipartimento di Patologia Umana, Università di Messina, 98100 Messina, Italy
[1]Dipartimento Farmaco-Biologico, Università di Messina, 98100 Messina, Italy
* Corresponding author: Tel.+39 090 716070, Fax +39 090 6782336, e-mail: sgangemi@eniware.it

ABSTRACT

Cichorium intybus var. *sylvestre* (Compositae family), a vegetable domestically known as red chicory (radicchio rosso), is a traditional Italian food. A few cases of IgE-mediated allergy following chicory (*Cichorium intybus)* ingestion have been described. We report the first case of a type-1 allergy following ingestion of Chioggia red chicory. A 26-year-old woman developed urticaria and facial angioedema after ingestion of red chicory. A prick-by-prick with fresh red chicory showed an in-

RIASSUNTO

Il radicchio rosso (*Cichorium intybus* var. *sylvestre)*, appartenente alla famiglia delle Compositae, è un tipico vegetale italiano. Poche sono le segnalazioni in letteratura di allergie IgE-mediate dopo ingestione di cicoria. Riportiamo il primo caso di allergia di tipo I dopo ingestione di radicchio di Chioggia. Una donna di 26 anni ha sviluppato orticaria e angioedema facciale dopo ingestione di radicchio. La paziente è stata sottoposta a prick by prick con radicchio fresco che ha mostrato una inten-

- Key-words: allergy, chicory, compositae, radicchio, urticaria -

tense reaction; on the contrary, prick-by-prick with fresh lettuce and fresh chicory was negative.

sa reazione; il prick by prick con lattuga e cicoria fresche non ha mostrato, invece, alcuna positività.

INTRODUCTION

Cichorium intybus var. *sylvestre* (Compositeae family) is a vegetable domestically known as red chicory (radicchio rosso). It is grown in the Veneto area (northeastern Italy) and is a traditional Italian food. It is known that plants in the Compositae family may become sensitizing not only through their pollen, but also through food. In fact, several cases of immediate-type allergy have been reported due to oral (besides inhalatory and cutaneous) exposure to vegetables belonging to the Compositae family, such as endive (*Chicorium endivia*) and lettuce (*Lactuca sativa*) (VILA *et al.*, 1998; FRANCK *et al.*, 2000; SAN MIGUEL-MONCIN *et al.*, 2003). A few cases of IgE-mediated allergy following chicory (*Cichorium intybus*) ingestion have been reported (CADOT *et al.*, 1996; 2003; ESCUDERO *et al.*, 1999). The systemic reactions observed in these patients included oral allergy syndrome, rhinoconjunctivitis, facial angioedema, generalized urticaria, nausea and dyspnoea.

CASE REPORT

We report the first case of allergy to Chioggia red chicory (*Cichorium intybus* var. *sylvestre*, "Rosso di Chioggia"), one of the most appreciated types of red chicory, cultivated in the area of Chioggia. A 26-year-old woman, with a history of seasonal rhinitis and asthma, came to the outpatient clinic of Allergy and Clinical Immunology for a reaction of urticaria and facial angioedema that arose after eating a salad with lettuce and Chioggia red chicory. She also asserted that she had eaten lettuce after this allergic episode without any adverse reaction. Skin prick tests for inhalant allergens were positive for *Parietaria judaica* (++++) and negative for house dust mites, moulds, grass pollen, *Betula verrucosa*, *Olea europaea*, *Corylus avellana* and *Artemisia vulgaris* pollen. Skin prick tests for food allergens showed positivity for sunflower (+++), apple (+++), peach (++++), hazelnut (+++) and walnut (+++). A prick-by-prick, performed with fresh leaves and cores of Chioggia red chicory showed an intense reaction (++++); on the contrary, prick-by-prick with fresh lettuce and chicory was negative. The patient asserted that she had avoided eating peaches for many years, because she experienced oral itching and swelling of the lips after eating this fruit; furthermore, a few months before the visit to our clinic she had experienced skin redness and swelling at the site of contact with this fruit. Finally she presented oral itching after eating hazelnut and walnut, but not after eating apple or sunflower oil.

To our knowledge, this is the first report of a systemic allergic reaction following ingestion of red chicory. In our opinion, this is particularly interesting because consumption of red chicory has increased in the last few years, and this vegetable is acquiring greater commercial interest. Besides being very popular in Italy, this vegetable is also appreciated in several European countries and is gaining popularity in the United States. It is eaten raw, mixed in a variety of salads, grilled or roasted or combined with other dish-

es. Chioggia red chicory is protected by the European Protected Geographical Indication (PGI).

Further studies are needed to identify the allergen responsible for the allergy to red chicory. Immunoblotting inhibition experiments carried out on serum from patients with allergic reactions to chicory or lettuce have shown that the pattern of IgE binding to extracts from these vegetables was variable among the patients, with protein bands recognized between 18 and 71 kDa (SAN MIGUEL-MONCIN *et al.*, 2003; VILA *et al.*, 1998). Furthermore SAN MIGUEL-MONCIN *et al.* (2003) recently demonstrated that a 9 kDa lipid transfer protein (LTP) may be a major allergen in patients suffering from lettuce allergy. The LTPs are panallergens weighing approximately 9-10 kDa and are potentially severe food allergens due to their high stability and resistance to pepsin digestion (GARCIA-SELLES *et al.*, 2002; DIAZ-PERALES *et al.*, 2000; ASERO *et al.*, 2002).

However, the present patient showed no allergic reaction to chicory and lettuce, which excludes that those already described Compositae allergens are involved in our case of red chicory allergy. Interestingly, the patient was allergic to Parietaria pollen. Therefore, it would be interesting to identify the allergen(s) of red chicory that are responsible for the observed allergic reaction in order to confirm or infer a case of pollen-related food hypersensitivity.

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SHORT COMMUNICATION

SPME-GC-MS ANALYSIS OF HORSERADISH (*ARMORACIA RUSTICANA*)

ANALISI SPME-GC-MS DEL RAFANO (*ARMORACIA RUSTICANA*)

M. D'AURIA*, G. MAURIELLO and R. RACIOPPI
Dipartimento di Chimica, Università della Basilicata,
Via N. Sauro 85, 85100 Potenza, Italy
*Corresponding author

ABSTRACT

The presence of allyl isothiocyanate, isobutyl isothiocyanate, butyl isothiocyanate, 4-isothiocyanato-1-butene, 3-methylbutyl isothiocyanate, pentyl isothiocyanate, 4-methylpentyl isothiocyanate, benzyl isothiocyanate and 2-phenylethyl isothiocyanate were determined in horseradish immediately after harvesting by SPME-GC-MS. After storage of the sample at 5°C for 12 h the analysis showed that 2-phenylethyl isothiocyanate was the main compound. Other new compounds (aldehydes and terpenes) were also found.

RIASSUNTO

Un campione di rafano è stato analizzato usando la tecnica SPME-GC-MS. Immediatamente dopo il taglio è stata osservata la presenza di allil isotiocianato, isobutil isotiocianato, butil isotiocianato, 4-isotiocianato-1-butene, 3-metilbutil isotiocianato, pentil isotiocianato, 4-metilpentil isotiocianato, benzil isotiocianato e 2-feniletil isotiocianato. Dopo conservazione del campione a 5°C per 12 h l'analisi ha mostrato che l'allil isotiocianato non era più il componente principale, bensì lo era il 2-feniletil isotiocianato. Sono, poi, stati osservati altri componenti quali aldeidi e terpeni.

- Key words: gas chromatography-mass spectroscopy, horseradish, isothiocyanates, solid phase microextraction -

Horseradish (*Armoracia rusticana*) is a component of some important foods in the oriental cuisine. GILBERT and NURSTEN (1972) used gas chromatography coupled with mass spectrometry to identify the flavour compounds of horseradish. They found seventeen compounds, including methyl, ethyl, isopropyl, 2-butyl, allyl, 4-pentenyl, and 2-phenetyl isothiocyanate and allyl thiocyanate. Fourteen isothiocyanates were used to test the aroma of wasabi (Japanese horseradish) and horseradish and ω-alkenyl isothiocyanates were identified as being responsible for the green odour of wasabi (MASUDA *et al.*, 1996). These compounds give horseradish antimicrobial properties (MASUDA *et al.*, 2001).

Recently we reported that solid phase microextraction (SPME) coupled with gas chromatography mass spectrometry can be used to determine the flavour components in olive oil (BENTIVENGA *et al.*, 2001; 2002a; b), truffles (MAURIELLO *et al.*, 2001; 2004), wine (D'AURIA *et al.*, 2003), saffron (D'AURIA *et al.*, 2004a), thyme (D'AURIA *et al.*, 2004b), oregano (D'AURIA *et al.*, 2004b) and lavender (D'AURIA *et al.*, 2004b).

In this study, the same technique was used to determine the minor compounds that contribute to the flavour of horseradish fresh and after twelve hours of storage. Horseradish was analyzed after twelve hours to check the effect of storage on the composition of the flavour.

MATERIALS AND METHODS

The analyses were performed on a sample of *Armoracia rusticana* Gaertner, Meyer et Scherb obtained from cultivations located in Basilicata, Italy. The sample (root) was chopped and 3 g was put into a vial and immediately analyzed. Another cut sample was maintained at 5°C for 12 h and then 3 g was put into another vial and analyzed.

An SPME fiber coated with 100 µm of nongrafted poly(dimethysiloxane) (PDMS) phase (Supelco 57300-U, mounted on a Supelco 57330 support, Supelco, Milan, Italy) was conditioned for 1 h at 250°C in a stream of helium. A single fiber was used for the complete study. A blank run was performed after the analysis to confirm that no residual compound was contaminating the fiber or the column. The headspace was generated from 3 g of sample cut roughly with a scissors and placed in a 20 mL flask in the presence of 10 mg of internal standard (n-propylbenzene). The flasks were sealed and heated for 20 min in an aluminium block maintained at 45°C (40°C in the flask). During this time, the fiber was kept over the sample. The fiber was then introduced into the injection port of an HP6890 plus gas chromatograph (Agilent, Milan, Italy) equipped with a Phenomenex Zebron ZB-5 MS capillary column (30 m x 0.25 mm ID x 0.25 µm film thickness). An HP 5973 Mass Selective Detector (mass range: 15-800 a.m.u.; scan rate: 1.9 scans/s; EM voltage: 1435) was used; helium at 0.8 mL/min was used as the carrier gas. The injection port, equipped with a glass insert (internal diameter 0.75 mm) was splitless at 250°C. A desorption time of 0.4 min was used. The detector was held at 230°C and the oven was kept at 40°C for 2 min and then the temperature was increased to 250°C (8°C/min) which was then maintained for 10 min. All the analyses were performed in triplicate. The chromatograms obtained from the total ion current (TIC) were integrated without any correction for co-elutions and the results are expressed in arbitrary surface units (asu). All the peaks were tentatively identified by comparing their mass spectra with spectra in the Wiley6N and NIST98 libraries.

RESULTS AND DISCUSSION

Eighteen compounds were identified in the horseradish sample. The main compounds were allyl isothiocyanate, 4-isothiocyanato-1-butene, and 2-phenylethyl isothiocyanate. Eleven compounds contained sulphur, nine of which were isothiocyanate. Allyl isothiocyanate was the largest peak in the chromatogram and accounted for ca. 81% of the overall integrated peaks (Table 1).

It is noteworthy that methyl, ethyl, and isopropyl isothiocyanates were not found. They have a lower volatility than thiobismethane and carbon disulphide, which, nevertheless, were found. Furthermore, allyl isothiocyanate and 4-isothiocyanato-1-butene were the only ω-alkenyl isothiocyanates detected, while 3-butenyl, 4-pentenyl, 5-hexenyl, and 6-heptenyl isothiocyanates were not found as reported by MASUDA *et al.* (1996). The presence of four aldehydes (3-methylbutanal, hexanal, E-2-hexenal, and nonanal) probably contributed to giving a more aromatic flavour bouquet.

Table 2 gives the results of the volatile organic compounds found in the cut horseradish kept at 5°C for 12 hours. Most of the volatile compounds diminished and 2-phenylethyl isothiocyanate became the most abundant compound. Relevant amounts of allyl isothiocyanate and benzyl isothiocyanate were also found. Twelve hours after the horseradish was cut several new non-isothiocyanate compounds were found that contributed to the new flavour of the horseradish. Some aldehydes (hexanal, *E*-2-hexenal, heptanal, benzaldehyde, phenylacetaldehyde, nonanal, 4-ethylbenzaldehyde, decanal, *trans,trans*-nona-2,4-dienal) were found as well as terpenes (menthol, junipene and italicene). Thiobismethane was also identified as a flavour component.

It is not clear whether the differences in the composition of the flavour are dependent on the decomposition/oxidation in the air of some non-volatile precursors or whether evaporation of the main compound (allyl isothiocyanate) allows the minor components, that could play a role in determining the flavour, to be identified.

Furthermore, the fact that the flavour composition of cut horseradish sam-

Table 1 - Volatile organic compounds found in the fresh horseradish sample.

Compound	r.t. (min)	ppm	Standard deviation
Thiobismethane	1.76	0.8	±0.2
Carbon disulphide	1.81	0.8	±0.2
3-Butenenitrile	2.38	7.8	±0.1
3-Methylbutanal	2.73	0.4	±0.2
2-Ethylfuran	2.78	1.2	±0.1
Hexanal	4.32	4.5	±0.1
E-2-Hexenal	5.42	1.6	±0.1
Allyl isothiocyanate	6.64	3,300	±2
Isobutyl isothiocyanate	7.85	15	±0.3
4-Isothiocyanato-1-butene	8.45	63	±1
Butyl isothiocyanate	8.74	13	±0.3
3-Methylbutyl isothiocyanate	9.98	8.6	±0.2
Pentyl isothiocyanate	10.78	3.7	±0.1
Nonanal	10.85	1.6	±0.1
4-Methylpentyl isothiocyanate	12.02	0.4	±0.1
Benzenepropanenitrile	13.49	0.8	±0.1
Benzyl isothiocyanate	15.67	14	±0.2
2-Phenylethyl isothiocyanate	17.44	400	±1

Table 2 - Volatile organic compounds found in the horseradish sample after 12 hours.

Compound	r.t. (min)	ppm	Standard deviation
Thiobismethane	1.76	1.4	±0.1
3-Methylbutanal	2.73	0.6	±0.1
2-Ethylfuran	2.78	5.8	±0.3
3-Methyl-1-butanol	3.16	1.4	±0.1
Toluene	3.69	0.8	±0.1
Hexanal	4.32	3	±0.3
E-2-Hexenal	5.42	3	±0.2
Heptanal	6.45	0.3	±0.1
Allyl isothiocyanate	6.64	175	±2
Benzaldehyde	7.78	0.5	±0.1
2-Pentylfuran	8.45	3	±0.3
2-Ethyl-1-hexanol	9.24	1.8	±0.2
Phenylacetaldehyde	9.60	0.3	±0.1
Nonanal	10.85	1.4	±0.2
4-Ethylbenzaldehyde	12.01	0.4	±0.1
L-(-)-Menthol	12.20	0.2	±0.1
Naphthalene	12.43	0.2	±0.1
Decanal	12.76	0.5	±0.1
trans,trans-Nona-2,4-dienal	12.95	0.2	±0.1
Benzenepropanenitrile	13.49	0.2	±0.1
Benzyl isothiocyanate	15.67	8.8	±2
Junipene	16.43	0.2	±0.1
Italicene	17.24	0.8	±0.1
2-Phenylethyl isothiocyanate	17.44	432	±3

ples changes is relevant when specifying the treatment of horseradish to obtain mustard.

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ABSTRACTS OF DOCTORAL RESEARCH IN FOOD SCIENCE

The 9th Workshop on Developments in Italian Doctoral Research in Food Science and Technology was held at the University of Parma from September 8-10, 2004. There were about 250 participants, including doctoral students (about 180), professors and representatives from the food industries.

The workshop was organized by Prof. Davide Barbanti and Prof. Roberto Massini (Coordinator of the doctoral program of Parma) with the help of the entire food technology research team at the Faculty of Agriculture in Parma. The Dean of the Faculty of Agriculture, Prof. Rosangela Marchelli officially opened the Workshop and gave the welcoming address.

The main objective of this workshop was to contribute to the scientific formation of the doctoral students and give the national and international scientific community an accurate picture of the research being carried out. During the workshop, the doctoral students in the Food Science and Technology sector presented the results of their research.

Keeping with "tradition", the 3rd year doctoral students presented their results in an oral presentation and the 2nd year doctoral students presented their results as posters. A plenary lecture was presented at the beginning of each of the sessions: "Building a Career as a University Scientist" (Prof. Paolo Fantozzi, University of Perugia); "Sourdough Biotechnology: Use of Selected Lactic Acid Bacteria to Decrease Human Cereal Intolerance" (Prof. Marco Gobbetti, University of Bari) and "Food Allergy: What We Know, What We Don't Know" (Prof. Michael Morgan, University of Leeds, UK).

The research topics addressed by the doctoral students spanned the field of Food Science and Technology through studies on new methods of food analysis, isolation aned characterization of microorganisms that are of interest to food science, optimization of technologies for food quality and safety and characterization of typical products.

For further information, contact Prof. Davide Barbanti, Dipartimento di Ingegneria Industriale, Parco Area delle Scienze 181/A, 43100 Parma (Italy); Tel. +39 0521 905706; Fax +39 0521 905705; e-mail: davide.barbanti@unipr.it.

ORAL COMMUNICATION ABSTRACTS

Study of toxicity and mutagenicity of urban wastewater for reuse, treated with alternative disinfection methods: UV rays, ozone and high-energy electron beams

Roberto Angrisani - Dept. of Biology, D.B.A.F., University of Basilicata, Potenza

In this research the antimicrobial action of different disinfection treatments

on urban wastewater treated in a plant in southern Italy was investigated. The conventional method, dosage of sodium hypochlorite, was compared with alternative methods: UV rays, ozone and ionizing radiation (in this case high-energy electron beams). Then, the antimicrobial action of the different methods was correlated with the possible formation of mutagenic and toxic by-products in the treated wastewater for reuse.

Development of an amplified ribosomal DNA restriction analysis (ARDRA) method for the identification of non-starter lactic acid bacteria from Pecorino marchigiano cheese

Lucia Aquilanti - Dipartimento di Scienze degli Alimenti, Università Politecnica delle Marche, Ancona

An amplified ribosomal DNA restriction analysis (ARDRA) method for the identification of LAB was developed by using basic bioinformatic tools on forty-four 16S rRNA gene sequences retrieved from the GenBank DNA database. This technique was subsequently applied to forty-five LAB reference strains and eighty-eight isolates from Pecorino marchigiano cheese. The discrimination capacity of the three enzymes selected by simulation was confirmed by the results obtained on reference strains. Afterwards, the identification of isolates was carried out through a new combined approach, based on the evaluation of ARDRA profiles and on cluster analysis, which also allowed strains with atypical profiles to be identified. On this basis, twenty-two isolates were assigned to the genus *Enterococcus*, and the remaining ones to the *sensu strictu* LAB group, with a prevalence of *Lactobacillus brevis*.

Biodiversity and technological potential of strains of *Yarrowia lipolytica* in relation to isolation source

Federica Badiali - Dipartimento di Protezione e Valorizzazione Agroalimentare (DIPROVAL), Sez. Chimica e Tecnologia degli Alimenti, Università di Bologna

Molecular characterization, lipolytic and proteolytic activity of 115 strains of *Yarrowia lipolytica* of different origin were performed in this study. Genetic biodiversity was evaluated through the application of random amplified polymorphic DNA-PCR (RAPD-PCR) analysis and different strains were grouped in relation to isolation source. However, the technological characteristics studied, such as proteolytic and lipolytic activities, do not seem to be associated with the same evolutive phenomena that produced the genetic biodiversity.

Denaturation of proteins: thermal and spectroscopic investigations

Alberto Barbiroli - Dipartimento di Scienze e Tecnologie Alimentari, sezione di Chimica, Università degli Studi di Milano

Quantification of the thermodynamic and kinetic parameters that describe the transition of a protein from the native to the unfolded state is relevant for the biochemist, biotechnologist, and food scientist. Protein-protein and protein-ligand interactions contribute to protein structural stability. Circular dichroism, fluorescence, and differential scanning calorimetry were used to investigate protein denaturation processes (induced by both thermal and chemical treatments), and to clarify the role of protein-protein and protein-ligand interactions with respect to the structural stability of model proteins characterized by different structural features.

Fumigation of foodstuffs: technical and ecological aspects

Angela Battaglia - Dipartimento di Scienza degli Alimenti, Università di Napoli "Federico II", Portici (NA)

Phosphine is a toxic gas, used for the fumigation of foodstuffs. If phosphine is released into the atmosphere it causes damage, in particular it may cause variations in the pH in acquatic ecosystems. In this research, the system to treat the phosphine with chemical oxidants, heat and the oxidation of phosphine to water and nonvolatile phosphates was investigated. For the industrial application, a scrubbing tower in counter-current with bichromate solution in sulfuric acid was used. A phosphine generator was developed, which accelerates the generation, and reduces the risks of fire and the danger from operating. The residual dust of the phosphide formulations, containing about 1-1.2% phosphide, were deactivated by lowering the concentration of P to under 0.1%, the limit for disposing the dust as non-toxic waste.

Pistachio nuts. Chemical characterisation and storage stability

Maria Giovanna Bellomo - D.O.F.A.T.A. Sez. Tecnologie Agroalimentari - Università degli Studi di Catania

Pistachio is a seed particularly appreciated for its flavour and emerald-green colour. The aim of this paper was to study the major colour compounds (anthocyanins, chlorophylls and carotenoids) in pistachio nuts of different origin. Moreover, the effect of a high gas-barrier film (EVOH, Ethylene Vinyl Alcohol), with or without oxygen scavengers, at two different temperatures (25° and 37°C), on the pistachio kernel storage stability was also observed. Pigment concentration (chlorophylls a-b and lutein), peroxide values and spectrophotometric index were analysed during nine months of storage.

Studies on retinoid transport in aquaculture fish: characterization of retinol-binding protein (RBP) secretion

Diana Bellovino - Unità di Nutrizione Sperimentale, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (INRAN), Roma

Characterization of retinoid transport and its use in fish is still largely unknown and is crucial to optimize the vitamin A requirement of fish grown in aquaculture. To study the absorption and transport of this vitamin in fish, we previously isolated and partially characterized its plasma carrier, the retinol-binding protein (RBP), in carp. Carp RBP has features that are unique among other RBPs: an uncleavable signal peptide and two carbohydrate chains at the N-terminus. In this work we investigated the function of these carbohydrates by creating mutants deprived of the glycosylations. In the absence of carbohydrates, RBP is not secreted, because translocation into endoplasmic reticulum membranes does not occur. The conclusion is that N-glycosylation is necessary for the secretion of this protein.

Improved utilization of broiler dark meat through chemical modifications: composition and chemical-physical properties of the extracts

Mirko Betti - Department of Food Science, Alma Mater Studiorum, University of Bologna, Cesena (FC)

Effects of several alkaline extraction procedures on the composition, colour characteristics, and water-holding capacity of broiler dark meat were determined. All evaluations were compared to control leg meat. Higher ($P<0.05$) protein and lower moisture and fat were found in meat subjected to chemical modifications. Extraction also significantly ($P<0.05$) increased lightness (L*). Water-holding capacity (expressible moisture)

was lower in the treated meat ($P<0.05$) compare to non-extracted dark meat, probably due to compositional changes, loss of protein, and partial denaturation. These results indicate that this technology could be a valid alternative for improving the utilisation of poultry dark meat in light-coloured poultry products.

Functional properties of breakfast cereals: results from an intervention study

Marta Angela Bianchi - Dipartimento di Sanità Pubblica, Università degli Studi di Parma

Phenolic antioxidants that are not absorbed in the upper gastrointestinal tract may reach the large bowel and exhibit antioxidant activity, opposing the action of reactive oxygen species and thus contributing to protection from oxidative damage-induced gastrointestinal diseases. The aim of this study was to evaluate the effects of supplementing the normal diet with wheat – and oat bran – based cereals on markers of gut health. Parameters related to colo-rectal cancer risk, such as faecal water, total antioxidant capacity (TAC), geno- and cytotoxicity, and short-chain fatty acid production, were evaluated. Results did not show any statistically significant difference between the two cereals tested indicating that against a background of a varied, healthy diet, the incorporation of a moderate portion of breakfast cereals does not have any effect.

Biochemical processes characterizing Asìno cheese

Marialuisa Biasutti - Dipartimento di Scienze degli Alimenti, Università degli Studi di Udine

Asìno is a traditional Italian cheese dipped in a special diluted salting brine, named “salmuerie”, that is never totally renewed, but is periodically mixed with salt, milk and milk cream. The study proved that osmotic exchange processes occur, mainly regarding the short-chain free volatile fatty acids and the biogenic amines, which migrate from the salmuerie to the cheese. Moreover, chemical, aromatic and sensory profiles that could be used to identify and characterize this peculiar traditional product are suggested.

Study of isolated yeasts from sausages

Francesca Bonifacio - Dipartimento di Scienze e Tecnologie Agro-Alimentari Ambientali Microbiologiche, Università degli Studi del Molise, Campobasso

Some yeast species (mainly *Debaryomyces hansenii*, and its imperfect form *Candida famata*) are usually present in meat, in mixtures, and in the working room. These halophilic species grow in the presence of 2.2/2.8% NaCl, the amount normally used in these kinds of products, and could contribute to the organoleptic characteristics and carry out adulterated action. The aim of the research was to isolate and identify different yeast strains associated with sausages using traditional microbiological criteria and molecular methods by electrophoretic karyotype analysis (pulse field gel electrophoresis) and by random amplified polymorphic DNA-polymerase chain reaction analysis genomic DNA. Further, studies investigated their lipolytic and proteolytic activities.

Separation and quantification of fatty acids by HPLC with an evaporative light scattering detector

Elisabetta Bravi - Dipartimento di Scienze degli Alimenti, Università di Perugia

A high-performance liquid chromatography (HPLC) technique with an evaporative light-scattering detector (ELSD) has

been developed to separate and quantify fatty acid methyl esters (FAMEs). Thirteen FAMEs were separated by using a methanol/water eluent mixture, and detected by ELSD. The retention times reflect the elution behavior of these compounds on C18 reversed-phase HPLC. The use of two columns in series allowed some fatty acid pairs (FAs) that are difficult to separate, to be separated.

Effect of the process parameters on the structural characteristics of extruded products with high proteic content

Barbara Filomena Carbone - Dipartimento di Scienze degli Alimenti, Facoltà di Agraria, Università di Foggia

A rice and chickpea (*Cicer arietinum* L.) blend was extruded in a co-rotating twin-screw extruder at various combinations of barrel temperatures, screw speeds and moisture content. Break strength and porosity of extrudates were evaluated. Structural properties were influenced mostly by extrusion temperature and dough moisture; the screw speed did not have a significant effect.

Selection of starter cultures and their use for producing traditional fermented sausages of the Campania region (Vallo di Diano)

Annalisa Casaburi - Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli "Federico II", Portici (NA)

In this research, 90 staphylococci and 10 lactobacilli isolated from three types of fermented sausages were studied for technological properties in order to select strains for starter culture formulation. Three strains of *Staphylococcus xylosus* (DS18, SS4, and BS1) and one strain of *Lactobacillus curvatus* (L25b) were selected on the basis of their proteolytic, lipolytic, acidifying and SOD activities to formulate four different starter cultures (S3, S4, S8 and S9) to use for producing fermented sausages of the Campania region (Vallo di Diano). This research was developed to investigate the effect of starter cultures on the physico-chemical, microbiological and sensory properties of fermented sausages in order to improve their typical features.

Validation of a procedure for quantifying platinum at sub-µg/L level in food dyes by catalytic adsorptive stripping voltammetry

Raffaella Cattaneo - Dipartimento di Produzione Vegetale, Università degli Studi di Milano

The potential toxicological properties of platinum group elements, often present in real matrices at sub-ng/g level, explain the increasing interest in developing specific and sensitive analytical methods. Hoppstock *et al.* developed one of the most sensitive methods for determining platinum and rhodium. In this work, a validation study was performed in order to ensure the fitness-for-purposes of a procedure developed for quantifying platinum in food dyes using this method. As suggested by EURACHEM, the work involved the estimation of selectivity, detection and quantification limits, linear range, accuracy (trueness plus precision), uncertainty of measurement, robustness and recovery. The results are discussed in light of the most recent literature findings.

Traceability in the brewing industries

Rolando Della Sera - Dipartimento di Scienze degli Alimenti, Università di Perugia

In light of the European Community legislation for mandatory traceabili-

ty for foods or foodstuffs, this work proposes a standard solution for the identification and traceability of all "ingredients" used in the beer production chain (in Italy). The European Commission has recognized an urgent need to regain consumer confidence in food products and therefore believes in fast tracing of food products throughout the supply chain. Consequently the European Parliament and Council have adopted a regulation on traceability: Regulation (EC) n. 178/2002.

Our traceability system is based on record keeping (closely linked to identity preservation (IP) and segregation of ingredients) and audit systems; in fact, traceability is established when information on a particular "food" is recorded.

Mathematical prediction of water activity in complex food

Antonio Derossi - Dipartimento di Scienze degli Alimenti, Facoltà di Agraria, Università di Foggia

A simple mathematical equation for water activity (a_w) prediction in food was studied. Three different mathematical models were used to evaluate the single and synergistic effects of humectants used in model systems and vegetable creams. The predicted values were compared with experimental data in aqueous solution, aqueous vegetable extracts and vegetable creams of mushrooms (cv. Champignon). Results showed that the proposed model had a high accuracy, in fact, the correlation coefficient (r) was always greater then 0.985.

The influence of packaging on the shelf-life of broccoli and cherry tomatoes

Vito Di Donna - Dip. di Biologia D.B.A.F., Università della Basilicata, Potenza

The aim of this research was to evaluate the shelf-life of broccoli and cherry tomatoes during storage with different packaging systems. Broccoli was stored at 6°C and 95% r.h. up to 35 days using different films, while tomatoes were stored at 7°C and 90% r.h. up to 50 days using different trays.

Characterization of virgin olive oil head space (HS). Comparison by three different methods: Solid Phase Micro Extraction-Gas Chromatography Mass Spectrometry (SPME-GC/MS), Electronic Nose and Proton Transfer Reaction-Mass Spectrometry (PTR-MS)

Sonia Esposto - Dipartimento di Scienze degli Alimenti, Div. di Tecnologia Alimentare e Biotecnologia, Perugia

The Head-Space Analysis (HSA) of the volatile compounds in olive oil can be performed using the Solid Phase Micro Extraction (SPME)-GC/MS, the Electronic Nose or the Proton Transfer Reaction (PTR) Mass Spectrometry (MS).

This paper reports a comparison of the methods to evaluate their application for the detection of the most important olive oil defects and to classify virgin olive oils according to variety, area of origin and olive ripening stage.

Improvement of wine starter cultures by metabolic and enzymatic characterization of starter strains

Concetta Fiore - Dip. di Biologia D.B.A.F., Università di Basilicata, Potenza

In this work 196 strains of *H. uvarum* and 50 strains of *S. cerevisiae* were studied for enzymatic activities, fermentation performance and production of secondary compounds. Ten selected strains of *S. cerevisiae* were used to carry out fermentation tests of 4 different grape musts: Aglianico Vulture, Merlot, Cabernet and Aglianico Campania on a pilot scale (10 L). The influence of strain

was evaluated in the final experimental wines, by determining the alcoholic level, by-product content and total polyphenols (PFT).

Influence of winemaking on ochratoxin A (OTA) content in red wines

Barbara Grazioli - Istituto di Enologia e e Ingegneria Alimentare, Università Cattolica del Sacro Cuore, Piacenza

In the winemaking process, OTA must be considered a major safety hazard because it might involve huge risks. The aim of this work was to study the OTA behaviour during the main stages of the winemaking process and the influence of the technological treatments on the OTA concentration in order to identify CCPs. The results show that no OTA is produced during winemaking, but each operation during winemaking can modify the OTA content.

The acetic acid bacteria from traditional balsamic vinegar

Maria Gullo - Università di Catania, Facoltà di Scienze Agrarie, DOFATA, Catania

Forty-eight strains of acetic acid bacteria (AAB) were isolated from "barrels" of traditional balsamic vinegar (TBV) and investigated for the phenotypic and molecular characteristics. Moreover the strains were tested for sugar tolerance. A large percentage were not able to grow in the presence of 30% glucose and this shows that the greatest hurdle to the growth of AAB in TBV is high sugar concentration. 16S-23S-5S rDNA PCR/RFLP analysis by RsaI endonuclease enzyme was performed on 13 type strains and on the isolated strains. On the basis of the results, showed that 32 strains belong to the *Gluconacetobacter xylinus* species, 2 strains to *Acetobacter pasteurianus* and one to *Acetobacter aceti*.

Origin and technology of "Soppressata" salami: effect on Calabria and Campania consumer acceptance

Teresita Iaccarino - BIOMAA, Università degli Studi Mediterranea di Reggio Calabria; DSA, Università degli Studi di Napoli "Federico II", Portici (NA)

Nine "Soppressata" salami types, were investigated: three samples were produced on an artisanal scale according to the traditional Campania technology, and three according to a traditional Calabria technology. The remaining three samples were made on an industrial scale. Groups of consumers from both Campania and Calabria evaluated the samples in blind and informed conditions. During the second test, prior to the sample presentation, information about the technology and origin were provided to the panelists. Next, the consumers tested all three types of salami each labeled according to its origin and manufacture. The "Soppressata" made on industrial scale received the lowest expected liking by both groups of consumers. Among the artisan-made samples, Campania consumers showed a higher preference for Campania-made salami than for the Calabria-made one; as expected, the opposite situation was observed for Calabria consumers.

Study of *Staphylococcus xylosus* lipase gene (gehM) expression

Lucilla Iacumin - Dipartimento di Scienze degli Alimenti, Università degli Studi di Udine

The most important microorganisms involved in the fermentation of traditional sausages of the Friuli Venezia Giulia region, Italy, are lactic acid bacteria, *Staphylococcus* and *Kocuria* spp. In particular, *Staphylococcus* spp. release lipases that are able to free short-chain fatty acids that are responsible for the aroma of fermented sausage. No studies have

been undertaken yet to elucidate the regulation of lipase gene expression by substrate molecules or products of lipolysis that may also be involved in the fine regulation of expression. The aim of this study was to evaluate the expression of the gehM gene of *S. xylosus*. The high concentration of substrate inhibits expression of the gene.

Sterol ester analysis in tetraploid and hexaploid wheats: comparison between chromatographic methods

Giovanna Iafelice - Dipartimento di Scienze e Tecnologie Agro-Alimentari Ambientali e Microbiologiche, Campobasso

The sterol ester composition of tetraploid and hexaploid wheats was evaluated. This paper describes chromatographic methods to assess sterol esters in cereal lipid fraction. The methods described could be valuable tools for distinguishing hexaploid and tetraploid free-threshing wheats (*Triticum aestivum* L. and *T. durum* Desf) and their respective ancestors (*T. spelta* L. and *T. dicoccon* Schrank). The results obtained confirmed the genetic affinity between hulled and free-threshing wheats and suggest that sterol ester composition can be used to detect authenticity or adulteration of emmer products with soft or spelt flour.

Evaluation of colour in whole wheat and semolina samples. Comparison between reflectance measurements and carotenoid content determined by different procedures

Mario Irano - Dipartimento di Scienze e Tecnologie Agro-Alimentari Ambientali e Microbiologiche, Università degli Studi del Molise, Campobasso

Colour is an important parameter involved in the definition of pasta quality. The yellow colour of pasta is mainly due to natural pigments (carotenoids) that are distributed differently in the kernel.

In this study, three different methodologies for determining the pigment content in whole meal and semolina samples have been applied and the results compared.

The results showed a good agreement between the lutein and β-carotene content and total carotenoids determined by the HPLC and WSB methods. The results also demonstrate that the HPLC procedure is a useful method for determining carotenoid content in cereal products.

Effect of high pressure homogenisation on plasmin and lipase activity in cow milk

Luciana Iucci - Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi di Bologna

High pressure homogenisation (HPH) is an effective sanitizing process that could be used as a milk pre-treatment in cheese production. Recent works showed that HPH treatment activates the lipolytic and proteolytic activities of cheese. These more precocious actvities can be attributed to an enhancement of endogenous or microbial enzymatic activities (7). The results of this work indicate that the HPH treatment has a direct effect on endogenous lipase in cow milk.

The role of oxygen in red winemaking

Milena Lambri - Istituto di Enologia e Ingegneria Alimentare Università Cattolica S. Cuore, Piacenza

In red winemaking oxygen is an important technological factor for defining the final wine quality (4, 5, 6). As a processing parameter, it must be carefully controlled, especially in the maceration phase.

Production of fungal laccases for agro-food industries

Stefania Lupino - Dipartimento di Agrobiologia e Agrochimica, Università della Tuscia, Viterbo

The white-rot fungus *Trametes trogii* 201 secretes several laccase isoforms into the medium, which are encoded by a family of closely related genes. Total laccase activity increases up to 50-fold when the fungus is grown in the presence of copper sulphate; under these conditions the transcription of three out of four lcc genes is strongly stimulated. We have cloned two laccase genes, lcc2 and lcc4, which encod minor isoforms of this enzyme. The cDNA sequence corresponding to lcc1 was expressed in yeasts, *Pichia pastoris* and *Saccharomyces cerevisiae*. The highest recombinant laccase production, 2520 U/l, was obtained in *P. pastoris*. Finally, we have demonstrated that the recombinant laccase can be used to decolourise synthetic dyes with and without mediators.

Innovative non conventional technologies in agri-food fields: effect of low electric current (LEC) during first phase of fresh grape must fermenting

Giuseppe Lustrato - DISTAAM, Università degli Studi del Molise, Campobasso

The aim of the research was to describe the effects of low electric current (LEC) on selected co-culture yeast cells (*Saccharomyces cerevisiae* 404, *Hanseniaspora guilliermodii* 465) during grape-must fermentation in laboratory and pilot plant scale winemaking. LEC (10, 30, 50 and 100 mA) was applied to fresh grape-must as an alternative to the conventional addition of SO_2. LEC decreased the survival time of *H. guilliermondii* 465 in co-culture, by increasing its death rate, while it did not affect the growth and survival rate of *S. cerevisiae* 404. A final comparison of the main physico-chemical parameters was made in wine obtained after the different tests.

Preparation, properties and employment of lamb rennet paste

Monica Assunta Madrau - Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agro-Alimentari, Università degli Studi di Sassari

An alternative preparation technology for lamb rennet paste was developed. Studies of several parameters affecting proteolytic and lipolytic activities led to the preparation of a rennet that is highly soluble in milk, has a higher and more balanced enzymatic content and reduced antidairy microflora compared to the traditional one. Experimental rennet enzymes were characterised by HPLC analysis. The technological suitability of the proposed rennet was verified on an industrial scale in experimental batches of Pecorino Romano cheese. Results showed an equilibrated action of proteolytic and lipolytic enzymes, particularly when supplied with a greater concentration of essential free amino acids in Pecorino Romano cheese.

Effect of membrane technology on the quality of tomato juice

Ombretta Marconi - Dipartimento di Scienza degli Alimenti, Università di Perugia

The results of the experiments for producing a new tomato juice using membrane technology are reported. The experiments were carried out in a pilot plant combining an ultrafiltration module (UF), a reverse osmosis module (RO) and a microfiltration module (MF). Acetate cellulose membranes with an average pore size of a micrometer was used in the UF stage to separate the clarified serum and the concentrated pulp. The clarified serum was concentrated by RO

and then remixed with the concentrated pulp. The MF stage was used to concentrate the tomato juice mix in order to formulate the new tomato juice of optimal consistency. The effect of this mild technology was discussed analysing the new tomato juice composition.

Different approaches to study in the colour of pesto

Francesca Masino - Dipartimento Interdisciplinare di Scienze Agrarie, Università degli Studi di Modena e Reggio Emilia, Reggio Emilia

The study of pesto colour was performed by means of three different approaches: HPLC pigment analysis, visual analysis, and image analysis. The HPLC analysis was concluded and showed a large variability of the main substances (Pheophytins, β-Carotene Lutein, and Chlorophylls). Currently, data of visual and image analyses are under investigation, and an innovative method for capturing image with a common digital camera and subsequent image processing with multivariate methods was set up.

Influence of phenolic compounds on the growth of *Oenococcus oeni*

Ferdinando Massarella - Dipartimento di Scienze e Tecnologie Agro-Alimentari Ambientali Microbiologiche, Università degli Studi del Molise, Campobasso

We studied the influence of some phenolic compounds (such as gallic, caffeic, ferulic and cinnamic acids, and flavonoids, such as catechin and quercetin) on the metabolism of 32 strains of *Oenococcus oeni*, selected for their good enological attributes (resistance to low pH, ethanol and SO_2 values). This study shows that the growth of *Oenococcus oeni* strains in a semisynthetic medium is affected by pH, total acidity and ethanol, and by phenolic compounds in different ways, depending on the type and concentration of phenolic compounds and on bacteria strains. The results obtained show the influence of these compounds on the metabolism of malolactic starter.

Mass spectrometry in the study of anthocyanins and their derivatives: differentiation of *Vitis vinifera* and hybrid grapes by HPLC-ESI/MS and MS/MS analysis

Pierluca Mazzuca - Dipartimento di Scienza degli Alimenti, Università di Napoli "Federico II", Portici (NA)

A mass spectrometric-based procedure for anthocyanin profiling has been set up to distinguish authentic *Vitis vinifera* from a hybrid red grapevine variety. The anthocyanin monoglucoside only occurred in *V. vinifera* whereas 3,5-O-diglucoside, variously substituted, diglucoside anthocyanins were the additional pigments of hybrid vines. Specifically, one red grape variety characterized by the presence of 3,5-O-diglucoside anthocyanins was classified as a hybrid. A second vine ecotype containing exclusively 3-O-monoglucoside anthocyanins was correctly included among the *V. vinifera* species. The anthocyanin profiling by MS could be the core of a chemotaxonomic procedure for distinguishing American and European vines based on the identification of anthocyanidin.

Identification and technological characterization of bacterial microbiota isolated from two pecorino cheeses

Dina Messina - Dipartimento di Biologia, Università della Basilicata, Potenza

Pecorino Filiano and Lucano are hard cheeses produced in Basilicata. In this study bacteria present in these Pecorini during ripening were typed, at the species level, using the molecular tools: PCR spe-

cies-specific, ARDRA-PCR and Spacer; and these bacteria were technologically characterized by evaluating their proleolytic and lipolytic activity and their ability to produce diacetile was also evaluated. Results showed that *Lb. paracasei/casei* represented 41% of the total bacteria isolated from Pecorino Lucano cheese, while *Leuc. mesenteroides* represented 23% of the total bacteria coming from Pecorino Filiano cheese. *B. linens*, isolated from both cheeses, showed major proteolytic activity, but few bacteria had a high production of diacetile. *Leuc. mesenteroides* showed a lower lipolytic activity than the other bacteria analyzed.

Effects of immature durum wheat on immune response and antioxidant status in rats

Romina Molinari - Dipartimento di Scienze Ambientali, Università della Tuscia, Viterbo

Analyses carried-out on immature grains have shown that the concentration of fructans and antioxidants in this growth phase is higher than in mature grains. Experiments in animals models indicate that fructans may modulate the systemic immune response and the serum lipids. The effects on intestinal immunity and on the mechanism involved have not been clarified. Our study investigated the effects of fructans and antioxidant compounds present in immature durum wheat on lymphocytes of mesenteric lymph nodes and antioxidant status. The results showed that the immature wheat is able to stimulate the local immune response without affecting the plasmatic and cellular antioxidant status.

Application of protective atmosphere packaging on processed broccoli: two study cases

Valeria Nicolais - Dipartimento di Ingegneria Agraria e Agronomia; Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli "Federico II", Portici (NA)

Modified atmosphere packaging offers the possibility of extending food shelf-life, by controlling chemical and biological processes that cause qualitative decay. In the two study cases, the quality changes in broccoli, a typical South Italian vegetable, were studied. Products, of known cultivation, were minimally processed in two different ways, packaged with various mixtures of gas and then stored at 4±1°C for 10 days. Microbial, colorimetric, gravimetric, chemical-physical analyses and sensorial profile evaluation were performed to monitor the qualitative decay in the samples during storage time.

Study of D-glucosidase in cellular and acellular form for use in winemaking applications

Rosa Palmeri - Dipartimento di Orto-FloroArboricoltura e Tecnologie Agroalimentari (DOFATA) Sezione Tecnologie Agroalimentari, Università di Catania

D-glucosidase activities (G, EC 3.2.1.21) from molds (*Aspergillus niger*), yeasts (*Hanseniaspora uvarum*, *Saccharomyces cerevisiae*, *Pichia anomala*) and lactic acid bacteria (*Oenococcus oeni*) were studied. In particular, three lactic acid bacteria strains, isolated during malolactic fermentation (FML) from wines of Valpolicella, were characterised in terms of chemical-physical and kinetics parameters, including a commercial starter culture as control. The results show that, malolactic bacteria strains should be included in the quality program for the organoleptic and sensory improvement of wines.

Biomolecular characterization of *Salmonella* spp. strains isolated from foods of animal origin

Paris Arianna - Dipartimento di Salute Animale, Università di Parma

Ninety-seven strains of *Salmonella* spp. were isolated from different food stuffs in the Emilian district. The serological typing of strains allowed 15 different serotypes to be identified. Genotypic characterization, by PCR, of some virulence factors (chromosomic and plasmidic) and of a drug-resistance factor, led to the identification of 4 genes (inva, phop, spvr, spvc) and of marrab operon. Every strain presented gene pair inva, phop, only *S. enteritidis* serotype was positive for spvc gene, while spvr factor was found in 12 different serotypes. The presence of a different number of genes, among the various serotypes, allowed 3 pathogenicity classes to be identified. A relatively high frequency of strains with drug-resistance features was observed.

Selection and characterization of *Lactobacillus* strains from fermented sausages for their potential use as probiotics

Carmela Pennacchia - Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli "Federico II", Portici (NA)

The aim of the study was to select potentially probiotic *Lactobacillus* (Lb.) strains from fermented sausages applying a rapid procedure to screen them at low pH and in the presence of bile salts, directly from the foods. The screened isolates were characterized to evaluate their specific probiotic properties. A proteomic approach was also applied to monitor the protein expression in conditions simulating the human gastro-intestinal (GI) tract.

Physico-chemical and microbiological changes in natural black table olives during brining

Ivo Pinna - Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agro-alimentari, Università degli Studi di Sassari

Evolution of physico-chemical and microbiological parameters of black table olives during brining was studied, with particular emphasis on the evolution of anthocyanic compounds during fermentation. Natural fermentation was mainly carried out by yeasts, while indigenous lactic acid bacteria (lab) were not found in brine. *Lactobacillus plantarum*, which was inoculated as a starter culture, grew until the 5th day of fermentation. Anthocyanic olive extract was analysed by HPLC-MS and some important changes in anthocyanic compounds at the 10th day of fermentation were found.

Mating type interconversion in flor *Saccharomyces cerevisiae* strains

Giorgia Pirino - Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agro- Alimentari, Università degli Studi di Sassari

Wild type strains of *Saccharomyces cerevisiae* can change mating type as often as once every generation. The Ho endonuclease initiates the process by cutting at MAT locus. This DNA break is then repaired by homologous recombination with one of two donor sequences, HMLα or HMRa. The different mating types a and α are biased in the selection of donor: a chooses HMLα, and α chooses HMRa. Interestingly, flor yeasts showed no canonic life cycles, called semi homothallic, compared with other wine yeasts. In this work we analyzed the mechanisms involved in mating type interconversion of five flor strains having no canonic life cycles.

Wine-making wastes: cellulose and hemicellulose recovery by mild methods

Tiziana Pizzorno - Istituto di Enologia e Ingegneria Alimentare, Università Cattolica del Sacro Cuore, Piacenza

Grape stalks are both a lignocellulosic material (LCM) and a wine-making waste. LCM are interesting raw materials for the chemical industry owing to their low price, renewable nature and large availability. In order to find an economic and easy way to recover this resource, two chemical fractionation systems reported in the literature were compared to separate and recover lignocellulosic components. Both methods involve a mild acid hydrolysis followed or preceded by an alkaline/oxidative step to remove lignin. Different times and temperatures were tested. Further trials were carried out to assess the influence of ultrasound and autoclave on cellulose and hemicellulose yield.

Hopanes confirming mineral oil contamination in foods and human milk: the relative hopane content determined by GC-MS with large volume on-column injection

Tiziana Populin - Dipartimento di Scienze degli Alimenti, Università degli Studi di Udine

GC-MS analysis of hopanes in the hydrocarbon fraction of foodstuffs was performed to investigate the origin of unresolved complex mixture (UCM) in GC-FID traces. If these compounds, generally recognized as petroleum markers, having been formed during geological times and under geological conditions, are found in every sample, it is conclusive evidence of mineral origin of the contamination. A parameter was defined: relative hopane content (RHC), i.e. the area ratio of the sum of the hopanes as determined by MS using m/z 191 and the paraffins in the same segment of the chromatogram determined by m/z 81. The RHC of the samples containing a UCM of hydrocarbons was in the same range as mineral oils, increasing the evidence of mineral origin of the contamination.

Food and packaging interactions: study and evaluation of new barrier materials

Maria Laura Puglisi - Dipartimento di Scienze Agrarie, Università degli Studi di Modena e Reggio Emilia, Reggio Emilia

Nanotechnology is still an emerging process for developing new structural materials based on nanotube composites. By using nanoparticles in composite material, it is possible to improve the polymer properties, such as mechanical, barrier, water absorption and so on, which are desired in food packaging material.

In this work, a new barrier material based on polymer/layered silicate (PLS) nanocomposites was investigated. To obtain this PLS nanocomposite, layered silicates were added to the polymer matrix. To enhance the properties of these new materials, the type of structure requested is exfoliated nanocomposites. In order to characterize the structure of nanocomposites in the polymer matrix and to verify the spatial distribution of layered silicate and the structural homogeneity in nanocomposites, WAXD (wide angle X-ray diffraction) analysis was used.

Purification, characterization and expression of an enetrococcal bacteriocin of potential use in food protection

Ileana Ramazzina - Dipartimento di Biochimica e Biologia Molecolare, Università di Parma

A wide spectrum bacteriocin, purified from *E. faecalis*, has been found to have a molecular weight, cyclization and amino acid sequence typical

of bacteriocin AS-48. In addition to the structural gene as-48A, the sequence analysis of the AS-48 gene cluster revealed the presence of several putative coding regions presumably involved in bacteriocin production and immunity. The results of DNA hybridization assays indicate that the AS-48 gene cluster and the gene pd78 are present on the same plasmid, which is likely to be the pheromone-dependent pPD1 plasmid. The expression by different bacteria of recombinant bacteriocin AS-48 has been attempted, using both pET and NICE systems.

Storage of highly perishable foodstuffs by innovative means

Anna Reale - Dipartimento di Scienze e Tecnologie Agro-alimentari, Ambientali e Microbiologiche, Università degli Studi del Molise, Campobasso

This study focused on the use of innovative means to improve the microbiological and sensory properties of fresh fish during storage, particularly *Dicentrarchus labrax*.

Innovative technologies such as modified atmosphere and irradiation treatments were assayed in an attempt to extend the shelf-life of fresh fish.

Proliferation of spoilage micro-organisms on fresh fish was measured during storage. The effects induced by treatments were also estimated by monitoring chemical and sensorial parameters (color, odor, texture...). Good results were obtained using modified atmosphere packaging.

Influence of pre-treatments on metabolism and wounding response of fresh cut potatoes evaluated with isothermal calorimetry

Pietro Rocculi - Dipartimento di Scienze degli Alimenti, Università di Bologna - Sede di Cesena

The anti-browning effects of acidificant and antioxidant substances in potatoes have been broadly studied in the literature but there is no information about their effects on the metabolism of wounded tissue. We have studied the influence of ascorbic and citric acid on the metabolism of fresh-cut potatoes. The metabolic heat production was measured in closed glass ampoules in a TAM Air isothermal calorimeter for 24 hours at 20°C. Surprisingly, the results showed that all the treatments accelerated the metabolism of the samples. Moreover we found that the effects of ascorbic and in particular citric acid principally affected normal respiration and not wounding response.

The functional role of lipids in dough development during the fermentation process

Annalisa Romano - BIOMAA, Università degli Studi Mediterranea di Reggio Calabria; DSA, Università degli Studi di Napoli "Federico II", Portici (NA)

The objective of this study was to investigate the influence of lipids on fermentation kinetics of wheat flour dough. For this purpose image analysis and rheological characterization of the dough behaviour in biaxial extension were performed. Mathematically, the fermentation process is a nonlinear and time-dependent process; thus kinetic models describing the process should also be nonlinear and time dependent. To find the best descriptive model of the variation of dough volume versus time (y = x(t)), fitting results of three models were compared. Lipid addition modifies the rheological behaviour of doughs and has a negative effect on dough volume development during leavening.

Use of a potentially probiotic *Lactobacillus rhamnosus* strain in silage production

Cristina Angela Romano - DISTAAM, Università degli Studi del Molise, Campobasso

The aim of the study was to characterize lactic acid bacteria (LAB) isolated from sorghum silage. A potentially probiotic strain of *Lactobacillus rhamnosus* was used as starter for lactic acid fermentation in order to study the effect of the starter addition on microbiological and chemical quality of silage. NMR spectroscopy was applied to detect lactic acid, acetic acid and glucose concentration.

Contribution to the study on cava wine browning

Elisa Sartini - Dipartimento di Scienze degli Alimenti, Università di Bologna, Facoltà di Agraria, sede di Cesena

To have a better knowledge of the sparkling cava wine browning, 6 series of wine (2 different categories of cava with and without yeast lees and a commercial type) each one conditioned with carbon dioxide, nitrogen and oxygen in the headspace, were analysed at different times of accelerated browning (37°C for 92 days). During the cava wine browning, absorbance changes (abs 280 and 420 nm) were evaluated and phenolic compound modifications were studied using HPLC-DAD.

Lipid composition and evolution during the ripening of Menaica salted anchovies

Maria Savarese - Dipartimento di Scienza degli Alimenti, Università "Federico II" di Napoli, Portici (NA)

In this study, the lipid fraction of "Menaica anchovies" and its modification during 145 days of ripening was studied. The samples of anchovies at different ripening times were collected from 3 Cilento's traditional farms. The capillary gas-chromatographic analysis was applied to study the composition of free, bound and total fatty acids. The liquid chromatographic light scattering detector analysis of lipid classes, was combined with the 1H NMR analysis of lipid extract and the evaluation of the degree of oxidation. This allowed the main processes (lipolysis, oxidation) that occur at the lipid fraction of this typical product during the ripening to be followed. The results suggest a rationalization of the ripening process with suitable temperature and humidity controls to obtain a greater commercial development of Menaica anchovies.

Evolution of microbial populations of Pecorino Abruzzese during ripening, with particular attention to enterococci

Annalisa Serio - Dipartimento di Scienze degli Alimenti, Università degli Studi di Teramo, Mosciano Stazione (Te)

In order to evaluate the evolution of the microbial populations, the typical Pecorino Abruzzese cheese was analysed during manufacturing and ripening in spring (2 batches) and in summer (2 batches). Lactic acid bacteria and enterococci were the dominant groups during the entire process. Seventy enterococci strains were isolated, phenotypically and genotypically identified, and characterized for some biochemical and technological properties. *Enterococcus durans* was the most abundant species in the batches manufactured during spring, while *Enterococcus faecalis* and *E. faecium* dominated in summer batches. Most enterococci showed proteolytic activity and amino acid decarboxylase activity, whereas they possessed low acidifying power. In addition, the presence of *Enterobacteriaceae* throughout ripening suggests that the hygienic quality of Pecorino Abruzzese should be improved.

Evaluation of lipoxygenase enzyme activity in virgin olive oil

Rosa Terminiello - Dipartimento di Scienza degli Alimenti, Università "Federico II" di Napoli, Portici (NA)

One of most important problems of olive oil quality is oxidative rancidity due to the oxygenation of polyenoic fatty acids and formation of undesiderable compounds that derive from these fatty acid hydroperoxides. Beside autoxidation, it has been shown that lipoxygenases (LOXs) are involved in this process. It has been shown that the LOX located at the oil body membrane of mature olive endosperms is a linoleate 13-LOX that is preferentially active on free linoleic acid. This enzyme activity could survive olive oil production and affect the quality of this fine food product during storage. The present work was designed to detect the survival of this lipoxygenase in virgin olive oil samples, and evaluate possible differences in enzymatic activity due to technological treatments, such as traditional and de-stoned olive paste processing, and clarification phase.

Interactions between *Staphylococcus xylosus* and *Kocuria varians* strains isolated from fermented meat

Patrizio Tremonte - Dipartimento di Scienze e Tecnologie Agro-alimentari, Ambientali e Microbiologiche, Università degli Studi del Molise, Campobasso

This study was undertaken to determine the stimulating or inhibiting action between *Staphylococcus xylosus* and *Kocuria varians* strains.

Interaction was assessed by agar spot test, agar well diffusion assay and by spectrophotometric analysis (od=580). Spectrophotometric analyses were performed on all single strain cultures (indicator and producer strains), on their combinations, on indicator strain cultures (K. varians) in combination with producer strain filtrates (*S. xylosus* strains) and on indicator strain cultures in combination with heated filtrates of producer strains.

The results showed an appreciable stimulating action between *Kocuria varians* AL12 and six strains of *Staphylococcus xylosus*. Whereas five strains of Staphylococcus xylosus showed an inhibitory action against the indicator strain *K. varians* AL12. Moreover, these stimulating or inhibiting actions between *K. varians* strains and filtered *S. xylosus* strains were observed. These results were absent between *K. varians* strains and heated filtered *S. xylosus* strains.

Characterization of fresh pasta produced in Calabria (Southern Italy)

Michele Valenzise - Dipartimento di Biotecnologie per il Monitoraggio Agro-Alimentare ed Ambientale (BIOMAA), Università degli Studi Mediterranea di Reggio Calabria

This aim of this study was to characterize fresh pasta produced in the Calabria region (Southern Italy). The analysed pasta types were of traditional shapes such as macaroni and extruded shapes. Chemical, physical and sensory analysis techniques were employed for this characterization.

Morphological changes in *Lactobacillus elveticus* cn1156 in response to exposure to metabolites having a possible signalling activity

Melania Vallicelli - Dipartimento di Protezione e Valorizzazione Agro-Alimentare, Alma Mater Studiorum, Università di Bologna

The aims of this work were to identify furanones, and other metabolites released in whey by the starter *Lactobacillus helveticus* following exposure to various stresses. The possible role of these molecules in cell survival, morphologi-

cal change and autolysis was evaluated in comparison with other commercial furanones such as HDMF (2,5-dimethyl-4-hydroxy-3(2H)-furanone), HEMFI (5-ethyl-3hydroxy-4-metyl-2(5H)-furanone) and HEMF (2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone).

Thermophysical and mechanical properties of melanoidins

Elena Venir - Dipartimento di Scienze degli Alimenti, Università degli Studi di Udine

Melanoidins, final products of Maillard reaction, were obtained from a model system and separated into high (HMW) and low molecular weight (LMW) fractions. Physical and structural properties of melanoidins were investigated through thermal and rheological analysis. Results showed a small size molecular structure, similar to disaccharides of LMW, while the lack of glass transition and the higher initial melting temperature for HMW were consistent with more complex, large polymers. Mixed or single LMW and HMW fractions were characterised respectively by weak gel and strong gel behaviour. Intrinsic viscosities were consistent with spherical hydrodynamic volumes of LMW and HMW, but not for their mixture.

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GUIDE FOR AUTHORS

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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, article number or thesis fulfillment may be included.

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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 Ingegneria e Tecnologie Agro-Forestali
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Dipartimento di Scienze degli Alimenti,
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Dipartimento di Scienze e Tecnologie Agroalimentari e
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