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PAPER

UNDERSTANDING CONSUMER PERCEPTIONS OF FERMENTED YOGHURT PRODUCTS USING CONJOINT AND GENERALISED PROCRUSTES ANALYSIS

COMPRENDERE LA PERCEZIONE DEI CONSUMATORI IN MERITO AGLI YOGHURT FERMENTATI MEDIANTE L'USO DELLE TECNICHE DI CONJOINT E PROCRUSTES ANALISI

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ABSTRACT

Two methods, Conjoint Analysis and Generalised Procrustes Analysis, were used to evaluate the acceptance of a novel category of functional foods: a yoghurt with the probiotic *Lactobacillus acidophilus* and a yoghurt fortified with calcium and vitamins C and E. Three attributes, function claims, method of production, and certification by an authority, were selected. The results indicated that the respondents attached importance mainly to the presence of the certification by an au-

RIASSUNTO

Sono state utilizzate due tecniche, Conjoint Analysis e Generalised Procrustes Analysis, per valutare l'accettazione da parte del consumatore di una categoria di alimenti funzionali: lo yoghurt con il *Lactobacillus acidophilus* e lo yoghurt arricchito con calcio e vitamine. Sono stati selezionati tre attributi tra quelli che potrebbero essere importanti per l'accettazione di tali prodotti, più precisamente il messaggio che informa della funzionalità del prodotto, la tecnica di produzione e la presenza

- Key words: conjoint analysis, functional foods, GPA, Repertory Grid Method -

thority. The genetic technology, used as process of production, was seen as a negative feature by the respondents, whereas they showed little interest in function claims.

di un marchio di controllo. Dall'analisi è emerso che il marchio di controllo è l'attributo più influente per la scelta dell'alimento funzionale. La tecnologia genetica, utilizzata come tecnica di produzione, veniva considerata negativamente dai rispondenti. I messaggi informativi sulla funzionalità, invece, non risultavano essere importanti per l'accettazione dell'alimento.

INTRODUCTION

The concept of food is changing from a past emphasis on health maintenance through Recommended Dietary Allowances of nutrients to an emphasis on use of foods to promote better health by helping to prevent and treat illness and disease (GOLDBERG, 1994).

The Institute of Medicine of the US National Academy of Sciences has defined a new category of foods, so-called "functional food" as those foods that "encompass potentially healthful products," including "any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains" (THOMAS, 1994).

This concept of functional foods has captured the interest of the research community which has long worked in this area of food and pharmaceutical industries because of the large, growing market, and of governments which are concerned with health care costs. Functional foods are of interest also for the general public ("consumers") who are becoming health conscious and are demanding more information on how to achieve health benefits through food (WRICK *et al.*, 1993; CHILDS and PORYZEES, 1998).

As a result of the growing body of scientific evidence linking diet and health and the public interest in nutrition, nutrition educators are joining public health officials and the food industry in advocating putting explicit health-related information on the food label (MILLER, 1991). Public attention is easily captured by claims and previous research has found that consumers have a positive attitude towards health claims on breakfast cereal labels, although they tend to misinterpret what these particular messages say overall (FULLMER *et al.*, 1991). Health claims in the food policy process are generally recognized as claims that make a statement connecting a food or a food nutrient to the state of a desired health condition (CHILDS and PORYZEES, 1998). The most common health claims, i.e. function claims, associate nutrients with the positive physical characteristic resulting from adequate intake of the nutrient.

The whole issue of functional foods essentially relates to ingredients which provide both nutritional and health benefits. Because there is an understanding that these ingredients must be obtained from natural sources, there is a substantial amount of research in the area of modern biotechnology oriented towards identifying a new generation of functional ingredients (EC, 1998). There has been a great deal of concern about public acceptance of new biotechnology (ZECHENDORF, 1994; SPARKS *et al.*, 1995). Some studies have shown that the majority of consumers are uncertain about genetically engineered foods (KUZ-

NESOF and RITSON, 1996), and that genetic modification is less acceptable as a method of producing novel foods (FREWER *et al.*, 1997).

Consumer research in the functional food area is still in its initial stage, but WRICK *et al.* (1993) examined the attitudes and beliefs of consumers towards this new food category and how they may influence future acceptance, and CHILDS and PORYZEES (1998) looked at the marketing implications of functional foods.

In the current project two studies were carried out in order to evaluate consumer acceptance of a novel category of milk-based products. Three attributes (function claim, certification and method of production) that could be important to consumers with respect to acceptance of these novel foods were selected. In the first study, Conjoint Analysis was applied to examine the relative importance of these attributes with respect to overall preference judgment of consumers[1]. Conjoint Analysis is a technique based on multi-attribute utility theory which has been widely used in consumer research (GREEN and RAO, 1971; STEENKAMP and van TRIJP, 1988; NESS and GERHARDY, 1994; VISTOLA, 1997; FREWER *et al.*, 1997). In the second study, the Repertory Grid Method (RGM) was applied to identify consumers' beliefs concerning different novel milk-based products. RGM has been widely used to obtain information about consumer perceptions of foods (RAATS and SHEPHERD, 1993; FREWER *et al.*, 1996; MONTELEONE *et al.*, 1997).

MATERIALS AND METHODS

Products

The criterion which was used to select the products included the identification of important attributes for consumer acceptance: type of functional food, function claim, certification by an authority and method of production. The attributes chosen reflected factors that could be evaluated at the time of purchase. Each attribute selected was represented with different features. The attributes and the features are shown in Table 1.

Two types of novel milk-based products, a yoghurt with probiotic *Lactobacillus acidophilus* and a yoghurt fortified with calcium and vitamins C and E were chosen. Three examples of function claims on food labels were selected from the marketplace. A product without a nutrition claim was included. Two authorities, the Association of Consumers and the Ministry of Health, were chosen as control authorities reported on the food label. A product not having the name of any authority was included. The last attribute considered was the information on the method of production. The features chosen were the traditional method and the use of genetic engineering. The last two attributes, the certification by an authority and the method of production are not, actually, information available at the time of the purchasing decision in the Italian marketplace.

The combination of these four attributes and their respective features gave rise to 48 (2x4x3x2) descriptions of the product. Clearly, it would have been unrealistic to ask individuals their preferences for so many products. Thus, a fractional factorial design (COCHRAN and COX, 1957), assuming that there were no interaction effects, was used to reduce these to 16 descriptions of products, called profiles. Each of the 16 profiles was presented to respondents on a separate card. The order of presentation of the cards was randomized. Each card was examined individually by the respondents recruited for the first study. Groups of three cards were randomly presented to respondents recruited for the second study. All respondents were given a

Table 1 - Specification of attributes and features.

Attribute	Features
Yoghurt with	- lactic cultures La1 (lactobacillus species of intestinal origin) - calcium, vitamin C, and vitamin E
Health claims	- none - strengthens the body's defense mechanism against intestinal infections - strengthens the body's defense mechanism against free radical action, most responsible for aging - helps strengthen bone functionality
Certification	- none - Ministry of Health - Association of consumers
Method of production	- Traditional method - Genetic engineering

short description of genetic engineering before starting the two studies[1].

Study 1

Subjects

Consumers, aged 18 years or more, were approached at random in two large supermarkets in Rome. A total of 120 subjects accepted taking part in the study about consumer preferences for the two types of novel milk-based products. They were visited in their own homes at a convenient time and asked to complete a questionnaire according to the methodology described below. The mean age of the respondents was 33 years (s.d. 12.0), 53% of which were female. 56% of the respondents were 18-29 years, 28% included respondents aged 30-49 years, and 16% were over 50 years. The educational level was rather high (65.9% had a high school education and 10% had a university degree).

Questionnaire

The respondents were asked to examine the description presented for each product and they then rated the 16 profiles on a 9-point scale ranging from "not at all acceptable" to "very acceptable". In addition, the respondents rated two product profiles (holdout cards) for evaluating the results of the study. The second part of the questionnaire included questions on socio-demographic data.

Conjoint Analysis

Conjoint Analysis is an extension of the general linear model which has been developed in mathematical psychology and psychometry (LUCE and TUKEY, 1964). This technique permits the evaluation of consumer preferences about some characteristics of a product. The first phase of Conjoint Analysis consists in defining different specific models, that is, the form of the relationships between overall preference judgments for a set of objects and the attributes of the objects (WITTINK *et al.*, 1994). The simplest and most frequently used form is the additive model, which assumes that the main effects of each characteristic of attribute, called part-worth (WITTINK *et al.*, 1994), are independent of each other.

[1] "Gene technology involves the transfer of genetic material form one living thing to another. These living things may be animals, plants or microorganisms. In food production the use of genetic technology could enable the transfer of desirable characteristics form one living thing to another, leading to disease resistance in plants, to less fatty meat production in animals, etc.".

Data analysis

In this study, Conjoint Analysis was applied. The final optimal independent variables scores (the part-worth utilities) were measures of how influential the attributes were in determining subject preference. Positive utilities were associated with more preferred levels and negative utilities with less preferred features. The relative importance of each attribute was calculated as the percentage contribution to the difference between the most and the least preferred feature of the attribute difference between the lowest and the highest utilities of the attribute). This difference represented the maximum impact that the attribute could contribute to the product.

Cluster analysis was applied in order to group respondents on the basis of the similarity of their part-worth utilities estimated by Conjoint Analysis. The clustering was performed on the basis of the two-stage clustering procedure (PUNJ and STEWART, 1983). Initially, a first approximation of a solution was obtained by using Ward's method (WARD, 1963), then the outliers were discarded and a *K*-means procedure was applied (MACQUEEN, 1967). Mean part-worth utilities for each of the resulting clustered segments were calculated.

Conjoint Analysis was performed using the SPSS statistical package (SPSS, 1990). Cluster Analysis was conducted using the SAS statistical package (SAS, 1990).

Study 2

Subjects

Fifteen subjects, nine females and six males, were recruited from two large supermarkets in Rome. None of them had participated in the first study. They were interviewed according the Repertory Grid method. One week after the first interview, they were visited in their own homes and asked to complete a personalized questionnaire. The mean age of respondents was 33 years (s.d. 10).

Repertory Grid Method

The RGM provides an efficient and relatively expensive interviewing procedure to aid the consumers in developing their own vocabulary to choose amongst foods.

In this study, the RGM was divided into two interview phases. The first phase consisted of construct elicitation, the second phase involved rating the functional food products for each construct. In the first phase of the interview, the 16 (*n*) product descriptions were randomly arranged in triads and presented to the respondents. A total of 7 triads ((*n*-1)/2) was presented to each respondent (MCEWAN and THOMSON and MCEWAN, 1988). For each triad, respondents were asked: "If given the choice between these three types of food products, which would you choose and why?" (RAATS and SHEPHERD, 1993). The respondents listed short phrases or sentences (constructs) describing why they preferred that product. Subsequently, the respondents were asked to score each of the sixteen products for each construct that they mentioned on 100 mm unstructured line scales anchored by "not" and "very". Responses were written down on the personalized questionnaire by the respondents themselves. Since respondents were able to generate individual constructs, a matrix of scores from each respondent was obtained, respondent x products x constructs.

Data analysis

Free-choice profile data obtained using RGM were analyzed by classical Generalized Procrustes Analysis (GPA) (GOWER, 1975). GPA is a multivariate statistical tool which allows the agreement or consensus between individuals on each object (food product) to be maximized. This analysis has been described by many authors (ARNOLD and WILLIAMS, 1986;

DIJKSTERHUIS and GOWER, 1991/92). GPA allows each individual to have a unique set of attributes by transforming the resulting data, in order to find an agreement or consensus among respondents. The consensus represents the average of all the transformed configurations, and interpretation of this consensus enables those constructs of greatest importance to be identified. To interpret the dimensions of the profile spaces derived from the GPA, the correlation coefficients obtained by correlating the scores on the principal components with each individual's original construct scores were examined (STEENKAMP and VAN TRIJP, 1988). In order to estimate the significance of the GPA results, a Permutation test (WAKELING *et al.*, 1992; MONTELEONE *et al.*, 1997) was performed using Senstools (OP&P, 1996). This test indicated a probability of less than 5% that the consensus generated in the main study could have arisen by chance (upper 5% of the total variance accounted for in the permutated data sets was equal to 40.5, that was less than the total variance accounted for in the real data set, 75.5).

In order to simplify the interpretation of the data, an arbitrary method was adopted to select the most important constructs. Only those constructs occurring three or more times with correlation coefficients $\geq +0.7$ or ≤ -0.7 were used for the data interpretation.

In this study, GPA was performed using SENSTOOLS software (OP&P, 1996).

RESULTS

Study 1

Certification by an authority was the most important attribute influencing the consumers' preference (the degree of relative importance was 44%). The method of production and the function claim appeared to be relatively important (degrees of relative importance were, respectively, 29 and 26%), whereas the type of novel milk-based product was not important (the degree of relative importance was 1%). Analysis of the holdout cards indicated that the goodness of fit of the additive model was good, with a correlation coefficient of 1.0 between the observed and estimated preferences[2].

Cluster analysis produced a solution with three clusters. Average part-worth utilities and the relative importance of the attributes for each cluster are shown in Table 2.

It is clear that the three clusters represent people with different preference criteria. The largest group, cluster 3, considered the certification by an authority as the most important attribute (the degree of relative importance was 74%). Labels from the Ministry of Health and the Association of Consumers were the attribute features with the highest part-worth utilities among the clusters.

The method of production was the most important attribute (86% of relative importance) for the subjects of cluster 1. The traditional method was the attribute feature with the highest part-worth utilities among the three clusters, whereas genetic modification was the attribute feature with the lowest part-worth utilities. This group of respondents preferred the health claim which set out the beneficial properties in the area of intestinal infections.

The respondents of the smallest group, cluster 2, were significantly affected by the function claim (the degree of relative importance was 65%). Claims setting out the beneficial properties in the area of intestinal infections or of bone mass were the attribute features having the highest part-worth utilities among the three clusters.

The socio-demographic picture of each of the three clusters was quite similar

[2] Some results of the conjoint analysis have been published in Rosati, S., Cotta, F., Saba, A. (1999). Alimenti funzionali: atteggiamenti e preferenze dei consumatori. La Rivista Italiana di Scienza dell'Alimentazione, 3: 227.

Table 2 - Average part-worth utilities and relative importance of the attributes of the functional foods by cluster.

Attributes and levels	Average Part-Worth Utilities		
	Cluster 1 (*N*=37) 31%	Cluster 2 (*N*=31) 26%	Cluster 3 (*N*=51) 43%
YOGHURT with			
Lactobacillus acidophilus La1	0.12	0.05	-0.02
Calcium, vitamin C, and E	-0.12	-0.05	0.02
Relative Importance	0%	3%	0%
FUNCTION CLAIM			
none	-0.37	-0.57	-0.95
strengthens the body's defense mechanism against intestinal infections	0.27	0.33	0.19
strengthens the body's defense mechanism against free radical action	-0.13	-0.53	0.46
helps strengthen bone functionality	0.23	0.78	0.29
Relative Importance	3%	65%	24%
CERTIFICATION			
none	-0.61	-0.21	-1.34
Ministry of Health	0.59	0.42	1.04
Association of Consumers	0.03	-0.20	0.31
Relative Importance	11%	26%	74%
METHOD OF PRODUCTION			
traditional method	1.63	0.25	0.47
genetic engineering	-1.63	-0.25	-0.47
Relative Importance	86%	6%	2%

(Table 3). The respondents in cluster 1 were mostly females (59% of subjects in cluster 1, 52% in cluster 2, and 51% in cluster 1) and were high school graduates (70% of subjects in cluster 1, 61% in cluster 2, and 65% in cluster 3). Cluster 2 included the highest frequency of respondents with a university degree (11% of subjects in cluster 1, 13% in cluster 2, and 8% in cluster 3).

Study 2

The number of constructs elicited from each respondent ranged from 7 to 16 (the mean number of constructs being 11). The constructs were allocated to one of 28 classes by two researchers, using a predetermined category system of reduction (Table 4). When disagreement occurred, the classification was discussed until agreement was reached.

An average group score for each of the 16 food products was obtained using the Generalized Procrustes analysis. Fig. 1

Table 3 - Socio-demographic variables for each cluster.

Variable	Cluster		
	1 (*N*=37)	2 (*N*=31)	3 (*N*=51)
Gender			
Male	41%	48%	49%
Female	59%	52%	51%
Age			
18-29	51%	48%	65%
30-49	32%	32%	24%
50 **and over**	16%	19%	12%
Education			
none	3%	0%	0%
Primary-school certificate	3%	3%	4%
Secondary-school certificate	14%	23%	24%
High-school diploma	70%	61%	65%
University degree	11%	13%	8%
Occupation			
Student	14%	13%	35%
Housewife	8%	13%	4%
Retired	0%	0%	4%
Employed	57%	68%	47%
Unemployed	22%	6%	10%

Table 4 - Total number of occurrences of each construct class in the data set and number of occurrences of each construct with high correlation coefficients on the first two principal axes (PA1 and PA2)[a].

	Construct	Total	PA1 (40%)		PA2 (18%)	
			+	-	+	-
1.	Certification ensures food product	25	23	0	0	0
2.	Certification ensures method of production	7	7	0	0	0
3.	Certification ensures health claim	4	4	0	0	0
4.	Trust in certification	5	4	0	0	0
5.	Health claim adds information	1	0	0	0	0
6.	Health claim is credible/truthful	9	0	0	0	0
7.	Interesting health claim	5	0	0	0	0
8.	Traditional method is more safe	11	0	0	8	1
9.	Trust in traditional method	4	0	0	4	1
10.	Traditional method is natural	2	0	0	1	1
11.	Beneficial food product	9	1	0	1	0
12.	Trust in food product	4	1	0	0	0
13.	Natural food product	1	0	0	0	0
14.	Well-known food product	3	0	0	1	0
15.	Complete information	2	0	0	0	0
16.	Genetic engineering is uncertain	11	0	1	0	7
17.	Genetic engineering is unacceptable	4	1	0	4	0
18.	Genetic engineering is unnatural	1	0	0	0	1
19.	Health claim not interesting	3	1	0	0	0
20.	Health claim doesn't add information	5	0	0	0	0
21.	Health claim not credible/truthful	10	0	0	0	0
22.	Mistrust in certification	6	5	0	0	0
23.	There isn't an ensuring certification	13	12	0	0	0
24.	Lack of information	15	0	3	0	3
25.	Information not understandable	3	0	0	0	0
26.	Mistrust in food product	3	2	0	0	0
27.	Artificial food product	1	0	0	0	0
28.	Food product not interesting	2	0	0	0	0
	Total	169	61	4	19	14

[a] Constructs with correlation coefficients ≤-0.7 or ≥+0.7 on the first two dimensions, that occur three or more times, have been included. A construct can have a high correlation on more than one axis.

shows how the respondents separated the different types of novel food products into four groups, each of which was judged similarly by the respondents. The first two principal axes of the consensus configuration (PA1 and PA2) together explained 58.0% of the variation in the data.

A first group of products (upper right quadrant) included only those novel foods that were produced traditionally, had function claims and were certified by an authority. A second group (lower right quadrant) included novel foods that were produced by genetic technology and were certified by an authority. A third group (lower left quadrant) included profiles of products produced by genetic technology and were not certified by an authority. The last group of novel foods (upper left quadrant) was produced traditionally and were not certified by an authority.

Fig. 2 reveals that the interpretation of the average configuration of the group is straightforward. For purpose of clari-

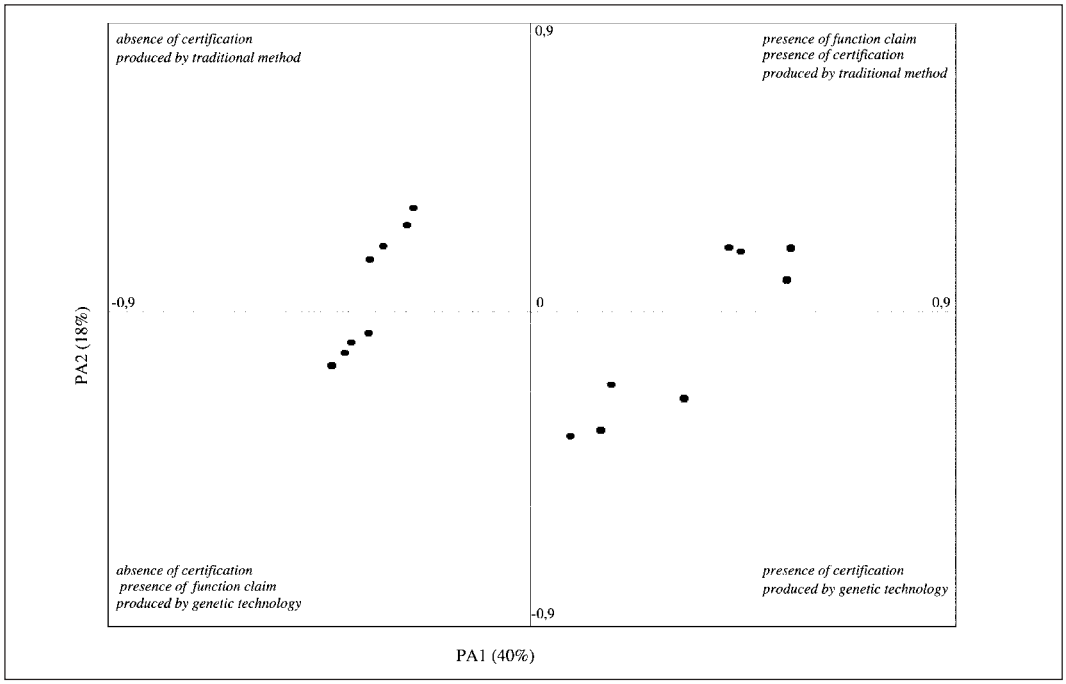


Fig. 1 - Generalized Procrustes Analysis: plot of the average group configuration for the 16 functional foods. Attributes in common with all the foods grouped in the same quadrant are identified in italics within the quadrant for which they have an explanatory value.

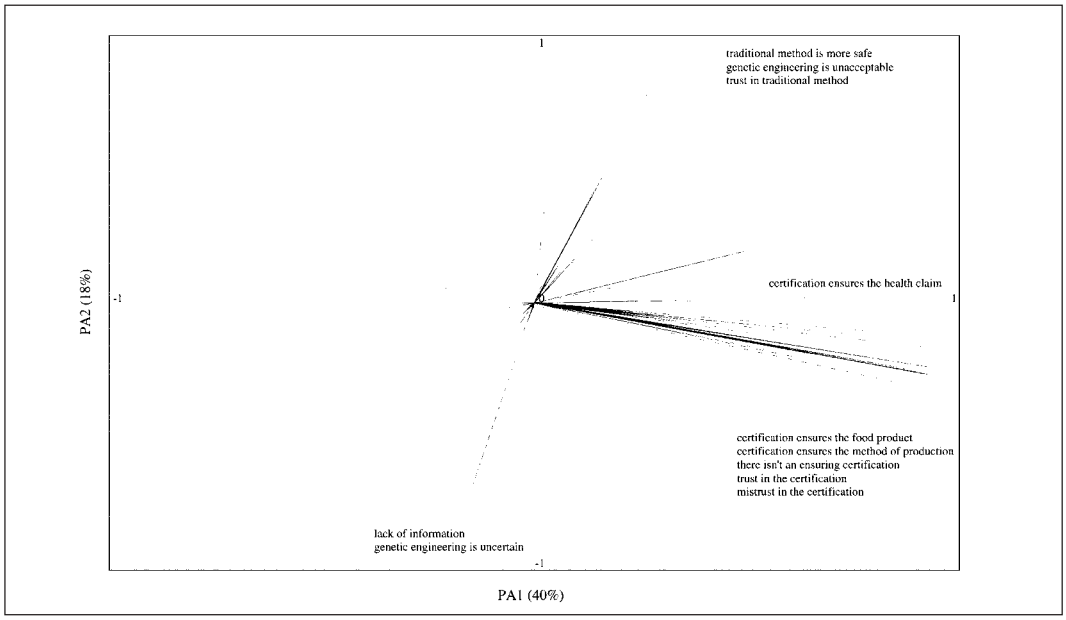


Fig. 2 - Generalized Procrustes Analysis: plot of the correlation ≤-0.7 or ≥+0.7 on the first two principal axes of the average group configuration.

ty, the vectors are not labelled individually, but labels are for groups of vectors associated with similar constructs. The terms used to describe the first dimension were "certification ensures the food product", "certification ensures the method of production", "certification ensures the health claim", "trust in the certification". Other terms used to describe this dimension were "mistrust in the certification" and "there isn't an ensuring certification". The second dimension referred to the method of production and to the information reported on the food label. The constructs "traditional method is safer", "trust in traditional method" and "genetic engineering is unacceptable" described the positive side of the second dimension, whilst the negative side of this dimension was described by the constructs "genetic engineering is uncertain" and "lack of information".

DISCUSSION

The Conjoint Analysis showed that the respondents attached importance mainly to the presence of the certification by an authority, such as the Ministry of Health or the Association of Consumers, followed by the method of production and by the function claim. Genetic technology was seen as a negative feature by the respondents. Studies have suggested that the acceptance of food produced by genic technology is likely to increase if there are direct benefits, in particular for consumers, resulting from that technological innovation (FREWER *et al.*, 1997). In this study we did not take into consideration interactions between the attributes, so it was possible to test how the certification by an authority is important in influencing the acceptance of novel food when the production is genetic technology. Function claims, expressing beneficial effects on the intestinal system or bone mass, were the most common messages of the respondents.

From the results of the RGM and GPA it was apparent that, whilst the content of a health message was not found to be important in accepting the novel food, the reactions of the respondents were positively influenced by the presence of a certification by an authority that reassured them about all information reported on the food label and the method of production. Concern about genetic technology appeared mainly to be associated with uncertainty and rejection of that innovative technology. Providing information is likely to influence attitude (HOBAN, 1996), however increased understanding does not necessarily facilitate acceptance of new technology (HAMSTRA, 1991).

RGM was confirmed as an efficient method to gain information about the reasons why the preference of these novel foods differed. However, a methodological consideration which should be made in interpreting the results from the two different studies is that the Conjoint Analysis revealed the relative importance of each pre-selected attribute for preference, whilst the RGM allowed the respondents to describe their preference using their own words. The results of the RGM, completed with the GPA, appeared to confirm in some way the findings of the Conjoint Analysis study, showing that the presence of a certification by an authority was the major concern. However, care must be taken when interpreting the results of this study, because the two small samples of respondents cannot be considered representative of the attitudes towards the functional foods.

In the present study, only respondents in cluster 2 showed interest in function claims. This could confirm, in some way, previous studies that found that consumers want more information about nutrients that may be associated with disease (RUSSO *et al.*, 1986; FULLMER *et al.*, 1991). FULLMER *et al.* (1991) sug-

gested that attitudes toward placing health messages on food labels were very positive when the information was not questioned by nutrition experts. Therefore, claims reported on the food label may play a role in public health education as they inform the consumers of a health advantage of the food. Thus, to the degree that the function claim influences consumption, it helps the consumer conform to public policy goals by assisting in choosing healthier foods.

A number of studies have presented data showing that probiotics and foods fortified with vitamins have significant potential in improving human health (HITCHINS and MCDONOUGH, 1989; GOLDBERG, 1994; GOLDIN, 1998;). The positive health effects of these products should be re-inforced by regulatory bodies, who must be seen to be proactive in taking appropriate regulatory measures to increase trust in information reported on the food label and also functional foods as a whole.

CONCLUSIONS

The results from the two studies showed that the respondents attached importance mainly to the presence of a certification by an authority, such as the Ministry of Health or the Association of Consumers, whereas the function claim was not found as important in accepting the novel food. Concern about genetic technology appeared to be associated with uncertainty and rejection of that innovative technology.

However, the lack of knowledge about novel food products, on the other hand, leads people to have lack of confidence in these products. An important issue, particularly if innovative biotechnology is the delivery mechanism for the health benefit of the food, is the role of labelling transgenic foods and how food biotechnology impacts the choice for food health claim labelling.

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PAPER

LACTOBACILLUS PARACASEI SUBSP. *PARACASEI* HETEROGENEITY: THE DIVERSITY AMONG STRAINS ISOLATED FROM TRADITIONAL GREEK CHEESES

ETEROGEINICITÀ DEL *LACTOBACILLUS PARACASEI* SUBSP. *PARACASEI*: DIVERSITÀ TRA CEPPI ISOLATI DA FORMAGGI TRADIZIONALI GRECI

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ABSTRACT

Twenty-seven strains isolated from traditional Greek cheeses and phenotypically identified as *L. paracasei* subsp. *paracasei* were studied for their diversity in genetic and some biotechnological characteristics. Strain identification was confirmed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of whole-cell proteins, except for one strain. Six SDS-PAGE clusters were distinguished at a similarity level of 68%. RAPD-PCR was also used for typing of the strains and this confirmed the re-

RIASSUNTO

Ventisette ceppi isolati da formaggi tradizionali greci e fenotipicamente identificati come *L. paracasei* subsp. *paracasei* sono stati studiati per la loro diversità in alcune caratteristiche genetiche e biotecnologiche. L'identificazione dei ceppi è stata confermata mediante elettroforesi SDS-PAGE delle proteine totali cellulari, con l'eccezione di un solo ceppo. Con valori di somiglianza pari al 68%, è stato possibile distinguere sei gruppi SDS-PAGE. La PCR RAPD, utilizzata per tipizzare i ceppi, ha permes-

- Key words: acidification, *L. paracasei* subsp. *paracasei*, plasmids, proteolysis, RAPD-PCR, SDS-PAGE -

sults of SDS-PAGE, concerning the identity of the strains. According to their genetic relatedness, three distinct clusters of *L. paracasei* subsp. *paracasei* strains with an internal correlation coefficient (r) ranging from 55.4 to 62.6% were formed. The strains differed in both the number and molecular weights of plasmids harboured. Biotypes were found which differed in terms of acidifying, caseinolytic and aminopeptidase activities.

so di riconfermare i dati del SDS-PAGE relativamente alla identificazione dei ceppi. In relazione alla loro familiarità genetica, sono stati creati tre distinti gruppi di ceppi di *L. paracasei* subsp. *paracasei* con un coefficiente interno (r) compreso tra il 55,4 ed il 62,6%. I ceppi differiscono tra loro sia nel numero che nel peso molecolare dei plasmidi presenti. Sono stati identificati biotipi che differiscono per attività acidificante, caseinolitica ed amminopeptidasica.

INTRODUCTION

Mesophilic lactobacilli constitute a significant part of the non starter lactic acid bacteria (NSLAB) present in traditional Greek cheeses (TZANETAKIS and LITOPOULOU-TZANETAKI 1992; PRODROMOU *et al.*, 2001) and become the dominant bacterial population in the maturing cheeses. Although the exact role of NSLAB in the development of cheese flavour is not completely understood, it is well known that mesophilic lactobacilli play an important role during the ripening of cheeses (LYNCH *et al.*, 1997) as they may be involved in lipolysis and proteolysis (WILLIAMS and BANKS, 1997). Selected adjunct strains of *Lactobacillus* spp. have been found to improve the quality of cheeses (LYNCH *et al.*, 1997). Selected *L. paracasei* subsp. *paracasei* strains used in adjunct combinations to make Cheddar cheese showed flavour improvement (CROW *et al.*, 2001). It appears therefore, that there is an obvious need to characterize, study and finally preserve interesting strains from a biotechnological point of view. Traditional Greek cheeses, particularly those made from unpasteurized milk, may be interesting sources for strain isolation and selection.

The study of microbial diversity is important for exploiting the peculiarities of microorganisms and allows the choice of biotypes with interesting biotechnological characteristics for the various fermentation processes. Thus, the diversity and perhaps the source of isolation of the wild-type strains may contribute to flavour differences and specific features detected for the same product in different areas or to differences between the various cheese varieties. The purpose of the present investigation was to study the genetic diversity as well as the heterogeneity in some biochemical activities of twenty-seven *Lactobacillus paracasei* subsp. *paracasei* wild isolates from four traditional Greek cheeses.

MATERIALS AND METHODS

Bacterial strains and growth conditions

A total of 27 strains phenotypically (API50CHL, Biomerieux, Marcy l'Etoile, France) identified as *L. paracasei* subsp. *paracasei* were analyzed; seven strains were isolated from white-brined cheese from raw goat milk (LITOPOULOU-TZANETAKI and TZANETAKIS, 1992), six from Feta cheese (TZANETAKIS and LITOPOULOU-TZANETAKI, 1992), six from Ladotyri of Mytelene (a PDO cheese) and eight from Orinotyri (PRODROMOU *et al.*, 2001). The strains NCDO 151 (National Collection of

Dairy Organisms, AFRC Institute of Food Research, Reading Laboratory, Reading, Berkshire, UK) and LMG 11459 (Culture Collection of the Laboratorium voor Microbiologie, Universiteit Gent, Gent Belgium) were used as reference strains of *L. paracasei* subsp. *paracasei*. *L. rhamnosus* LMG 6400, *L. paraplantarum* LMG 16673, *L. plantarum* ATCC 14917 (ATCC, American Type Culture Collection, Rockville, Maryland, USA), *L. pentosus* NCFB 363 (NCFB, National Collection of Food Bacteria, AFRC Reading Laboratory) were also included as reference strains. The strains were grown in MRS broth (Merck 64271 Darmstadt, Germany) at 30°C for 24h. Stock cultures were maintained at -80°C in MRS broth plus glycerol (70:30) and working cultures were prepared from frozen stocks through two transfers in MRS broth.

SDS-PAGE of whole-cell protein extracts

Activated strains were grown in 20 mL MRS broth at 30°C for 24h. Cells were collected from the growth medium by centrifugation (4,000 g, 10 min, 4°C). The pellet was washed three times with 10 mM K phosphate buffer (pH 7.0). The washed cells were disrupted by grinding with Alumina (Type A-5, Sigma chemical Co, St Louis, MO 63178, USA) in a mortar and pestle. The extracts were suspended in 1 mL 10 mM K phosphate buffer (pH 7.0) and Alumina and cells were separated by centrifugation (12,000 g, 10 min, 4°C). The supernatant fluids obtained were designated as crude cell-free extracts. The extracts were subjected to SDS-PAGE according to LAEMMLI (1970). Recording of the protein electrophoretic patterns, normalization of the densitometric traces, grouping of strains by the Pearson product moment correlation coefficient (r) and UPGMA (Unweighted Pair Group Method using Average linkages) cluster analysis were performed by the techniques described by POT *et al.* (1994) using the software package Gel-Compar (Version 4.0, Applied Maths, Kortrijk, Belgium). Identification of the isolates was performed by comparison of their protein patterns to the fingerprints of reference strains.

RAPD-PCR analysis

The strains were grown in MRS broth at 30°C for 24h. The total DNA of the strains was obtained by phenol-chloroform extraction as described by DE LOS REYES- GAVILAN *et al.* (1992) and quantified by electrophoresis on 0.8% (w/v) agarose gels. Amplification was performed in a PTC-100 Thermo Cycler (MJ Research Inc. Massachusetts, USA). The reaction mixture had a total volume of 25 µL and consisted of 5 µL of chromosomal DNA, 1.5 mM $MgCl_2$, 200 µM dNTPs, 2.5 µL 10x PCR buffer (Gibco-BRL Life Technologies, Gaithersburg, U.S.A.) and 2 units of Taq polymerase. Two single arbitrary primers M13 (5'-GAGGGTGGCGGTTCT-3') and G1 (5'-GAAGTCGTAACAAGGTAGCC-3') were used, at concentrations of 1 µM and 2 µM, respectively. The reaction mixture was cycled through the following temperature profiles: 1 cycle at 94°C (1 min) and 35 cycles at 94°C (1 min), 45°C (20 sec) and 72°C (2 min). Amplification reactions were followed by cooling at 6°C. Amplification products were analyzed by electrophoresis in 1.5% (w/v) agarose (GIBCO) gels and staining with ethidium bromide. PCR fingerprinting profiles of photo positives were scanned with an AGFA studiostar scanner (AGFA-GEVAERT N.V., Morstel, Belgium). The densitometric traces of the band profiles were analyzed with the pattern analysis software package Gel-Compar (Version 4.0). Calculation of similarity of the band profiles was based on the Pearson correlation coefficient r. A dendrogram was deduced from the similarity matrix by using UPGMA clustering algorithm (VAUTERIN and VAUTERIN, 1992).

Plasmid profiling

The plasmid profile analysis was performed on 4 mL of a 24 h incubated culture in MRS broth, as previously described by ANDERSON and McKAY (1983). The samples were subjected to electrophoresis (0.6% agarose gels in 40 mM Tris-acetate, 1 mM EDTA, pH 8.0 buffer) at 100 V. A molecular weight marker of 125-23,130 bp (l DNA/Hind III Fragments, GibcoBRL) was used.

Aminopeptidase activity of intact cells

The aminopeptidase activities of the strains were tested on the substrates L-alanine p-nitroanilide, L-leucine p-nitroanilide and L-lysine p-nitroanilide, as suggested by REQUENA *et al.* (1991) with modifications. Thus, cell suspensions at 4 on the McFarland scale were used and the reactions were terminated after 4h at 30°C.

Acidification ability

The strains were initially grown in MRS broth and then in sterile reconstituted skim milk supplemented with yeast extract (0.3%) and glucose (0.2%) for two successive subcultures. Sterile reconstituted skim milk (100 mL) was inoculated with 1% of a 24h activated culture and pH changes were measured and automatically recorded by pH meter (glass electrode; HANNA Instruments, Padova, Italy) during incubation at 30°C for 24h. At this time the proteolytic activity of the strains was also estimated.

Proteolytic activity

The proteolytic activity of the strains grown in milk for 24h at 30°C was measured by the o-PA (o-pthaldialdehyde) method (CHURCH *et al.*, 1983) and results are expressed in L-glycine equivalent. Their caseinolytic activity was also estimated after 7 days of growth in milk by UREA-PAGE, according to ANDREWS (1983). The gels were scanned and peak areas were estimated by Gel-Compar version 4.0.

RESULTS

Genetic diversity

According to the SDS-PAGE results, shown in Fig. 1, the phenotypic identification was confirmed for all strains of *L. paracasei* subsp. *paracasei* except for strain L3.3, which showed a close resemblance to *L. plantarum*/*L. paraplantarum*. The results of the numerical analysis of the SDS-PAGE protein patterns showed a high heterogeneity of the *L. paracasei* subsp. *paracasei* strains. Thus, three main clusters (I, II, and V) were distinguished at a similarity level of 68%. Cluster I (mean correlation r = 0.85) comprised seven strains, five of which were isolated from Orinotyri. Cluster II (r = 0.86) was composed of four strains from goat milk cheese and three from Ladotyri and also included the reference strain LMG11459. Three strains from Ladotyri, one from Feta and the type strain *L. paracasei* subsp. *paracasei* NCDO151 formed cluster V (r = 0.79). Clusters III, IV and VI delineated at correlation levels r of 0.95, 0.88 and 0.87, respectively, contained 3,2 and 2 strains. Strain R30.5 from Feta cheese had an overall profile different from the other strains, but this did not affect its identity. The intragel reproducibility was always above r = 0.96.

PCR fingerprinting profiles are presented in Fig. 2. The relatedness of the strains studied was evaluated by computer-based comparisons of their M13 and G1 RAPD fingerprints. UPGMA dendrograms were derived from the patterns generated by each primer. The use of the M13 primer generated patterns with PCR products ranging from 300 to 2,322 bp. A common band of ~350 bp was observed as the main PCR product from all but

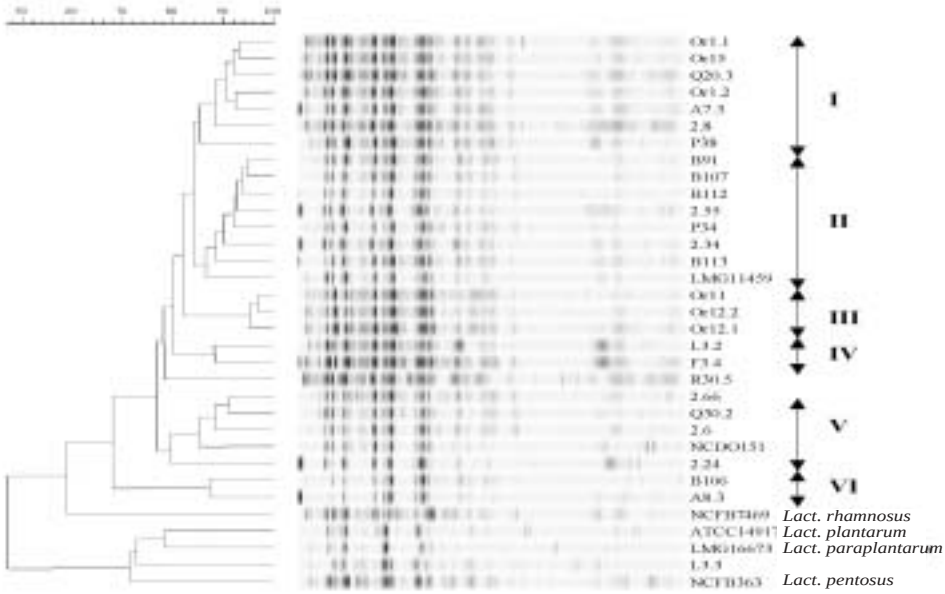


Fig. 1 - Dendrogram of the digitized and normalized protein patterns after SDS-PAGE of cell-free extracts of *L. paracasei* subsp. *paracasei* isolates and reference strains. Pearson moment correlation coefficient r was used and clustering analysis was performed by the UPGMA method. NCDO151 and LMG11459 are reference strains of *L. paracasei* subsp. *paracasei*. P34, P38, B91, B106, B107, B112 and B113 were isolates from white-brined raw goat milk cheese; F3.4, L3.2, L3.3, R30.5, Q30.2 and Q20.3 were isolates from Feta cheese; 2.55, 2.6, 2.66, 2.8, 2.24 and 2.34 were isolates from Ladotyri; Or1.1, Or1.2, Or11, Or12.1, Or12.2, Or15, A7.3 and A8.3 were isolates from Orinotyri.

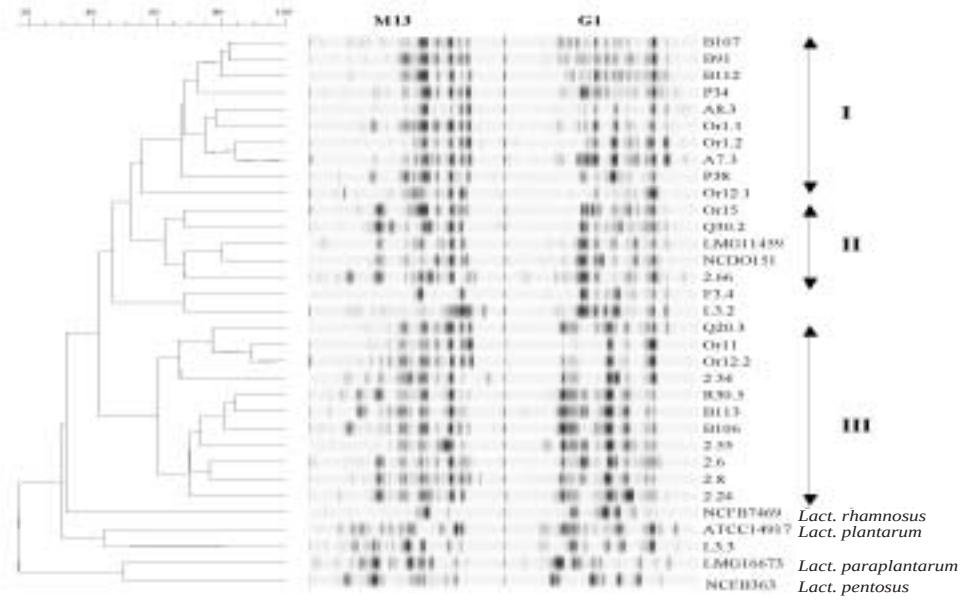


Fig. 2 - Dendrogram obtained by using RAPD profiles for 27 strains of *L. paracasei* subsp. *paracasei* from two primers, followed by evaluation using the Pearson product moment correlation coefficient (r) and the unweighted pair group algorithm with arithmetic averages (UPGMA). Reference and test strains, as explained in Fig. 1.

two strains (L3.2, L3.3); it was also present in the two *L. paracasei* subsp. *paracasei* referense strains. PCR patterns using the G1 primer consisted of DNA bands sized between 200 and 2,322 bp. A common band of ~350 bp for all strains, including the two reference strains, was also observed. The numerical analysis of the combined patterns using both M13 and G1 profiles showed three distinct clusters with internal correlation coefficients 0.55, 0.63 and 0.61, for cluster I, II and III, respectively. Two isolates (F3.4 and L3.2) from Feta cheese gave profiles different from the strains grouped in clusters I and/or II. Strain L3.3, which showed a close resemblance to *L. plantarum*/*L. paraplantarum* by SDS-PAGE seemed to be related to *L. plantarum* reference strain according to RAPD-PCR results.

The diversity of RAPD products generated by M13 was higher than that generated by the G1 primer and this subdivided clusters I, II and III into two subclusters, each; strains P38 and Or12.1 had profiles different from each other and the rest of the strains of group I. Each cluster contained strains that were at least 80% similar. The two *L. paracasei* subsp. *paracasei* reference strains were 80% similar and their percentage similarity with the three test strains found in group II was 63%. The intragel reproducibility was always above r = 0.94.

No plasmid was detected in two strains (P34 and B106). As summarized in Table 1, the number of plasmids

Table 1 - Plasmid profiles of *L. paracasei* subsp. *paracasei* strains from various traditional Greek cheeses.

Bacterial strains	Number of plasmids	Plasmid size (kbp)
P34	0	
P38	6	14, 10.7, 7.2, 5.2, 3.3, 1.8
B91	3	19.1,17.1,8.5
B106	0	
B107	2	19.4, 11.2
B112	1	0.8
B113	9	22.1, 18.8, 14.9, 12, 6.9, 5.7, 3.8, 2.1, 1
F3.4	3	19.5, 17.8, 9.2
L3.2	1	20
L3.3	10	20.4, 17.2, 13.8, 9.4, 8.8, 6.6, 4.3, 2.1, 1.3
R30.5	4	12.2, 10.4, 5.8, 4.9
Q30.2	1	18.6
Q20.3	3	19.2, 12.3, 5.8
2.55	8	18.4, 11.6, 9.4, 7.7, 6.1, 5.4, 3.3, 3.1
2.6	3	19.2, 11.6, 4.7
2.66	2	3.5, 1.9
2.8	3	18.5, 12.2, 5.8
2.24	2	18.4, 10.7
2.34	8	18.7, 11.1, 9.7, 4.9, 2.4, 2, 1.4, 1
Or1.1	3	19.4, 3.7, 2
Or1.2	3	19.1, 6.3, 3.1
Or11	5	18.4, 9.6, 8.2, 4.5, 3.8
Or12.1	5	19.7, 9.5, 7.8, 4.4, 3.7
Or12.2	9	20.4, 17.2, 13.8, 9.4, 8.8, 6.6, 4.3, 2.1, 1.3
Or15	7	18.6, 14, 12.3, 9.4, 7.2, 6, 4.4
A8.3	7	18.8, 13.5, 10.6, 7.5, 5.1, 3.2, 1.9
A7.3	7	19.2, 12, 10.7, 7, 5.2, 3.7, 1.9

present in each of the 25 strains varied between 1 and 10 ranging from 0.8 to 22.1 kb in size; all these strains seemed to have unique plasmid profiles, but there were strains with common bands. Moreover, most of the strains had large plasmids, but low molecular weight plasmids predominated over the high molecular weight plasmids. Concerning the high number of plasmids harboured by some strains, the presence of OC and L forms of the same plasmid molecule can not be excluded.

Diversity in biochemical activities.

With respect to the acidifying activity of the test strains, shown in Table 2, two main groups with various degrees of acid development were observed. These groups were distinguished as slow and medium strains, according to their ability to reduce the pH more or less rapidly.

Slow strains showed slower rates of acid development; after 24h, the pH decrease (ΔpH 24h) was less than one unit. It is interesting to note that the strains of this cluster were the raw goat milk cheese isolates.

Medium strains showed a ΔpH 24h ranging from 1.17 to 2.56 pH units. In this group, two subgroups of strains can be distinguished, A and B. Subgroup A included strains which were slower than B throughout incubation and which de-

Table 2 - Acidifying activity of the *L. paracasei* subsp *paracasei* strains. Means of two trials (x±SD).

Cluster		Strains	ΔpH				pH	
			6h	12h	18h	24h	6h	24h
Slow activity		P34	0.34±0.02	0.32±0.00	0.46±0.00	0.74±0.00	5.94±0.03	5.54±0.00
		B91	0.33±0.04	0.49±0.00	0.52±0.00	0.59±0.00	5.91±0.07	5.67±0.00
		B106	0.37±0.07	0.61±0.08	0.66±0.27	0.52±0.00	5.77±0.03	5.51±0.00
		B107	0.32±0.07	0.42±0.00	0.4 ±0.09	0.51±0.17	6.05±0.07	5.83±0.03
		B112	0.35±0.11	0.44±0.01	0.49±0.13	0.60±0.23	5.89±0.00	5.65±0.12
Medium activity		P38	0.35±0.04	0.92±0.00	1.52±0.00	1.91±0.00	5.99±0.01	4.45±0.00
		B113	0.34±0.13	0.85±0.25	1.30±0.23	1.63±0.33	5.92±0.01	4.62±0.18
		F3.4	0.11±0.08	0.61±0.00	1.23±0.00	1.76±0.05	6.30±0.01	4.65±0.12
		L3.2	0.20±0.08	0.50±0.25	0.96±0.01	1.55±0.45	6.04±0.16	4.69±0.38
		R30.5	0.25±0.01	0.87±0.09	1.43±0.21	1.89±0.25	6.03±0.01	4.39±0.23
		2.6	0.32±0.02	0.89±0.06	1.42±0.02	1.85±0.04	6.11±0.18	4.57±0.16
	A	2.24	0.33±0.04	0.89±0.03	1.12±0.05	1.59±0.15	6.00±0.18	4.74±0.36
		A7.3	0.26±0.03	0.68±0.06	1.02±0.11	1.41±0.17	5.95±0.13	4.80±0.01
		Or1.1	0.32±0.02	0.96±0.13	1.51±0.32	1.90±0.28	5.86 +0.00	4.23±0.25
		2.34	0.29±0.04	0.76±0.01	1.30±0.01	1.79±0.03	6.01±0.05	4.51±0.06
		2.55	0.28±0.03	0.64±0.06	0.89±0.18	1.17±0.20	5.99±0.14	5.10±0.31
		A8.3	0.39±0.21	0.95±0.30	1.47±0.31	1.73±0.16	5.84±0.15	4.50±0.10
		Or12.2	0.24±0.03	0.67±0.01	1.24±0.01	1.70±0.01	6.16±0.11	4.71±0.15
		Or1.2	0.28±0.04	0.75±0.20	1.16±0.36	1.53±0.54	5.85±0.02	4.60±0.53
		Or12.1	0.22±0.02	0.70±0.08	1.41±0.11	1.99±0.00	6.03±0.09	4.25±0.23
		L3.3	0.52±0.07	1.15±0.18	1.55±0.37	2.18±0.21	5.72±0.21	4.03±0.07
		Q30.2	0.30±0.04	0.92±0.05	1.74±0.33	2.13±0.21	5.95±0.22	4.12±0.38
		Q20.3	0.31±0.07	1.02±0.13	1.84±0.12	2.28±0.06	5.85±0.01	3.83±0.03
	B	2.8	0.40±0.02	1.11±0.01	1.89±0.04	2.36±0.04	5.96±0.02	3.99±0.04
		2.66	0.38±0.04	1.05±0.22	1.72±0.31	2.05±0.25	5.80±0.06	4.13±0.28
		Or15	0.43±0.09	1.20±0.19	1.90±0.21	2.25±0.08	5.71±0.21	3.89±0.20
		Or11	0.47±0.08	1.27±0.04	1.93±0.11	2.22±0.05	5.87±0.02	4.12±0.05

termined a ΔpH at 18h of 0.89-1.52; their ΔpH 24h was < 2 units. Subgroup B had a faster acidification rate with ΔpH at 18h between 1.55 and 1.93 pH units; their ΔpH 24h was >2.0 pH units.

Fig. 3 shows the values for aminopeptidase activity of whole cells of the test strains for three peptide derivatives. In general, there was higher activity of leu aminopeptidase than either lys or ala aminopeptidase activity. *L. paracasei* subsp. *paracasei* strains deriving from white brined goat milk cheese exhibited the highest aminopeptidase activity. In addition, for the strains from goat milk cheese, leu aminopeptidase > lys aminopeptidase > ala aminopeptidase activity with ranges of activities of 4.19-5.58, 1.82-4.64 and 1.21-1.90 units, respectively, were obtained. It was also observed, that in general strains from feta cheese exhibited leu aminopeptidase > ala aminopeptidase > lys aminopeptidase activities. The activities of strains from the hard cheeses Orinotyri and Ladotyri on ala and/or lys p-nitroanilide were strain dependent.

As shown in Fig. 4, the amount of amino acids (in L-glycine equivalent) accumulated in the milk after 24h by the various strains varied greatly. The range of proteolytic activity was 3.24 (strain L.3.3) - 105.97 (strain B113) ppm of L-glycine equivalent. Fourteen strains displayed low activity (<50 ppm glycine). A higher activity (50-<100 ppm glycine) was recorded for 12 strains, while only one strain isolated from raw goat milk cheese exhibited a rather high (>100 ppm glycine) proteolytic activity after 24h of incubation at 30°C. Moreover, the data reported here on proteolytic activity suggest that the amount of proteolysis was not related to the source of the strain isolation nor to the final pH of the cultures (Table 2).

The caseinolytic activity of the test strains after growth for seven days in milk, obtained by UREA-PAGE, was significant, as suggested by results of Table 3. The strains degraded preferentially a_s-casein; strain 2.34 from Ladotyri degraded the whole amount of a_s-casein, while strain 2.55, also from the same

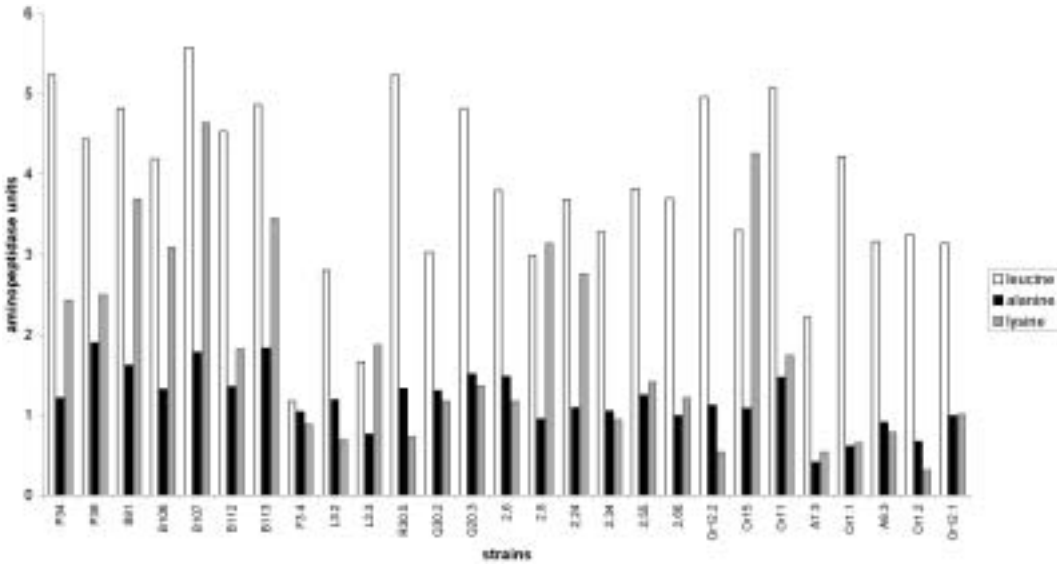


Fig. 3 - Aminopeptidase activities of whole cells of *L. paracasei* subsp. *paracasei* from traditional Greek cheeses (means of three trials). Test strains as explained in Fig. 1.

cheese, left a_s-casein completely unattacked. One strain from Orinotyri (A8.3) did not degrade β-casein. Strains R30.5, A7.3, Or11 and 2.66 did not exhibit any activity on either a_s-or β-casein after seven days of growth in milk.

DISCUSSION

The comparison of whole-cell protein patterns obtained by highly standardized sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) proved to be extremely reliable on the species and/or subspecies level (POT *et al.*, 1994). The taxonomic information obtained by SDS-PAGE for the 27 *Lactobacillus* isolates from four different Greek cheeses was the confirmation of their phenotypic (API50CHL) characterization as *Lactobacillus paracasei* subsp. *paracasei* for all strains, except strain L3.3; this strain showed a close resemblance to *L. plantarum*/*L. paraplantarum*. Apparently, the comparison of the protein patterns revealed a satisfactory similarity between the strains. Nevertheless, wild strains of NSLAB isolated from various habitats may show interstrain variability due to environmental pressures, as a result of minor or major changes of the phenotype and genotype. Genotypic distinctiveness between strains was reflected in their protein profile and it was possible by SDS-PAGE to discriminate between strains; thus several groupings of *L. paracasei* subsp. *paracasei* strains were obtained.

The numerical analysis of the RAPD-PCR profiles confirmed the phenotypic identification of the strains and clarified the taxonomic position of strain L3.3; this strain was characterized as *L. plantarum* by RAPD-PCR. The use of G1 primer produced patterns suitable for differentiating strains (ANDRIGHETTO *et al.*, 1998). Primer M13, which has been used for strain typing of *Lactobacillus* species by DNA-DNA hybridization (MITEVA *et al.*, 1992) was also used in this study to obtain strain specific patterns of lactobacilli. The RAPD-PCR data were analysed by UPGMA dendrograms,

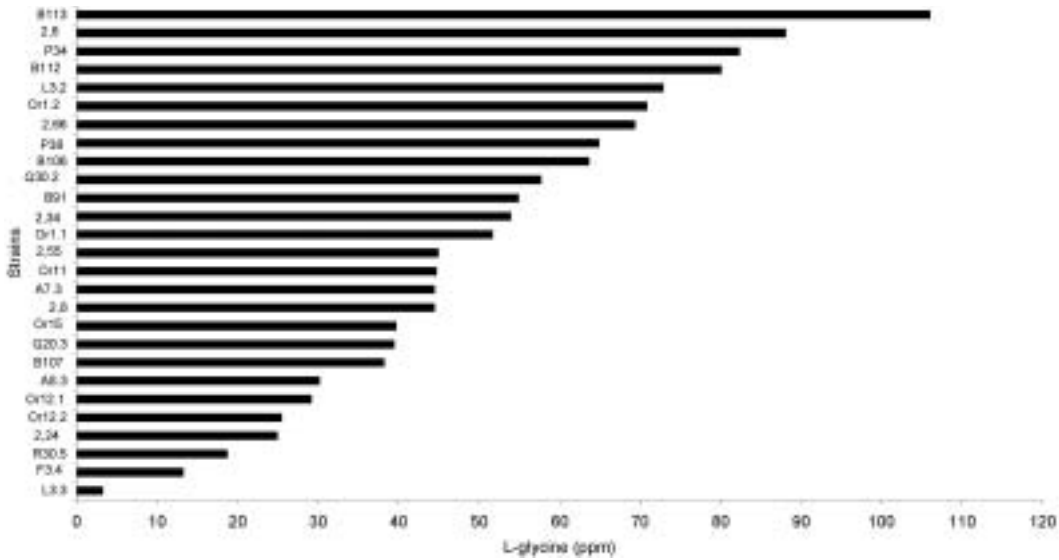


Fig. 4 - Proteolytic activity of *L. paracasei* subsp. *paracasei* strains after growth in milk for 24h at 30°C (o-PA method; means of three trials). Test strains as explained in Fig. 1.

Table 3 - Hydrolysis of a_s- and β-caseins by *L. paracasei* subsp. *paracasei* strains. The extent of hydrolysis (% reduction) was assessed via the ratio of intensities of the corresponding bands at 7 days to those of the initial time.

Strains	Caseins	% Reduction
P 34	β-	48.45
	α_s-	76.17
P 38	β-	10.04
	α_s-	40.06
B 91	β-	67.17
	α_s-	93.62
B 106	β-	3.09
	α_s-	16.81
B 107	β-	29.65
	α_s-	47.36
B 112	β-	65.22
	α_s-	89.29
B 113	β-	57.29
	α_s-	83.29
F 3.4	β-	46.88
	α_s-	69.19
L 3.2	β-	45.98
	α_s-	82.35
L 3.3	β-	35.16
	α_s-	67.74
R 30.5	β-	0
	α_s-	0
Q 30.2	β-	14.36
	α_s-	48.26
Q 20.3	β-	3.22
	α_s-	39.68
2.6	β-	11.86
	α_s-	52.53
2.8	β-	12.51
	α_s-	23.16
2.24	β-	12.6
	α_s-	23.21
A 7.3	β-	0
	α_s-	0
OR 1.1	β-	43.27
	α_s-	64.57
A 8.3	β-	0
	α_s-	16.77
OR 12.2	β-	12.34
	α_s-	37.55
OR 15	β-	50.88
	α_s-	99.34
OR 11	β-	0
	α_s-	0
2.34	β-	74.8
	α_s-	100
2.55	β-	3.83
	α_s-	0
2.66	β-	0
	α_s-	0
OR 1.2	β-	13.99
	α_s-	24.26
OR 12.1	β-	46.07
	α_s-	64.1

enabling the simultaneous comparison of both primers. At a similarity level of 42% the *L. paracasei* subsp. *paracasei* group comprised three distinct clusters. Quite low correlation levels within the three clusters, as a consequence of considerable strain heterogeneity was observed. However, there were strains with very similar profiles (coefficient of similarity $r > 0.80$). Strains with profiles more than 80% similar are considered to be extremely close genetically and perhaps identical (TAILLIEZ *et al.*, 1998). Thus, very similar strains, (for example Or1.1 with Or12.1, both from Orinotyri and B113 with B106 from raw goat milk white-brined cheese) could be multiple isolates of the same strain since they were isolated from the same cheese. A further subdivision according to the different ecological niches used as sources of the strain isolation was not possible. Nevertheless, two subgroups of cluster I, delineated at $r = 0.73$ and 0.75 respectively, consisted of four strains each, deriving from raw goat milk cheese the former and from Orinotyri the latter.

There were two strains lacking plasmids detectable by the isolation and separation conditions used. Plasmid analysis did not group the isolates and each strain had a unique plasmid profile. Moreover, strains having a common band did not fall into a group or subgroup formed by RAPD-PCR. The above results could suggest that patterns obtained by this method are not influenced by the presence of plasmids; results also suggest that this technique is unable to detect variations in the genetic extrachromosomal complements (MANNU *et al.*, 1999). In addition, the different plasmid profiles in *L. paracasei* subsp. *paracasei* strains did not allow the characterization of the strains. Since we do not know what these plasmids encode they must remain regarded as cryptic at present. However, plasmids may have a role in the beneficial characteristics of the lactobacilli (McKAY, 1985) and it is

thus necessary to continue research on the plasmid biology of these bacteria.

With regard to acidifying activities, groups of strains characterized by different acidifying abilities were observed. It seems, that none of *L. paracasei* subsp. *paracasei* strains can be characterized as fast as they did not reach a pH of 5.0±0.2 in 6h at 30°C (HUGGINS and SANDINE, 1984). The amount of acid formed in the milk after 24h of growth was higher than that of *L. plantarum* (XANTHOPOULOS *et al.*, 2000). *Lactobacillus casei* and *L. plantarum* may ferment lactose through a b-galactosidase activity, but some strains also show a b-phospho-galactosidase activity (HERRERA *et al.*, 1996).

With respect to proteolytic and peptidase activities, biotypes characterized by a very different casein breakdown ability and by different peptidase activities were observed. This peptidase activity may be related to the cell wall or to the outer surface of the cell membrane or to a possible intracellular enzyme leakage (REQUENA *et al.*, 1991). The uptake of the peptide derivatives through the cell envelope (THOMAS and PRITCHARD, 1987) might also be a possibility. EL-SODA *et al.* (1978) did not find cell-wall-bound peptidase in *L. casei*. However, results of REQUENA *et al.* (1991) suggest extracellular exopeptidase activity for *L. casei*. NSLAB appear to possess the enzymatic potential, like the lactococci, to be involved in the release of amino acids during cheese ripening (WILLIAMS and BANKS, 1997). Nevertheless, only a limited number of peptidase activities in lactobacilli have so far been confirmed (BOCKELMANN, 1995; LAW and HAANDRIKMAN, 1997). Concerning the relationship of the proteolytic and acidifying abilities, our results suggested that these two characteristics are not related (the highly proteolytic strains are not necessarily highly acidifying at the same time), as was also observed for *L. plantarum* (XANTHOPOULOS *et al.*, 2000). For example, strains B113 and R30.5 differed greatly in respect of proteolytic activity but had similar pH after 24h of growth in milk. Strains 2.55 and Or11 which exhibited the same proteolytic activity differed greatly at their final pH. The appropriate selection of strains in terms of the function of proteolytic and acidifying abilities may improve the cheese making process if *L. paracasei* subsp. *paracasei* strains are used as adjunct starters. Hydrolysis of casein by the test strains after 7 days of growth in milk was strain dependent and a preference towards a_s-casein was observed. REQUENA *et al.* (1991) recorded higher hydrolysis of b- than a-casein for a strain of *L. casei*, whereas EL-SODA *et al.* (1986) demonstrated a_s- and β-casein hydrolysis with intracellular enzymatic extracts from *L. casei* NCDO151. That there were strains with no activity suggests that their enzymes may require specific conditions for optimum activity (REQUENA *et al.*, 1991) and casein is not necessarily the preferred substrate for their enzymes (MILLS and THOMAS, 1978).

In conclusion, the results obtained in this research showed a considerable genetic variability in *L. paracasei* subsp. *paracasei* species. Results also suggest, that Greek traditional cheeses can be sources for isolation of strains with considerable differences in respect to acidifying, caseinolytic and peptidolytic activities. The use of such strains in the dairy industry could offer great potential for new applications or for existing processes. The variability seems to be strain dependent and unrelated to the source of isolation of the strains. Nevertheless, there were cases (as for example aminopeptidase activity) where the particular habitat could play a role in the isolation of biotypes with properties of biotechnological interest. Their effective use as adjunct cultures in the manipulation of flavour development seems promising and deserves further studies.

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PAPER

CHARACTERISTICS OF GOAT MILK PRODUCED IN THE ASPROMONTE MASSIF (CALABRIA, ITALY)

CARATTERISTICHE DEL LATTE CAPRINO PRODOTTO IN ASPROMONTE

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ABSTRACT

Physico-chemical parameters – pH (mean 6.55-6.81), fat (mean 3.95-5.68%), protein (mean 3.57-4.30%), lactose (mean 4.63-4.95%), and dry matter (mean 14.02-15.28%) – microbiological parameters – somatic cell (mean 5.1-5.7 Log mL^{-1}) and total microbial count (mean 4.0-5.0 Log CFU mL^{-1}) – and coagulation parameters – rennet coagulation time (r - mean 13'03"-17'22"), curd firming rate (k_{20} - mean 1'37"-2'32"), and curd firmness after 30 min (a_{30} - mean 33.13-53.67) – were determined in 300

RIASSUNTO

Sono stati determinati, in 300 latti individuali di capre autoctone, i parametri fisico-chimici, pH (media 6,55-6,81), grasso (media 3,95-5,68%), proteina (media 3,57-4,30%), lattosio (media 4,63-4,95%) e sostanza secca (media 14,02-15,28%), i parametri microbiologici, cellule somatiche (media 5,1-5,7 Log mL^{-1}) e conta microbica totale (media 4,0-5,0 Log CFU mL^{-1}) ed i parametri lattodinamografici, tempo di coagulazione (r - media 13'03"-17'22"), velocità di formazione del coagulo (k_{20} -

- Key words: goat milk, microbial groups, milk coagulation, raw milk -

individual native goat milk samples. Six bulk milk samples were also analysed. Most of the individual milk samples conformed to the European Community and Italian standards, but the bulk milk quality must be improved.

media 1'37"-2'32") e consistenza del coagulo a 30 min (a_{30} - media 33,13-53,67). Sono stati analizzati anche sei latti massali. La maggior parte dei latti individuali esaminati rientra nei parametri stabiliti dalla legislazione italiana ed europea, mentre la qualità dei latti massali necessita di interventi migliorativi.

INTRODUCTION

In the Mediterranean basin the raising of goat herds has increased in recent years. The ability of goats to use relatively poor food resources and, even thrive on marginal lands that would otherwise be unsuitable for animal production, is creating an ever-increasing interest in this type of livestock. This is particularly true in areas where the traditional rural system uses almost exclusively the native goat breeds. The increase in the knowledge about the composition and quality of goat milk is certainly important (CERUTTI, 1997) in helping to safeguard a distinctive Mediterranean product that is part of the local history and culture. Goat milk is the third most produced milk in the world (KLINGER and ROSENTHAL, 1997) and its production has been rising steadily, partially because of its good nutritional value (GOMES and MALCATA, 1998). However, unlike cow milk, which is subjected to stringent hygiene and quality regulations, the microbiological standards for the production and distribution of goat milk are less severe. The specific microflora (CASLA *et al.*, 1996; ZÁRATE *et al.*, 1997) and the microbiological problems connected with the poor hygienic standards for goat milk are well described (CLEMENTI *et al.*, 1998; THAM *et al.*, 1990). Isolation of bacteria responsible for human infections, such as brucellosis (WYATT, 1999) and listeriosis (ABOU-ELEININ *et al.*, 2000), from raw goat milk is often reported. In particular, *Listeria monocytogenes* was identified in 17 out of 35 Listeria-positive samples; *Listeria* isolation rates were markedly higher during winter (14.3%) and spring (10.4%), compared to autumn (5.3%) and summer (0.9%).

On the Calabrian Aspromonte Massif, in an area overlooking the Ionian coast in the province of Reggio Calabria, milk is almost exclusively produced from native populations of goats. These goats are characterised by high adaptability, morphological heterogeneity and an ability to exploit all the vegetative resources available in this desolate environment. The area, situated prevalently between 300 and 800 m asl, is largely inaccessible to humans and is therefore used almost exclusively for goat production. Each dairy farm has 180-200 goats and a few sheep, all half-wild. The pasture is primarily natural, with over 50 forage species. Lactation lasts about 200 days – from January to July – and the daily production of goat milk in the whole area is 10 tons. Each goat is milked by hand twice a day in the early-morning and the evening and gives from 0.5 to over 1 L per day.

On the Calabrian Aspromonte Massif goat milk is usually processed to make the distinctive Caprino d'Aspromonte cheese. It is manufactured in small dairies which do not have adequate techni-

cal and scientific knowledge for large-scale controlled production.

To date no research has ever been conducted on the goat milk produced on the Aspromonte Massif. Therefore, the purpose of the present work was to study the physico-chemical, microbiological, and coagulation characteristics of the raw milk. These characteristics were used to identify the hygienic problems connected with the raw milk, because the milk quality is fundamental and preliminary to the success of the cheese-making process and, therefore, to good cheese quality. The influence of the season, the farm, and the interaction season/farm were also studied.

MATERIALS AND METHODS

Three dairy farms, denominated I, II, and III, located at three altitudes in the province of Reggio Calabria, were chosen as representative of the production area. A total of 300 individual milk samples were collected by milking by hand directly into sterilised vessels in the early morning. Of these samples, 150 were simultaneously collected in the spring of 2000 (50 from each farm) and 150 were simultaneously collected in the winter of 2001 (50 from each farm). Each time the individual samples were collected, one bulk milk sample was also collected, giving a total of six bulk milk samples. The bulk milk samples were obtained by combining the bulk milk from the evening before, left at room temperature, with the early-morning bulk milk. The milk samples were transported to the laboratory in sterile, refrigerated containers (4°C) and maintained at this temperature for a maximum of 24 h.

The 300 individual milk samples were analysed using a pH-meter (model 355, Mettler Toledo, Greifensee, Switzerland), a Milkoscan (model 134, FossElectric, Hillerød, Denmark) and a Bactoscan (model 8000, FossElectric, Hillerød, Denmark), for the following parameters: pH, dry matter, fat, protein, lactose, somatic cell count and total microbial count. The rennet coagulation properties of individual milk samples were determined according to DURANTI (1995). Rennet coagulation time (r in min), curd firming rate (k_{20} in min) and curd firmness measured 30 min after the addition of rennet (a_{30} in mm) were determined using a lactodynamograph (Maspres, Firenze, Italy) in 10 mL of milk at 35°C with the addition of 200 μL of standard calf rennet (CHR Hansen, Corsico, Italy) with an original strength of 160±8 IMCU mL^{-1}, diluted to 1.6%.

The six bulk milk samples were also analysed for the above-listed physico-chemical parameters as well as for the following microbiological parameters: total microbial count, according to APHA (1978), on Plate Count Agar (Oxoid LTD, Hampshire, England) with the addition of 1 g L^{-1} of skim milk (Merck, Darmstadt, Germany) and incubated aerobically at 32°C for 72 h; coliforms, according to APHA (1978), on Violet Red Bile Agar (Fluka, Buchs, Switzerland) incubated aerobically at 32°C for 24 h; *Escherichia coli*, according to NMKL (1993), on 3M Petrifilm for *E. coli* and coliforms (3M Microbiology Products, St. Paul, USA) incubated aerobically at 37°C for 48 h; mesophilic and thermophilic lactobacilli, according to DE MAN *et al.* (1960), on MRS Agar (Biokar Diagnostics, Beauvais, France) acidified to pH 5.4 with acetic acid and incubated anaerobically, using a gas-pack catalyst (Oxoid LTD, Hampshire, England), at 22°C for 96 h or at 45°C for 48 h; mesophilic and thermophilic coccal-shaped lactic acid bacteria, according to TERZAGHI and SANDINE (1975), on M17 Agar (Fluka, Buchs, Switzerland) incubated anaerobically at 22°C for 96 h or at 45°C for 48 h; ente-

rococci, according to NMKL (1992), on Slanetz-Bartley Agar (Biokar Diagnostics, Beauvais, France) incubated aerobically at 45°C for 48 h; yeasts, according to IDF (1990), on Yeast Glucose Chloramphenicol Agar (Fluka, Buchs, Switzerland) incubated aerobically at 22°C for 96 h. The spread plate technique (0.1 mL) was used for all media, except Violet Red Bile Agar where the pour plate technique (1 mL) with overlay was used. Results are expressed as a logarithm (log) of colony forming units (CFU) mL^{-1} of milk.

All the analytical determinations were repeated twice and the reported values are the means of two analyses. The effect of season, farm, and the interaction between season and farm was statistically evaluated by ANOVA analysis (SAS system), with the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where Y_{ijk} is the middle experimental value, μ is the general mean, α_i is the season effect, β_j is the farm effect, $(\alpha\beta)_{ij}$ is the effect of the interaction between season and farm, and eijk is the residual error. The significance of the paired comparison was determined by the Student *t* test.

RESULTS AND CONCLUSIONS

The physico-chemical and microbiological parameters determined on the 300 individual milk samples are reported in Fig. 1-3. The significance of the comparisons, the significant effects, and the least square means are reported in Tables 1 and 2.

The pH of the milk (Table 2), which is indicative of the hygienic state of the goat and the milking procedure, was always lower in the spring (mean 6.55-6.60) than in the winter (mean 6.80-6.81). The spring values fell within the acceptable range for goat milk (ANIFANTAKIS and KANDARAKIS, 1980; KALANTZOPOULOS, 1993; SIMOS *et al.*, 1991; VEINOGLOU *et al.*, 1982; VOUTSINAS *et al.*, 1990). The second (6.40<pH<6.59) and third (6.60<pH<6.79) frequency classes were the most representative in spring, and the fourth (pH≥6.80) was most representative in winter (Fig. 1). A comparison among farms did not show significant differences, while a comparison between seasons showed significantly lower pH levels (P<0.01) in the spring.

Fat content (Table 2) varied significantly (P<0.01) with seasonal variations being lower in the winter (mean 3.95-4.98%) and higher in the spring (mean 4.20-5.68%). Similar levels have been reported for Greek goat milk (KALANTZOPOULOS, 1993; SIMOS *et al.*, 1991; VEINOGLOU *et al.*, 1982), while GRAPPIN *et al.* (1981), JOUBERT (1973) and PIERRE *et al.* (1998) reported lower values. The second frequency class (Fig. 1) (3.50%<fat<4.99%) was the most numerous. A comparison among farms, for each sampling period, was significant (P<0.05-0.01), except between farm I and farm III in the winter. The milk with the highest fat content was always produced on farm II.

The protein content (Table 2) was not statistically significant between spring (mean 3.57-4.11%) and winter (mean 3.74-4.30%). Analogous values have been reported for goat milk produced in Sardinia (COSSEDDU and PISANU, 1979), India (QURESHI *et al.*, 1981), and Libya (GNAN *et al.*, 1985); while, other authors have reported notably lower values (MBA *et al.*, 1975; MENA and ESCAMILLA, 1977; PRIMATESTA, 1979). The second frequency class (Fig. 1) was the most representative (3.50%<protein<4.99%). The comparison among farms was significant (P<0.01), with higher protein content in the winter milk from farm I and in the spring milk from farms I and II. This explains the significant season x farm interaction.

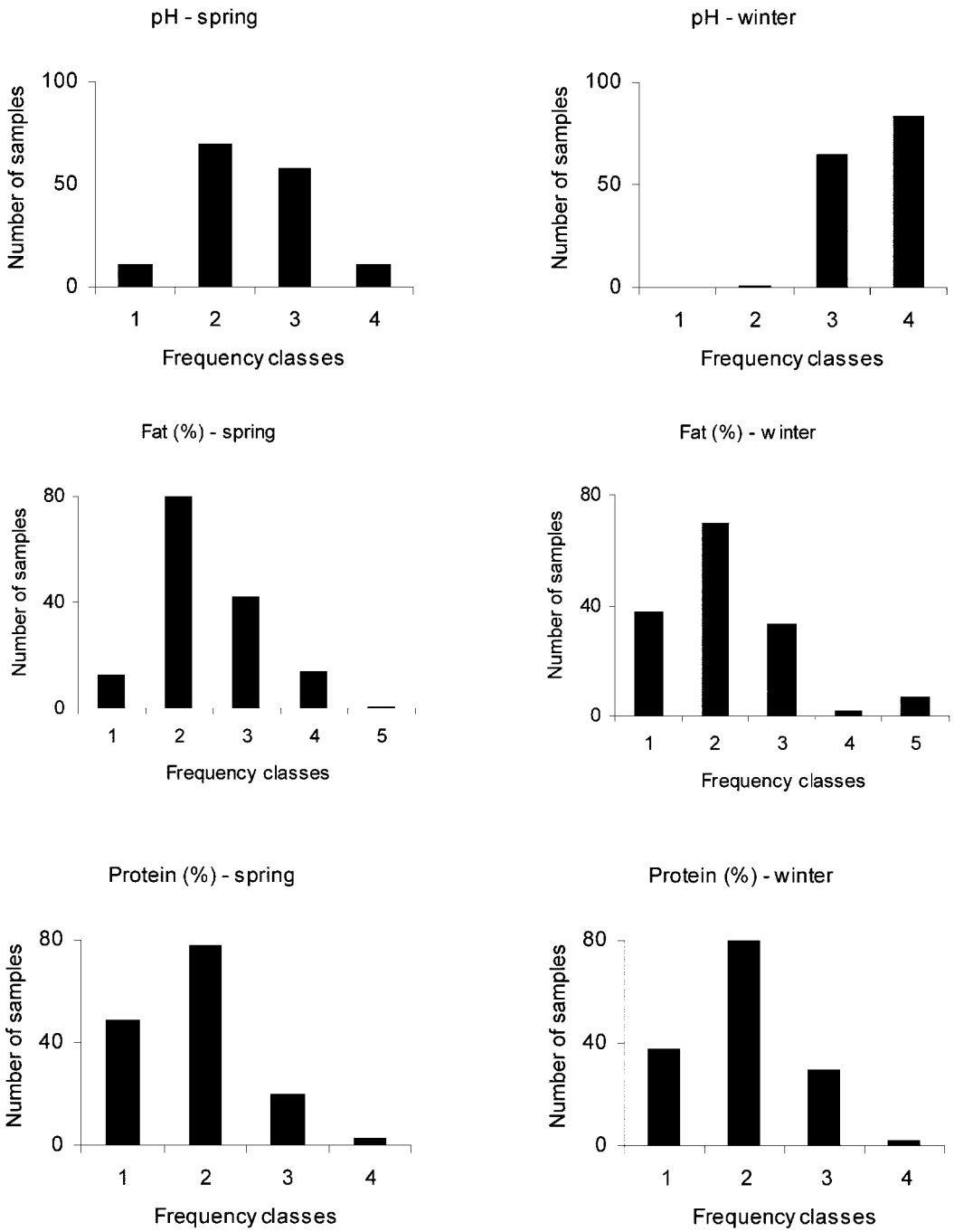


Fig. 1 - Trend of pH, fat, and protein in individual milk samples in the spring and winter. Frequency classes – pH: 1 (6.20-6.39), 2 (6.40-6.59), 3 (6.60-6.79), 4 (≥6.80); fat: 1 (2.00-3.49), 2 (3.50-4.99), 3 (5.00-6.49), 4 (6.50-7.99), 5 (≥8.00); protein: 1 (2.50-3.49), 2 (3.50-4.49), 3 (4.50-5.49), 4 (5.50-6.49).

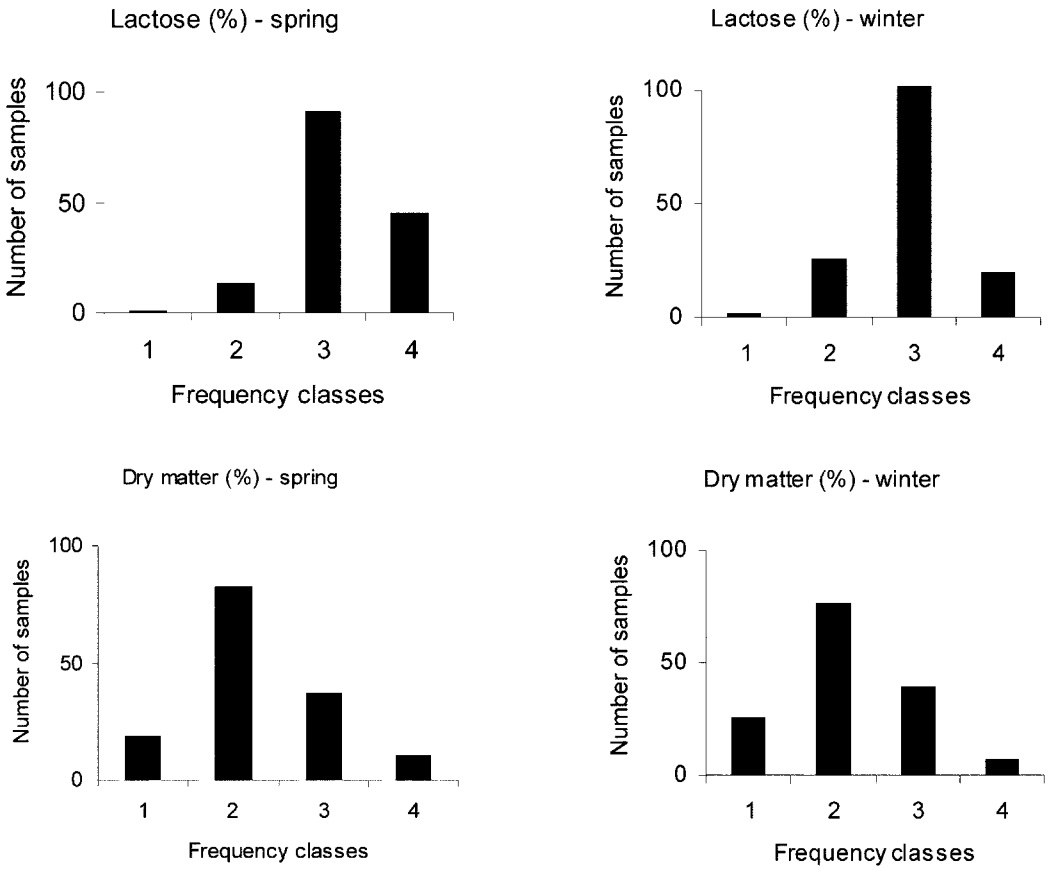


Fig. 2 - Trend of lactose and dry matter in individual milk samples in the spring and winter. Frequency classes – lactose: 1 (3.50-3.99), 2 (4.00-4.49), 3 (4.50-4.99), 4 (≥5.00); dry matter: 1 (11.00-12.99), 2 (13.00-14.99), 3 (15.00-16.99), 4 (17.00-18.99).

The average lactose values (Table 2) varied from 4.63 to 4.95% and were very similar to those reported in Greece for the Metsovo breed (SIMOS *et al.*, 1991) and in Italy for the Maltese breed (CLEMENTI *et al.*, 1998). The third frequency class (Fig. 2) was the most numerous (4.50%<lactose<4.99%). Significant differences ($P<0.01$) were noted between seasons and, for each season, among farms. This is quite important, considering that all the milk is processed into cheese. The season x farm interaction was also significant ($P<0.05$), with variability in the lactose content of the milk.

The very high dry matter values (mean 14.02-15.28%) (Table 2) are in agreement only with those reported for the Metsovo breed (SIMOS *et al.*, 1991). In contrast, the values reported for the British Alpine breed (DEVENDRA, 1972), the Saanen breed (CHANG and KIM, 1978; PILLA *et al.*, 1980) and for goat milk in general (MERIN *et al.*, 1988; MIDDLETON and FITZ-GERALD, 1981; SAWAYA *et al.*, 1984) are much lower. The second frequency class (Fig. 2) was the most representative (13.00%<dry matter<14.99%). Only the differences among farms for each season were significant. Compared to the others, farm

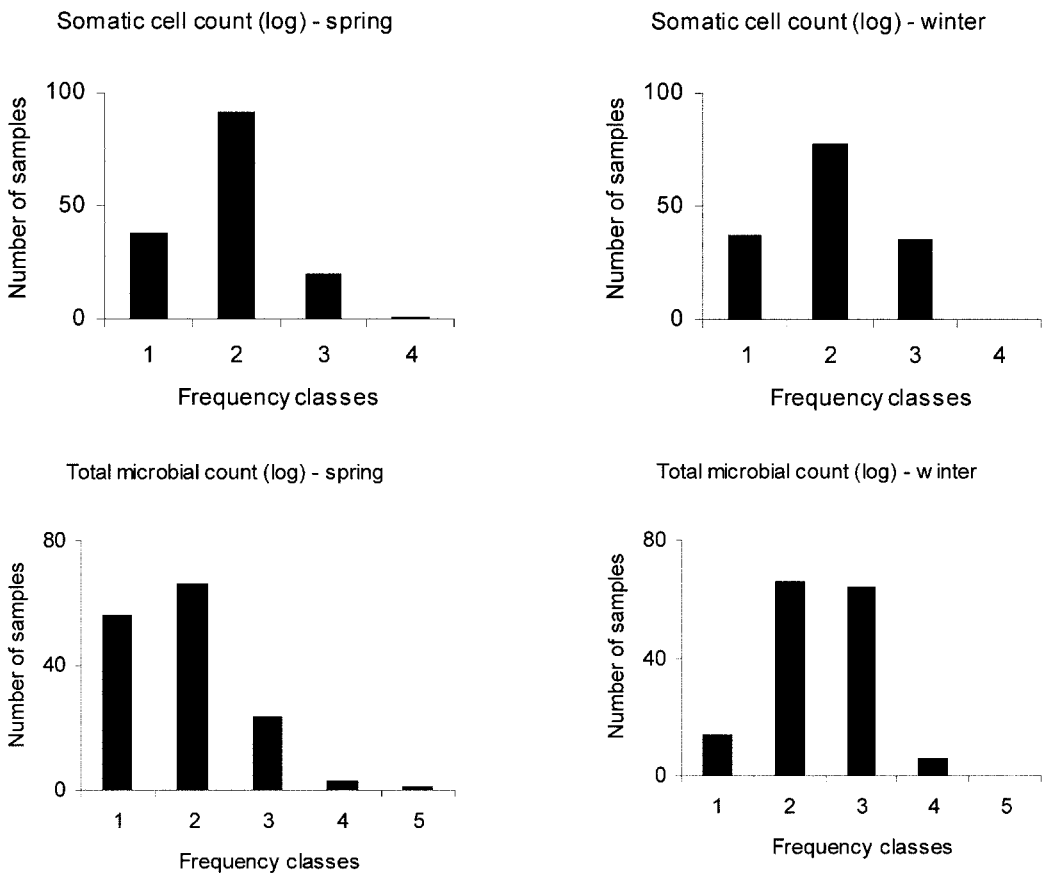


Fig. 3 - Trend of somatic cell count and total microbial count in individual milk samples in the spring and winter. Frequency classes – somatic cell count: 1 (4.00-4.99), 2 (5.00-5.99), 3 (6.00-6.99), 4 (≥7.00); total microbial count: 1 (3.00-3.99), 2 (4.00-4.99), 3 (5.00-5.99), 4 (6.00-6.99), 5 (≥7.00).

Table 1 - Significant effects regarding the physico-chemical and microbiological parameters determined on the 300 individual goat milk samples.

Significance effects	SED[a]	Season			Farm			Season x farm		
		MS[b]	F[c]	Pr[d]<F	MS	F	Pr<F	MS	F	Pr<F
pH	0.120	3.905	270.40	0.0001	0.023	1.61	0.2008	0.012	0.82	0.4412
Fat (%)	1.129	14.075	11.04	0.0010	40.558	31.81	<0.0001	1.479	1.16	0.3150
Protein (%)	0.597	0.231	0.65	0.4200	5.789	16.25	<0.0001	3.086	8.66	0.0002
Lactose (%)	0.249	1.393	22.43	<0.0001	1.330	21.42	<0.0001	0.238	3.83	0.0228
Dry matter (%)	1.390	0.900	0.47	0.4954	24.859	12.87	<0.0001	5.737	2.97	0.0529
Somatic cell count (log/mL)	0.498	0.523	2.11	0.1479	7.767	31.26	<0.0001	0.149	0.60	0.5503
Total microbial count (log/mL)	0.596	17.442	49.05	<0.0001	8.505	23.92	<0.0001	3.216	9.04	0.0002

[a] Standard Error Deviation; [b] Mean Square; [c] Fisher F; [d] Probability.

Table 2 - Least square means regarding the physico-chemical and microbiological parameters determined on the 300 individual goat milk samples. (a, b, c: P<0.05; A, B, C: P<0.01 indicate the significance of the comparison in the same row and by season).

Season	Spring						Winter					
Farm	I		II		III		I		II		III	
pH	6.573		6.604		6.555		6.813		6.807		6.796	
Fat (%)	4.199	Aa	5.685	B	4.689	Ab	3.952	A	4.977	B	4.345	A
Protein (%)	3.971	A	4.114	A	3.567	B	4.299	A	3.773	B	3.747	B
Lactose (%)	4.694	A	4.950	B	4.880	B	4.639	A	4.841	B	4.636	A
Dry matter (%)	14.024	A	15.282	B	14.294	A	14.066	a	14.636	b	14.294	ab
Somatic cell count (log/mL)	5.532	A	5.079	B	5.445	A	5.665	A	5.074	B	5.569	A
Total microbial count (log/mL)	4.948	A	4.028	aB	4.319	Bb	5.046		4.837		4.858	

II always produced milk with higher dry matter content.

The somatic cell count of milk depends on the health of the udder, on milking hygiene and on many stress factors. WHITE and HINCKLEY (1999) reported that the detection of pathogen-free goat milk samples that contain more than 10^6 somatic cells/mL demonstrate that elevated somatic cell counts alone are not a valid indication of mammary infection in goats. Although reference values for goat milk have not yet been established, a comparative study was recently conducted on the effects of the testing laboratory, counting method, storage and shipment on somatic cell counts in goat milk (ZENG *et al.*, 1999). The somatic cell counts of these samples (Table 2) fell (mean 5.1-5.7 Log mL^{-1}) within the normal range for milk from healthy goats (HUNTER, 1984; POUTREL *et al.*, 1997; ROGUINSKI *et al.*, 1980; SCHULZ, 1994). The second frequency class (Fig. 3) was the most representative (5.00%<Log somatic cell count<5.99%). The differences among farms for this parameter were highly significant (P<0.01), showing lower somatic cell counts in both seasons in milk samples from farm II.

Regarding the total microbial count, the European Community Directive (CD, 1992) and Italian law (DPR, 1997) dictate that raw goat milk used to manufacture dairy products is permitted to have a maximum of $5.0x10^5$ microorganisms/mL at 30°C. These laws, however, do not take into account the important differences among the types of microorganisms present. The majority of the milk samples (Fig. 3) comply with this limit in both seasons. Total microbial count (Table 2) was significantly different between spring (mean 4.0-4.9 Log CFU mL^{-1}) and winter (mean 4.8-5.0 Log CFU mL^{-1}) and among farms, only in the spring. This explains the result of the season x farm interaction. In spring (Fig. 3) the vast majority of the milk samples were included in the first two frequency classes (3.00<Log total microbial count<4.99), while in winter most of the milk samples were included in the second and the third frequency classes (4.00<Log total microbial count<5.99).

According to CHIOFALO and MICARI (1987), the analysis of goat milk using a lacto-dynamograph shows five different types of tracings. Among these, type C corresponds to the tracing of a milk with average dairy quality, with r-values around 12 min, k_{20} about 6 min, and a_{30} around 32 mm. Type E corresponds to the tracing of a milk with a dairy quality

poorer than type C; effectively, its coagulation parameters are r-values around 19 min, k_{20} about 7 min, and a_{30} around 28 mm. The coagulation properties were determined on the individual milk samples in spring 2000 and in winter 2001 (Table 3); the three tested parameters showed normal behaviour in both seasons (CASTAGNETTI *et al.*, 1984; PIERRE *et al.*, 1998; STORRY *et al.*, 1983). The a_{30} parameter was lower in the spring milk samples than in the winter samples. This demonstrates that in spring there are more milk samples with lower coagulation ability, although most milk samples coagulated after the addition of rennet, both in winter and in spring (97% for farm I, 95% for farm II, and 92% for farm III, averaging the values of the two seasons). The coagulation profiles for these milk samples fell within types C and E, because they produced a soft clot, which was slow to compact, and was prone to spontaneous syneresis. The shorter coagulation time of the spring milk compared to the winter milk can be primarily attributed to the lower pH of the milk, as often described (REMEUF *et al.*, 1991).

The results of the physico-chemical and microbiological analyses, performed on the bulk milk samples in spring 2000 and in winter 2001, are reported in Table 4. The physico-chemical parameters and the somatic cell counts of the bulk milk were analogous to those of the 300 individual milk samples. It is interesting to note that three of the six bulk milk samples had low total microbial counts (5.2-5.7 Log CFU mL^{-1}), similar to the data reported by ESPIE and MULLAN (1987) and TIRARD-COLLET *et al.* (1991). On the contrary, the other three samples had very high total microbial counts (6.3-7.3 Log CFU mL^{-1}), similar to counts reported for goat milk used to manufacture different varieties of cheese (ABO-ELNAGA *et al.*, 1985; GUTIÉRREZ *et al.*, 1988; MAS MAYORAL *et al.*, 1991). The coliform content in four of the six samples (3.2-5.0 Log CFU mL^{-1}) was similar to other reports (LOPEZ-DIAZ *et al.*, 1995; MEDINA *et al.*, 1992), whereas the remaining two samples had notably higher values (6.6-7.0 Log CFU mL^{-1}). These alarming results are due to a few, easily identifiable causes. Firstly, in the production area, by tradition, the early-

Table 3 - Mean, minimum and maximum of the coagulation parameters determined on the 300 individual goat milk samples.

	I			II			III		
Farm	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Spring									
Rennet coagulation time (r in min)	13' 03"	7' 15"	24' 30"	15' 17"	10' 30"	23'	13' 19"	6'	23'
Curd firming rate (k_{20} in min)	1' 46"	1' 15"	9' 45"	2' 32"	1' 30"	8' 15"	2' 08"	1' 15"	8'
Curd firmness after 30 min (a_{30} in mm)	34.94	6.50	66.50	37.76	17.88	64.44	33.13	12.12	63.42
Winter									
Rennet coagulation time (r in min)	16' 42"	8' 45"	23'	15' 41"	10' 15"	22' 45"	17' 22"	12' 45"	21'
Curd firming rate (k_{20} in min)	1' 37"	45"	3' 45"	1' 41"	1' 30"	4'	2' 16"	1' 30"	4' 45"
Curd firmness after 30 min (a_{30} in mm)	53.67	34.86	62.20	47.26	18.24	67.42	47.05	29.72	63.82

Table 4 - Physico-chemical and microbiological parameters determined on the six bulk milk samples.

Season	Spring			Winter		
Farm	I	II	III	I	II	III
pH	6.38	6.45	6.39	6.67	6.64	6.70
Fat (%)	5.39	5.14	5.46	4.85	4.99	5.02
Protein (%)	4.39	4.12	3.66	4.28	3.63	4.73
Lactose (%)	4.75	5.00	4.99	4.66	4.84	4.88
Dry matter (%)	15.38	15.11	14.96	14.98	15.26	15.07
Somatic cell count (log/mL)	5.777	5.415	5.401	6.289	5.394	6.239
Total microbial count (log/mL)	7.322	5.643	5.204	6.322	6.978	5.716
Coliforms (log/mL)	7.000	4.964	3.176	3.934	6.568	4.398
Escherichia coli (log/mL)	4.934	3.380	2.301	2.778	6.568	1.845
Mesophilic lactobacilli (log/mL)	5.982	3.477	3.919	5.114	5.301	3.505
Thermophilic lactobacilli (log/mL)	4.041	2.462	2.580	3.255	4.041	4.000
Mesophilic coccal-shaped lactic acid bacteria (log/mL)	6.556	4.886	4.949	5.892	6.806	5.255
Thermophilic coccal-shaped lactic acid bacteria (log/mL)	6.633	5.602	3.845	4.857	6.633	4.114
Enterococci (log/mL)	3.968	3.973	3.778	3.255	4.602	4.079
Yeasts (log/mL)	3.643	2.580	2.380	3.699	2.114	1.954

morning bulk milk is added to the bulk milk of the evening before, which has been left unrefrigerated overnight in non-sterilised containers, in an environment that is far from aseptic. Secondly, the hand milking is performed in unhygienic conditions. By simply addressing these two factors, however, and improving the hygienic-sanitary conditions of the milking and management environment, the total microbial count and the coliform numbers could be lowered to meet acceptable levels. Among the lactic acid bacteria, the mesophilic coccal-shaped lactic acid bacteria predominated in winter (5.3-6.8 Log CFU mL^{-1}) and the thermophilic coccal-shaped lactic acid bacteria predominated in spring (3.8-6.6 Log CFU mL^{-1}). The number of coccal-shaped lactic acid bacteria was 1-2 Log units higher than the lactobacilli, as reported for other goat milk samples (FATICHENTI *et al.*, 1979; MAS MAYORAL *et al.*, 1991; TORNADIJO *et al.*, 1995). The number of enterococci (3.3-4.6 Log CFU mL^{-1}) was within the range observed for other goat milk samples (CLEMENTI *et al.*, 1998; FONTECHA *et al.*, 1990; ZARATE *et al.*, 1997). The number of yeasts was rather low (1.9-3.7 Log CFU mL^{-1}), around 30% of them fermented lactose with production of gas.

The results of this study show that the qualitative characteristics of most of the individual milk samples from the three farms conformed to the standards laid down by the Italian and European directives. Implementation of improved hygienic-sanitary practices in response to the needs manifested in the present study, together with a selective breeding program, may improve the quality of goat milk produced in the Calabrian Aspromonte Massif.

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PAPER

CHARACTERIZATION OF BOTTLED WINES FROM THE TENERIFE ISLAND (SPAIN) BY THEIR METAL ION CONCENTRATION

CARATTERIZZAZIONE DI VINI IMBOTTIGLIATI DELL'ISOLA DI TENERIFE (SPAGNA) IN FUNZIONE DEL LORO CONTENUTO DI METALLI

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ABSTRACT

K, Na, Ca, Mg, Fe, Cu, Zn, Mn, Sr, Li and Rb concentrations were determined in bottled wines from the Tenerife Island belonging to the denominations of origin of Abona, Valle de Güímar, Valle de La Orotava, Tacoronte-Acentejo and Ycoden-Daute-Isora in order to characterize them. Analyses were performed by flame atomic absorption spectrophotometry with the exception of Li and Rb for which flame atomic emission spectrophotometry was used. The greater part of the mean

RIASSUNTO

Ai fini della loro caratterizzazione, in alcuni vini imbottigliati dell'isola di Tenerife appartenenti alle denominazioni di origine di Abona, Valle de Güimar, Valle de La Orotava, Tacoronte-Acentejo e Ycoden-Daute-Isora sono state determinate le concentrazioni di K, Na, Ca, Mg, Fe, Cu, Zn, Mn, Sr, Li e Rb. Le analisi sono state effettuate con spettrofotometria di assorbimento atomico ad eccezione del Li e del Rb per i quali è stata utilizzata la spettrofotometria di emissione atomica di fiamma. La mag-

- Keywords: LDA, metals, PCA, Tenerife Island, wines -

content for the different parameters (K=688 mg/L, Ca=64.3 mg/L, Mg=83.8 mg/L, Fe=2.25 mg/L, Cu=0.24 mg/L, Zn=0.68 mg/L, Mn=1.09 mg/L, Sr=0.54 mg/L, Rb=1.94 mg/L, Li=6.39 μg/L) fell within the ranges described in the literature, except for sodium (88 mg/L) whose higher content may be due to the effect of marine spray. For copper and zinc most samples were below the maximum amount recommended for these elements by the OIV. Differences in mean content according to vintage, type of wine and denomination of origin were determined for the different metals. Principal Component Analysis grouped wines according to their type and denomination of origin. Stepwise linear discriminant analysis gave about 80% correct classification of samples according to their denomination of origin.

gior parte dei valori medi dei parametri analizzati (K=688 mg/L, Ca=64,3 mg/L, Mg=83,8 mg/L, Fe=2,25 mg/L, Cu=0,24 mg/L, Zn=0,68 mg/L, Mn=1,09 mg/L, Sr=0,54 mg/L, Rb=1,94 mg/L, Li=6,39 μg/L) sono compresi negli intervalli descritti in letteratura, ad eccezione del sodio (88 mg/L), i cui valori più elevati possono essere dovuti all'effetto dell'aerosol marino. Per il rame e lo zinco la maggior parte dei campioni presentano contenuti inferiori alle quantitá massime raccomandate da l'OIV per questi stessi elementi. Sono state stabilite per i differenti metalli le differenze medie di contenuto tenendo presente la tecnologia di trasformazione, il tipo di vino e la denominazione di origine. L'Analisi delle Componenti Principali segnala la tendenza di aggregazione di vini secondo il loro tipo e la loro denominazione di origine. L'analisi lineare discriminante indica che circa l'80% della classificazione dei campioni si accorda con la denominazione di origine.

INTRODUCTION

The authentication of food products in general, and of wine in particular, is of great interest to prevent fraud and to guarantee their origin and quality. Thus it is very important to determine the chemical composition of wines and to study how factors such as vintage, grape variety, wine-making technology, area of production, etc. may affect them.

Knowledge of the metal ion concentration of wines is of interest not only because of their influence on the wine making process where metals such as potassium, calcium, iron and copper can produce precipitates, but also because of the low acceptance levels for some metals set by the international community. They are important in nutrition, may have potentially toxic effects, and can be used to characterize and typify wines.

Moreover metals seem to be the best way to identify the geographical origin due to their direct correlation with soil composition (MEDINA, 1996). It should also be taken into account that the metal ion content of wine is influenced not only by the metal content of the soil where the vine was grown but also by growing practices (fertilization, phytosanitary treatments, etc.), enological practices (machinery, piping, use of fining agents, additives, etc.), environmental contamination (cars, factories, etc.) and fraudulent practices (use of forbidden chemicals) (ESCHNAUER and NEEB, 1988).

When dealing with a high number of data coming from the analysis of many samples of wines, multivariate statisti-

cal techniques, especially Pattern Recognition techniques, are imperative for processing the information, and characterising the geographic origin (FORINA *et al.*, 1986). They have been used to differentiate wines from Catalonia (LARRECHI *et al.*, 1987), Valencia (LIZAMA *et al.*, 1997) and Galicia (LATORRE *et al.*, 1994). Furthermore, they have been used to specify three production areas of La Rioja (GONZÁLEZ-LARRAINA *et al.*, 1987), and to differentiate the wines of Galicia from those of other regions in Spain (HERRERO-LATORRE and MEDINA, 1990), and to discriminate between sweet wines of the Canary Islands (FRÍAS *et al.*, 2001).

Wines from the Canary Islands have several peculiarities because they are produced with native varieties which cannot be found elsewhere in the world. In addition, the Canary Island vineyards have never been attacked by phylloxera and the soils are volcanic (LOPEZ *et al.*, 1993).

No bibliographic data are available on the mineral content of bottled wines from the Tenerife Island bearing the certified labels of denomination of origin. The aim of this work was to determine the concentration of eleven metals: K, Na, Ca, Mg, Fe, Cu, Zn, Mn, Sr, Li and Rb in these wines in order to correlate the levels of these metals with the vintage, type of wine and denomination of origin and to typify these wines using univariate and multivariate techniques.

MATERIALS AND METHODS

Samples

One-hundred-twenty-five samples of wines were used. They were from two consecutive harvests (57 samples from 1995 and 68 samples from 1996), three types of wines (52 white, 22 rosé and 51 red), and five denominations of origin (DOs), Abona (12), Valle de Güímar (18), Valle de La Orotava (27), Tacoronte-Acentejo (32) and Ycoden-Daute-Isora (36), from the Island of Tenerife (Canary Islands, Spain). Their distribution is given in Table 1. All samples were provided by the Certification Denomination of Origin Council, to ensure their geographic origin; they include practically all the wines marketed.

Reagents

All chemicals were of analytical grade. Stock solutions of Na, K, Ca, Mg, Fe, Cu, Zn, Mn, Sr, Li, and Rb 1,000±0.002 mg/L were from Panreac (Barcelona, Spain), and working standard solutions were obtained by suitable dilution. Lithium chloride (99%) used as ionic suppressor was from Fluka (Buchs, Switzerland). Lanthanum chloride (99%) used as molecular suppressor was from Riedel-de Haën (Seelze, Germany). Nitric acid (65%) was from Panreac. Ultrapure water from a Milli-Q system (Millipore, Bed-

Table 1 - Number of wine samples analysed from two vintages from the Tenerife Island.

	White		Red		Rosé	
Denomination of Origin	Vintage 1	Vintage 2	Vintage 1	Vintage 2	Vintage 1	Vintage 2
Abona	4	4	1	1	1	1
Valle de Güímar	4	5	2	4	1	2
Valle de La Orotava	6	6	6	7	1	1
Tacoronte-Acentejo	4	4	9	9	3	3
Ycoden-Daute-Isora	6	9	5	7	4	5
Total	24	28	23	28	10	12

ford, MA, USA) with a conductivity of 18 MΩ· cm was used throughout.

Apparatus

A CEM MDS-81D microwave oven was used for wine sample digestion.

A Varian Spectra AA-10Plus spectrophotometer (Victoria, Australia) equipped with a deuterium arc background correction system was used for metal determination involving both atomic absorption spectrometry (AAS) and atomic emission spectrometry (AES).

Analytical procedures

Na, K, Ca, Mg, Fe, Cu, Zn, and Mn were determined by AAS using air/acetylene flame, and Sr using acetylene/nitrous oxide flame, and Li and Rb were measured by AES. Major elements (Na, K, Ca, Mg) were analysed by direct dilution of the wine samples. In order to determine minor and trace elements (Fe, Cu, Zn, Mn, Sr, Li and Rb), the samples were first treated with nitric acid in a microwave oven due to the matrix effect. Results are the average of three replicate determinations.

Univariate analysis

Mean values obtained for the variables studied in the different groups were compared by One-Way ANOVA assuming that there were significant differences among them when the statistical comparison was $p < 0.05$.

Multivariate analysis

Data processing

A matrix was constructed with rows representing wine samples and columns corresponding to the metals Na, K, Ca, Mg, Fe, Cu, Zn, Mn, Sr, Li and Rb. In order to avoid differences in units of measurement the data were first autoscaled (KOWALSKI and BENDER, 1972).

Principal Component Analysis (PCA)

PCA is a non-supervised technique that reduces the dimensionality of the original data matrix retaining the maximum amount of variability. It allows the relationship between variables and observations to be studied and the data structure to be recognized (MARDIA *et al.*, 1979).

Linear Discriminant Analysis (LDA)

LDA is a supervised method used for classification purposes. LDA renders a number of orthogonal linear discriminant functions equal to the number of categories minus one. This method maximises the variance between categories and minimises the variance within categories (WOLD *et al.*, 1984). A basic problem in LDA is deciding which variables should be included in the analysis. This may be achieved with a stepwise-LDA using Wilk's lambda as selection criterion, and an F statistic to establish the significance of the changes in lambda when a new variable is tested (POWERS and KEITH, 1968).

Leave-one-out cross validation

During this cross validation test, a sample is removed from the dataset. The classification model is rebuilt and the sample removed is classified in this new model. All the samples of the data set are sequentially removed and reclassified. Finally, a percentage of good classification is given (RAMIS and GARCÍA, 2001).

Software

Univariate and multivariate analysis were performed using the statistical software package STATGRAPHICS Plus for Windows 4.0 from Statistical Graphics Corporation and PARVUS 1.3 (FORINA *et al.*, 1994).

RESULTS AND DISCUSSION

One-hundred-twenty-five wines samples were analysed for eleven elements, K, Na, Ca, Mg, Fe, Cu, Zn, Mn, Sr, Li and Rb, giving a final data set of 125x11 data points. The influence of vintage, type of wine and denomination of origin were studied and the results are reported in Tables 2, 3 and 4 respectively. There were significant differences among the mean values for several elements for the three types of wine and the different denominations of origin (Table 5).

Concentration of elements

According to their mean concentrations, the elements can be arranged in the sequence K > Na > Mg > Ca > Fe > Rb > Mn > Zn > Sr > Cu > Li, for the samples grouped by vintage and by type of wine.

The mean concentrations of these metals in Tenerife wines are consistent with the values reported in the literature for those metals in other wines (AMERINE and OUGH, 1976; RIBEREAU-GAYON *et al.*, 1980; CABANIS and CABANIS, 2000), with the exception of sodium which was higher than that described in most of the literature (GALLEGO *et al.*, 1981; FERNANDEZ *et al.*, 1987; MUÑOZ *et al.*, 1987; OLALLA *et al.*, 1993; LIZAMA *et al.*, 1997; PEÑA *et al.*, 1999). The normal concentration of sodium is in the range 3-30 mg/L (CABANIS and CABANIS, 2000), while in the wines from Tenerife it ranged from

Table 2 - Mean values, standard deviations and minimum and maximum values (in parentheses) for the different elements according to the vintage.

Metal mg/L	First vintage (n = 57) Average ± S.D. Minimum - Maximum	Second vintage (n = 68) Average ± S.D. Minimum - Maximum	Both vintages (n = 125) Average ± S.D. Minimum - Maximum
K	**761±331** (255-1604)	**627±193** (301-1200)	688±272 (255-1604)
Na	92.9±43.6 (39-227)	83.9±32.8 (26-154)	88±38.2 (26-227)
Ca	**69.2±16.9** (41-106)	**60.2±13.8** (29-89)	64.3±15.9 (29-106)
Mg	84.9±13.6 (62-115)	82.8±12.7 (55-118)	83.8±13.1 (55-118)
Fe	2.22±1.03 (1-5.1)	2.28±0.84 (0.9-4.6)	2.25±0.93 (0.9-5.1)
Cu	**0.34±0.33** (0.06-1.19)	**0.16±0.09** (0.03-0.54)	0.24±0.25 (0.03-1.19)
Zn	0.71±0.34 (0.3-1.9)	0.66±0.25 (0.2-1.2)	0.68±0.29 (0.2-1.9)
Mn	1.15±0.45 (0.6-2.6)	1.03±0.42 (0.4-2.2)	1.09±0.43 (0.4-2.6)
Sr	0.57±0.22 (0.3-1.3)	0.52±0.19 (0.2-1.3)	0.54±0.21 (0.2-1.3)
Li*	5.94±2.9 (2-14.6)	6.77±3.04 (2.3-15.7)	6.39±2.99 (2-15.7)
Rb	1.91±0.82 (0.9-4.2)	1.96±0.68 (0.7-3.6)	1.94±0.75 (0.7-4.2)

* μg/L; S.D. = Standard Deviation, Numbers in bold indicate significant mean differences ($p<0.05$).

Table 3 - Mean values, standard deviations and minimum and maximum values (in parentheses) for the different elements according to the different types of wine.

Metal mg/L	White (n = 52) Average ± S.D. Minimum - Maximum	Rosé (n = 22) Average ± S.D. Minimum - Maximum	Red (n = 51) Average ± S.D. Minimum - Maximum
K	522±128 (255-806)	606±175 (336-1039)	893±284 (386-1604)
Na	82.1±37 (26-177)	88.5±36.3 (38-163)	93.8±40 (49-227)
Ca	69.2±13.1 (43-97)	69.2±18.5 (39-106)	57.2±14.8 (29-93)
Mg	75±9.3 (62-107)	85.3±8.8 (67-107)	92±12.4 (55-118)
Fe	1.83±0.84 (0.9-5)	2±0.63 (1.1-3.8)	2.8±0.87 (1-5.1)
Cu	0.26±0.27 (0.03-1.18)	0.31±0.35 (0.06-1.19)	0.19±0.15 (0.06-0.78)
Zn	0.73±0.27 (0.3-1.8)	0.73±0.3 (0.3-1.6)	0.61±0.31 (0.2-1.9)
Mn	0.98±0.43 (0.4-2.6)	1.07±0.35 (0.6-1.9)	1.2±0.44 (0.6-2.2)
Sr	0.44±0.13 (0.2-0.8)	0.54±0.29 (0.3-1.3)	0.65±0.18 (0.3-1.1)
Li*	6.46±3.12 (2-14.6)	6.75±3.32 (2.3-15)	6.17±2.75 (2.7-15.7)
Rb	1.48±0.48 (0.7-2.8)	1.74±0.45 (0.9-2.7)	2.5±0.72 (1.3-4.2)

* µg/L; S.D. = Standard Deviation.

26 to 227 mg/L; 92% of the samples ranged from 40 to 150, and 70% had more than 60 mg/L, considered by OIV (1990) to be the maximum limit for sodium. However, the values found here are similar to those obtained using wines made from vines exposed to a considerable marine influence, as in the present case (UREÑA *et al.*, 1987; SALAHA and VOUDOURI, 1991), so this higher sodium content in the wines from Tenerife can also be attributed to the effect of marine spray.

The iron concentration in 115 samples (92%) of the wines ranged from 0.9 to 3.6 mg/L and was always below 5.1 mg/L. These values are far below 10 mg/L considered to be the minimum necessary to produce ferric precipitate (OUGH, 1992).

The copper concentration in 114 of the 125 wines (91.2%) was below 0.6 mg/L, and eight samples had copper concentrations between 0.6-1.0 mg/L. Only three wines were above the maximum limit of 1 mg/L as recommended by the International Office of Vine and Wine (OIV, 1990). Ten of the eleven wines which had a copper concentration higher than 0.6 mg/L came from the same vintage and from wineries with modern equipment. The copper contamination can be attributed to a particular phytosanitary treatment rather than to technological contamination. Hence, the majority of the samples did not present a risk of cupric precipitate (OUGH, 1992).

The zinc concentration for 120 wines (96% of samples) ranged from 0.3 to 1.2 mg/L, and all the samples were below 1.9 mg/L, far below the maximum limit of 5 mg/L recommended by the OIV (1990).

Table 4 - Mean values, minimum and maximum values (in parentheses) for the different elements according to the different denominations of origin from the Tenerife Island (n = number of wines).

Metal m/L	Abona (n = 12) Average ± S.D. Min. - Max.	Valle de Güímar (n = 18) Average ± S.D. Min. - Max.	Valle de La Orotava (n = 27) Average ± S.D. Min. - Max.	Tacoronte-Acentejo (n = 32) Average ± S.D. Min. - Max.	Ycoden-Daute-Isora (n = 36) Average ± S.D. Min. - Max.
K	649±195 (429-1093)	742±298 (314-1200)	648±206 (265-1028)	748±338 (362-1604)	651±260 (255-1350)
Na	90±25 (49-125)	63±25 (26-127)	59±12 (40-83)	87±43 (44-227)	123±26 (64-176)
Ca	55±9 (41-71)	67±16 (42-93)	67±14 (39-92)	67±19 (29-106)	62±15 (39-97)
Mg	84±8 (75-101)	78±11 (62-102)	81±14 (62-107)	82±11 (55-102)	90±14 (68-118)
Fe	1.91±0.49 (1.20-2.70)	2.61±0.92 (1.40-5.00)	2.25±1.01 (0.90-4.70)	2.63±1.11 (1.10-5.10)	1.86±0.58 (1.00-3.30)
Cu	0.42±0.32 (0.06-0.81)	0.32±0.35 (0.03-1.18)	0.22±0.27 (0.06-1.19)	0.2±0.12 (0.06-0.58)	0.2±0.2 (0.08-1.15)
Zn	0.49±0.17 (0.30-0.80)	0.66±0.24 (0.30-1.20)	0.64±0.33 (0.30-1.90)	0.68±0.27 (0.20-1.30)	0.79±0.31 (0.30-1.80)
Mn	0.93±0.3 (0.60-1.50)	0.8±0.24 (0.50-1.60)	1.03±0.26 (0.60-1.60)	1.58±0.46 (0.60-2.60)	0.89±0.22 (0.40-1.30)
Sr	0.54±0.14 (0.30-0.80)	0.7±0.28 (0.40-1.30)	0.59±0.18 (0.30-0.90)	0.53±0.19 (0.30-1.00)	0.44±0.17 (0.20-0.90)
Li*	6.06±2.12 (2.90-8.90)	5.43±2.53 (2.00-11.70)	5.26±1.42 (3.10-8.30)	4.48±1.91 (2.30-11.00)	9.52±2.76 (4.10-15.70)
Rb	1.14±0.31 (0.70-1.70)	1.88±0.72 (0.90-3.30)	2.5±0.83 (1.20-4.20)	1.7±0.48 (0.90-2.70)	2.03±0.67 (0.90-3.40)

* µg/L; S.D. = Standard Deviation.

Vintage

The results obtained for the two vintages are given in Table 2. There were significant differences ($p < 0.05$) between both vintages only for potassium, calcium and copper (higher mean concentration in vintage 1). These results can be explained by differences in climatic conditions, fertilizing and phytosanitary treatments, that can vary with the year of production.

Type of wine

The results obtained for the different types of wines (white, rosé, red) are reported in Table 3. There were no significant differences in mean concentrations of sodium, copper, zinc and lithium among the three types of wines (Table 5). On the contrary, the concentrations of strontium and magnesium were significantly different. Red wines had significantly higher mean manganese concentrations than white wines. Red wines had mean concentrations significantly higher for potassium, iron and rubidium, than white and rosé wines and significantly lower for calcium. These differences are related to differences in the wine making process. For red wines, the must is macerated longer with the grape skins, which results in the extraction of higher amounts of some elements such as potassium, magnesium or rubidium from the skins. Cold stabilization of white and rosé wines can precipitate elements such as potassium and calcium. Likewise, the lower con-

Table 5 - ANOVA, using LSD as criterion to compare mean values by type and denomination of origin.

	Type of wine			Denomination of origin		
Metal	F	p	Sig Dif.	F	p	Sig. Dif.
K	41.24	0.000	1-3; 2-3	-	-	
Na	-	-		22.45	0.000	1-2; 1-3; 1-5; 2-4; 2-5; 3-4; 3-5; 4-5
Ca	9.77	0.001	1-3; 2-3	-	-	
Mg	33.37	0.000	1-2; 1-3; 2-3	4.22	0.003	2-5; 3-5; 4-5
Fe	19.33	0.000	1-3; 2-3	4.39	0.002	1-2; 1-4; 2-5; 4-5
Cu	-	-		-	-	
Zn	-	-		2.78	0.03	1-5; 3-5
Mn	3.44	0.035	1-3	28.22	0.000	1-4; 2-3; 2-4; 3-4; 4-5
Sr	17.44	0.000	1-2; 1-3; 2-3	5.69	0.001	1-2; 2-4; 2-5; 3-5
Li	-	-		26.54	0.000	1-4; 1-5; 2-5; 3-5; 4-5
Rb	40.41	0.000	1-3; 2-3	10.83	0.000	1-2; 1-3; 1-4; 1-5; 2-3; 3-4; 3-5; 4-5

Type of wine: 1 = white, 2 = rosé, 3 = red.
Denomination of origin: 1 = Abona, 2 = Valle de Güímar, 3 = Valle de La Orotava, 4 = Tacoronte-Acentejo, 5 = Ycoden-Daute-Isora.
F = between-group variance / within group variance.
Sig. Dif. = Statistically significant difference at the 95% confidence level.

centration of some metals such as copper in red wines may be due to the generation of sulfhydric acid during the wine making process which can thus precipitate such metals. However, differences were not significant.

Denominations of origin from Tenerife

The results about the elements analysed according to the different denominations of origin are shown in Table 4. Among the different denominations of origin studied significant differences were found in the mean concentrations of all metals except potassium, calcium and copper (Table 5). The Ycoden-Daute-Isora DO had significantly higher mean sodium and lithium concentrations than the other DOs. The Tacoronte-Acentejo DO had a significantly higher mean manganese concentration than the other DOs. Abona and Valle de La Orotava DOs had significantly lower and higher mean rubidium concentrations respectively than the other DOs. Among all the elements, sodium, manganese, lithium and rubidium had the greatest ability to discriminate among the different DOs. The differences among the various denominations of origin may be attributed to differences in soil composition and the effect of marine spray, in the case of sodium.

As can be seen in Table 5, although type of wine is more discriminating than Denomination of Origin, with the exception of Rb, the most discriminating metals according to type of wine were different from those most discriminating according to DO.

Likewise, it should be noted that the differences in the mean concentrations of the various metals among the different denominations of origin in Tenerife were lower than the differences between Tenerife and the other Canary Islands (ESTÉVEZ, 2002). These differences are of great interest for discriminating wines according to their geographic origin.

Principal component analysis

Although the metallic parameters studied gave significant differences, the differentiation of the wines according to vintage, type of wine or DO of production is quite difficult to establish by looking directly at the data. Principal Component Analysis (PCA) allows the information of the data set to be visualised in a few factors, retaining the maximum possible variability within that set. When PCA was applied to the data matrix, a total of four Principal Components (PC) were extracted with eigenvalues >1, which account for 63.3% of the total variance of the system. Table 6 shows the loading of each variable on each principal component. It can be seen that PC-1 (24% total variance) is mainly associated with potassium, magnesium and rubidium; PC-2 (17.6%) gave the highest loadings for lithium and sodium; PC-3 (11.8%) gave the highest loading for calcium, iron, copper and zinc, while PC-4 (10%) had the highest loading for manganese. Taking into account only the variables strongly associated with each PC, one can think of PC-1 as being related to type of wine, PC-2 to soil and marine spray, PC-3 to the equipment used in the wine making process, and PC-4 mainly to soil composition.

The plot of scores in the plane defined by PC-1 and PC-2 (Fig. 1) shows that PC-1 separates samples according to the type of wine. Thus, red wines with positive scores tend to be separated from white wines, with negative scores, and rosé wines fall between the red and white wines, but are more overlapped with white wines. This may be due to similarities in producing the white and rosé wines.

When the scores were plotted on the plane defined by PC-2 and PC-4 (Fig. 2), samples coming from Ycoden-Daute-Isora (higher lithium and sodium concentration) fall on the left of the plot on PC-2, well separated from the other samples. It can also be seen that PC-4 separated wines from Tacoronte-Acentejo (highest manganese concentration), found on the bottom right of the plot. Thus, both PC-2 and PC-4 contributed to the differentiation of some denominations of origin.

The latent structure of the first four components describes samples according to type of wine and origin.

Table 6 - Loading of the variables, eigenvalues, percentage of variance and cumulative variance for each principal component.

Element	PC 1	PC 2	PC 3	PC 4
K	0.498	0.088	0.071	0.042
Na	0.216	-0.584	0.022	-0.074
Ca	-0.19	-0.028	0.459	-0.232
Mg	0.489	-0.197	-0.009	-0.026
Fe	0.264	0.108	0.472	-0.088
Cu	-0.19	-0.012	0.454	0.468
Zn	-0.126	-0.377	0.421	0.03
Mn	0.075	0.146	0.242	-0.764
Sr	0.352	0.26	0.312	0.355
Li	0.096	-0.595	-0.052	0.027
Rb	0.412	0.121	-0.137	0.023
Eigenvalue	2.626	1.938	1.301	1.097
% variance	23.875	17.615	11.83	9.973
% cumulative	23.875	41.489	53.319	63.293

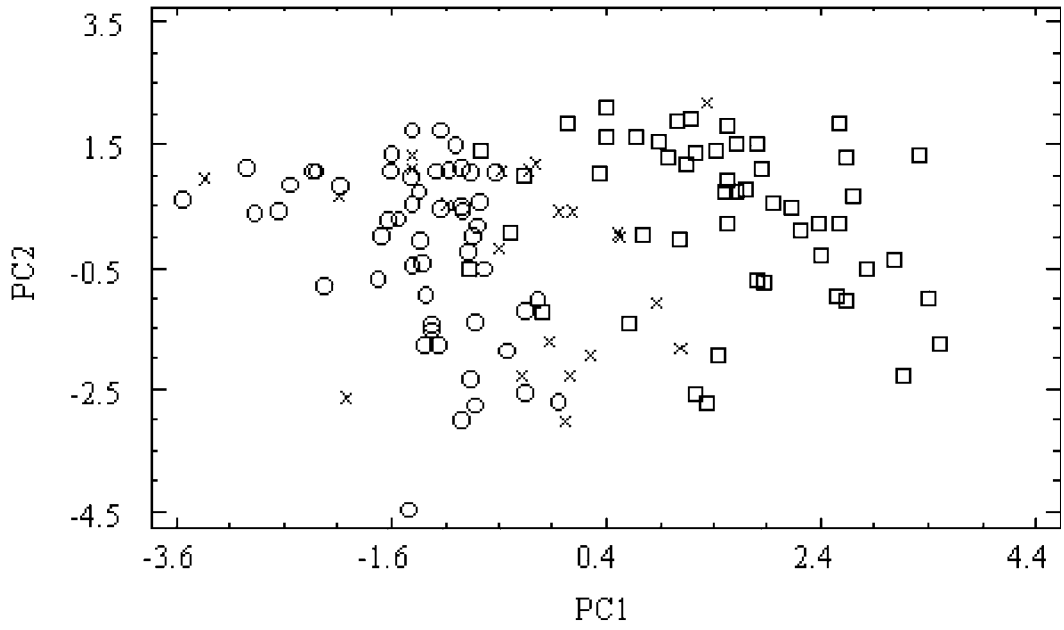


Fig. 1 - Wine sample scores on the first two principal components (41.5% of the total variance). O = white; X = rosé; □ = red.

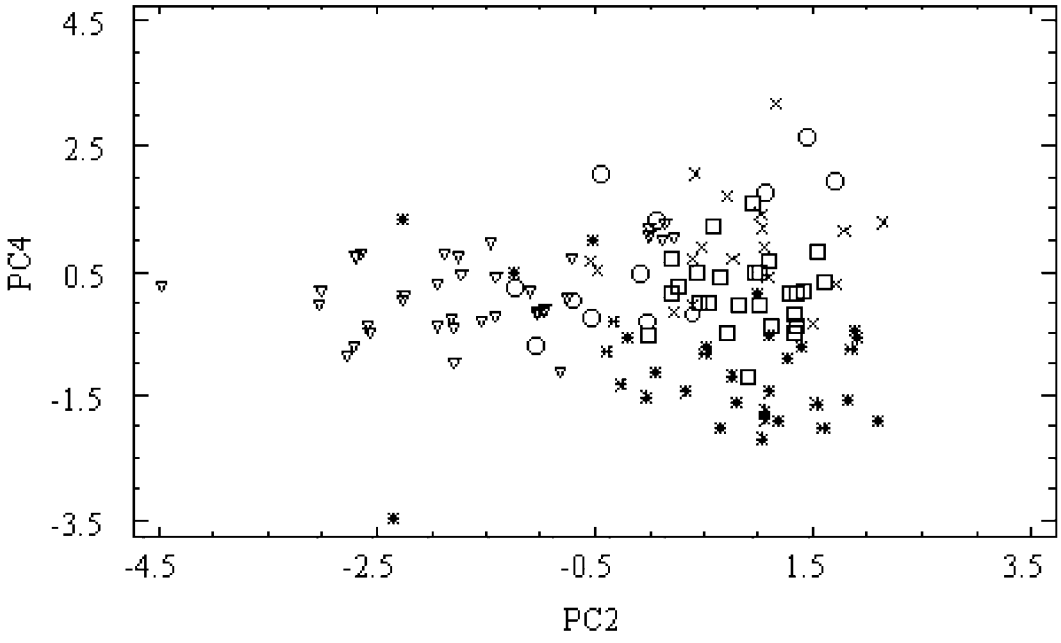


Fig. 2 - Wine sample scores on the plane of the second and fourth principal components (27.6% of the total variance). O = Abona; X = Valle de Güimar; □ = Valle de La Orotava; ✱ = Tacoronte-Acentejo; ▽ = Ycoden-Daute-Isora.

Linear discriminant analysis (LDA)

This technique is a widely used parametric method for classification purposes; it assumes a priori knowledge of the number of classes and the sample class membership. The classification was performed according to the denomination of origin. Stepwise linear discriminant analysis (POWERS and KEITH, 1968) is a method for seeking the subsets of variables (metals) that are most useful to discriminate among categories. In this case the forward selection approach was used, but all the variables were selected. Four discriminant functions, three of which were statistically significant ($p < 0.05$), were obtained. The Wilk's Lambda and standard F values for each variable are presented in Table 7, which shows that manganese, lithium, rubidium and sodium were the most discriminant elements. The classification results were validated by using a leave-one-out cross-validation. The results obtained for all the samples and separated for white and red wines are reported in Table 8. With all the samples, the best results were obtained for Abona and Ycoden-Daute-Isora. When samples were separated according to type of wine, in general, the recognition ability increased. The best prediction abilities were obtained for white wines from Abona, Tacoronte-Acentejo and Ycoden-Daute-Isora and for red wines from Ycoden-Daute-Isora.

Table 7 - Stepwise discriminant analysis according to the Denomination of Origin. Wilk's Lambda and standard F values for each variable.

Element	Wilk's Lambda	Standard F
Mn	0.515	28.2
Li	0.269	27.6
Rb	0.192	22.5
Na	0.145	19.8
Fe	0.113	17.9
K	0.096	16
Ca	0.085	14.5
Mg	0.075	13.3
Sr	0.066	12.4
Cu	0.062	11.4
Zn	0.058	10.6

CONCLUSIONS

The concentrations of eleven metals were determined in 125 bottled wine samples belonging to two consecutive harvests from Tenerife (Canary Islands, Spain), bearing certification of the denomination of origin. There were significant differences in the mean potassium, calcium and copper concentrations according to vintage, thus environmental factors influenced their concentration. The mean K, Ca, Mg, Fe, Mn, Sr and Rb concentrations showed signifi-

Table 8 - Recognition and prediction abilities for the different denominations of origin, using stepwise discriminant analysis.

Categories	White + Rosé + Red All variables		White Without Mg and Cu		Red Without Ca	
	Recognition ability	Prediction ability	Recognition ability	Prediction ability	Recognition ability	Prediction ability
Abona	100	91.7	100	100	-	-
Valle de Güimar	66.7	61.1	88.9	55.6	83.3	50
Valle de La Orotava	81.5	66.7	91.7	75	84.6	76.9
Tacoronte- Acentejo	84.4	78.1	100	87.5	94.4	77.8
Ycoden-Daute-Isora	91.7	83.3	86.7	86.7	100	83.3

cant differences according to the type of wine, indicating that their concentration depends on the wine making process. All metals with the exception of potassium, calcium and copper gave significant differences in mean concentration among the different denominations of origin, with manganese, lithium, sodium and rubidium being the most important. Ycoden-Daute-Isora had higher mean concentrations of Li and Na than the other DOs, while Tacoronte-Acentejo had a higher mean concentration of manganese compared with the other DOs. These differences are related to soil composition and marine spray. When PCA was applied, four principal components could be extracted, accounting for 63.3% of the total variance. The principal components are related to type of wine, marine spray, wine-making technology and soil composition. The first PC grouped samples according to type of wine, and the second and fourth PCs grouped according to the denomination of origin. Stepwise linear discriminant analysis showed an acceptable degree of recognition and prediction abilities for the denominations of origin of Abona, Tacoronte-Acentejo and Ycoden-Daute-Isora.

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PAPER

DETERMINATION OF COPPER (II), LEAD (II), CADMIUM (II) AND ZINC (II) IN VIRGIN OLIVE OILS PRODUCED IN SICILY AND APULIA BY DERIVATIVE POTENTIOMETRIC STRIPPING ANALYSIS

DETERMINAZIONE DEL RAME (II), DEL PIOMBO (II), DEL CADMIO (II) E DELLO ZINCO (II) IN OLI VERGINI D'OLIVA PRODOTTI IN SICILIA E PUGLIA MEDIANTE ANALISI POTENZIOMETRICA DERIVATIVA IN STRIPPING

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ABSTRACT

The presence of Cu (II), Pb (II), Cd (II) and Zn (II) was evaluated by derivative potentiometric stripping analysis in 82 virgin olive oil samples produced in Sicily and Apulia in the crop years 1999-2000 and 2000-2001. The sample pretreatment involved extraction of heavy metals from the oily matrix by hot concentrated hydrochloric acid. Lead (II), copper (II) and zinc (II) were found in all samples, with maximum values of 55.3 ng g^{-1} for Cu (II), 67.6 ng g^{-1} for Pb (II) and 610.1 ng g^{-1} for Zn (II), and min-

RIASSUNTO

È stata valutata la presenza di Cu (II), Pb (II), Cd (II), e Zn (II) in 82 campioni di oli vergini di oliva, prodotti in Sicilia e in Puglia nelle annate 1999-2000 e 2000-2001, mediante analisi potenziometrica derivativa in stripping. Il trattamento del campione consiste in un'estrazione a caldo dei metalli, dalla matrice oleosa, mediante acido cloridrico concentrato. Rame (II), piombo (II) e zinco (II) sono stati trovati in tutti gli oli, con valori massimi di 55,3 ng g^{-1} per il Cu (II), di 67,6 ng g^{-1} per il Pb (II)

- Key words: cadmium; copper; derivative potentiometric stripping analysis; lead; virgin olive oils; zinc -

imum values of 8.2, 15.4 and 53.9 ng g^{-1}, respectively. Cadmium (II) was found only in two samples from Apulia, with a maximum value of 2.1 ng g^{-1}. In order to investigate the relationship between heavy metal concentration and olive oil production techniques, the results were correlated with olive picking method (manual or mechanical), and harvesting and milling dates (different crop years). Sicilian olive oils produced in 1999/00 had a higher mean content of Pb (II) and Cu (II) than samples from 2000/01. Amounts of Pb (II) and Cu (II) in Apulian olive oils were similar to those found in Sicilian oils in the same crop year. Metal concentration was related to olive oil production techniques: Sicilian oils produced in the two crop years from olives harvesred mechanically had a Pb (II) content higher than oils produced from olives picked manually.

e di 610,1 ng g^{-1} per lo Zn (II), e valori minimi di 8,2, 15,4 e 53,9 ng g^{-1}, rispettivamente. Il cadmio (II) è stato trovato solo in due campioni pugliesi, in quantità massima di 2,1 ng g^{-1}. Inoltre al fine di studiare la correlazione tra la concentrazione dei metalli negli estratti e le tecniche di produzione dell'olio d'oliva, i risultati ottenuti sono stati messi in relazione al metodo di raccolta delle olive, manuale o meccanico, data di raccolta e lavorazione. Da questo studio è stato evidenziato che la concentrazione media di Pb (II) e Cu (II) negli oli Siciliani dell'annata 1999/00 è più alta rispetto a quella riscontrata negli oli dell'annata successiva, inoltre la concentrazione di Pb (II) e Cu (II) nei campioni Pugliesi dell'annata 1999/00 è simile a quella dei campioni Siciliani della stessa annata. la concentrazione dei metalli negli oli è stata correlata ad alcune tecniche agronomiche: gli oli siciliani delle due annate prodotti da olive raccolte meccanicamente hanno un contenuto di Pb (II) superiore rispetto a quello riscontrato in oli prodotti da olive raccolte manualmente.

INTRODUCTION

Analytical testing for metals in food is of great importance in order to estimate toxicological parameters and guarantee good food quality (ROJAS *et al.*, 1999; CERRUTI, 1993). Metals can be found in fatty foods such as edible oils that are often subjected to refining, bleaching, and deodorization, during which time they inevitably come into contact with metallic surfaces (MARTIN-POLVILLO *et al.*, 1994). Trace levels of some metal ions like Cu (II) could catalyse the oxidation reaction of fatty acid chains, with a consequent deleterious effect on oil flavor and shelf-life (FEDELI, 1968; POKORNY, 1968; CALAPAJ *et al.*, 1988).

Metal contamination of olive oil could be due to the presence of metals in the soil, water and, particularly for lead, in the air (PAOLETTI *et al.*, 1999). Many studies have been carried out in which the presence of trace metals in olive oils have been correlated to agronomical techniques, harvesting methods, oil extraction and packaging procedures (DE LEONARDIS *et al.*, 1997; SOLINAS *et al.*, 1984-87; DI BATTISTA *et al.*, 1993; GARRIDO *et al.*, 1994; MARTIN-POLVILLO *et al.*, 1994).

Testing for metals in edible oils has always been very problematic, because they are present in very small quantities within a complex organic matrix. Sample preparation is a critical step in the

whole analytical procedure. The commonly used classical methods are wet digestion, dry ashing, acid extraction, closed-vessel and focused open-vessel microwave dissolution, dilution (CROSBY, 1977; GARRIDO *et al.*, 1994; ALLEN *et al.*, 1998) and basic alcoholic solubilization (WAHDAT *et al.*, 1995). The analytical techniques frequently used for the subsequent determinations are emission and absorption spectrophotometric techniques as well as electroanalytical techniques (CALAPAJ *et al.*, 1988; HENDRIKSE *et al.*, 1991; IBRAHIM, 1991; WAHDAT *et al.*, 1995).

In this paper derivative potentiometric stripping analysis (dPSA) was used to determine copper (II), lead (II), cadmium (II) and zinc (II), in samples of virgin olive oils from Sicily and Apulia (Italy).

MATERIALS AND METHODS

Samples

Oil samples used in this study were produced in Sicily during two consecutive crop years, 1999-2000 and 2000-2001 and in Apulia during the crop year 2000-2001. The harvesting dates and olive picking methods of all the Sicilian oils were known, while only the harvesting dates of Apulian oils were known. Oil samples were stored in dark glass bottles at 4°C until the time of analysis.

Chemicals

All glassware was rinsed with 10% (v/v) nitric acid. Ultra-pure water was obtained from a Pure Lab RO and a Pure Lab UV system (USF, Ransbach-Baumbach, Germany). Ultra-pure and certified hydrochloric acid and pure mercury (II) chloride (Carlo Erba, Milan, Italy), were used. A 1,000 ng μL^{-1} copper (II) standard solution, 0.5 N HNO_3, a 1,000 ng μL^{-1} lead (II) standard solution, 0.5 N HNO_3, a 1,000 ng μL^{-1} cadmium (II) standard solution, 0.5 N HNO_3, a 1,000 ng μL^{-1} zinc (II) standard solution, 0.5 N HNO_3 and a 1,000 ng μL^{-1} gallium (III) standard solution, 0.5 N HNO_3 (Panreac Quimica, Barcellona, Spain) were used. By diluting each of the standard metal solutions with water, solutions containing 1.0 ng μL^{-1} of copper (II), 1.0 ng μL^{-1} of lead (II), 1.0 ng μL^{-1} of cadmium (II), 1.0 ng μL^{-1} of zinc (II) and 1.0 ng μL^{-1} of gallium (III) were prepared.

Apparatus

Determinations were carried out using a PSA ION^3 potentiometric stripping analyzer (Steroglass, S. Martino in Campo, Perugia, Italy), connected to an IBM-compatible personal computer. The analyzer operated under the control of the NEOTES software package (Steroglass). The analytical procedure can be completely controlled by this program, as already described in previous papers (LO COCO *et al.*, 1999; 2000 a, b).

Electrodes and electrochemical cell

The three-electrode system and the electrochemical cell utilized were already described in previous papers (LO COCO *et al.*, 1999; 2000 a, b).

Preliminary sample processing

A 19.2 g (20 mL) sample and a 20 mL volume of concentrated hydrochloric acid were poured into a teflon beaker and the extraction was carried out for about 45 min at 50°C under magnetic stirring. The sample was cooled for about 5 min to favour the separation of the two phases, then the lower acidic layer was directly transferred to a 40 mL volumetric flask. A second extraction procedure was carried out on the organic layer for 5 min with 20 mL of water under the same conditions described above and the wash-

ings were added to the volumetric flask. The aqueous solution was cooled if necessary and brought to the mark with water. To eliminate possible traces of organic compounds, the acid solution was then passed through a carbon column (Supelclean ENVI-Carb SPE Tubes, 0.5 g, 6 mL, Supelco, Bellefonte, PA, USA), previously activated with 2 mL of methanol followed by 2 mL of ultrapure water; the first drops of the eluate were discarded. The resulting solution was then analyzed to determine the levels of copper (II), lead (II), cadmium (II) and zinc (II)

Recovery test

To determine the metal recovery levels, appropriate volumes of 1 ng μL^{-1} copper (II), lead (II), cadmium (II) and zinc (II) nitrate diluted in aqueous solutions were added to a sample of olive oil. To favour mixing of the spiked aqueous solution and the oil, the mixture was stirred for 24 h.

Potentiometric determination

Copper (II), lead (II), cadmium (II) and zinc (II) levels were determined in 82 samples of virgin olive oil produced in Sicily and Apulia. Metal ions were extracted with hot concentrated hydrochloric acid. The subsequent analysis was carried out by dPSA. Cu (II), Pb (II), and Cd (II) levels were determined simultaneously in each sample, by adding 5.0 mL of the solution obtained as described previously to 15.0 mL of water and 1.0 mL of a mercury (II) chloride solution containing 1,000 ng μL^{-1} of mercury (II) ion in 1 M hydrochloric acid in the electrochemical cell. The electrolysis time was 300 s and the potential -0.9 V; the potential of the electrodes was monitored every 300 µs. Cu (II), Pb (II), and Cd (II) were oxidized at approximately -0.26, -0.43 and -0.64 V, respectively, vs. the reference electrode. A quantitative analysis was carried out using the method of standard additions by adding twice 200 µL of a solution containing 1.0 ng μL^{-1} of copper (II), 200 µL of a solution containing 1.0 ng μL^{-1} of lead (II) and 200 µL of a solution containing 0.1 ng μL^{-1} of cadmium (II). The zinc (II) levels were determined separately at pH values not lower than 1.8 in order to avoid H_2 from forming at the working electrode. An excess of gallium (III) was used to prevent the formation of Cu (II)-Zn (II) intermetallic compounds on Hg film by promoting the formation of the much more stable Cu(II)-Ga (III) intermetallic complex (PSARUODAKIS and EFSTATHIOU, 1987, 1989). A 0.5 mL volume of the solution obtained as described in the preliminary sample processing section was put into the electrochemical cell together with 19.5 mL of water, 0.5 mL of a mercury (II) chloride solution containing 1,000 ng μL^{-1} of mercury (II) ion in 1 M hydrochloric acid and 100 µL of a solution containing 1.0 ng μL^{-1} of gallium (III). The rest of the procedure was the same as that described except (i) the electrolysis potential was -1.25 V, (ii) the electrolysis time was 240 s and (iii) the two standard additions ranged from 100 to 600 µL of a solution containing 1.0 ng μL^{-1} of zinc (II). At the above described condition Zn is oxidized at approximately -0.90V.

Before each analysis, the working electrode was coated with a thin mercury film by electrolyzing a mercury (II) chloride solution of a concentration equal to that added to the sample at -0.9 V against the reference electrode (silver/silver chloride // saturated potassium chloride) for 1 min.

RESULTS AND DISCUSSION

In carrying out the potentiometric determination of Cu (II), Pb (II), Cd (II) and Zn (II), good linearity was obtained in the range of concentrations examined, as shown by the equations reported in Table 1 and the corresponding determination coefficients. Recovery tests were carried

Table 1 - Calibration equations, determination coefficents, detection limits, recovery (%) and repeatability (%) for copper (II), lead (II), cadmium (II) and zinc (II).

	Cu	Pb	Cd	Zn
Calibration equations(y=)	$6.9 \cdot 10^7 x + 2.1 \cdot 10^5$	$4.2 \cdot 10^7 x + 1.9 \cdot 10^5$	$2.0 \cdot 10^7 x + 1.5 \cdot 10^5$	$1.7 \cdot 10^8 x + 8.5 \cdot 10^4$
Determination coefficients %	100	98.8	99.2	99.9
Detection limit (ng g^{-1})	3.6	5.9	1.2	14.3
Recovery %	96-100	98-101	88-92	92-96
Repeatability %	5.3	1.3	13.6	4.1

out as described above and are reported by LA PERA *et al.* (2002). Both the spiked and unspiked samples were analyzed in triplicate; the results are also reported in Table 1. Theoretical instrumental detection limits (Table 1) were evaluated using the expression $3\sigma/S$ (MASSART *et al.*, 1978; LO COCO *et al.*, 1999) where σ is the peak threshold (set at 200) and S is the sensitivity obtained from the calibration equation of each analyte, also shown in Table 1. Repeatability of the method was evaluated by carrying out the determination three times on the same sample of olive oil. The values obtained were subjected to statistical analysis using the same software as that used for the analytical steps. The average concentrations were 7.6 ng g^{-1} for copper (II), with a standard deviation of 0.4 ng g^{-1}; 7.6 ng g^{-1} for lead (II), with a standard deviation of 0.1 ng g^{-1}; 2.6 ng g^{-1} for cadmium (II), with a standard deviation of 0.4 and 14.5 ng g^{-1} for zinc (II), with a standard deviation of 0.6 ng g^{-1}**.** The confidence intervals of the mean value, corresponding to a probability of 99%, were ±2.3, ±0.6, ±2.3, and ±3.4, respectively. The repeatability for each analyte, expressed as relative standard deviation, is reported in Table 1.

Sicilian oils from the crop year 1999-2000

The concentrations of the four metals in the 36 oil samples from Sicily are reported in Table 2 together with variety, location, harvesting method and harvesting date. The highest average concentration was Zn (II). The maximum mean value (252.3 ng g^{-1}) was found in the variety Tonda Iblea from Chiaramonte-Gulfi (Ragusa), the minimum (170.3 ng g^{-1}) in the Biancolilla variety from Caltabellotta (Trapani). The oil with the highest concentration of Zn (II) (437.8 ng g^{-1}) was from the Biancolilla variety from Delia (Caltanissetta); the Pb (II) and Cd (II) concentrations in this sample were lower than 50 ng g^{-1}.

The highest average concentration of Pb (II) (50.5 ng g^{-1}) was found in the Nocellara del Belice variety from Castelvetrano (Trapani), the lowest (23.5 ng g^{-1}) in the Tonda Iblea variety. The Nocellara del Belice variety also had the highest mean quantity of Cu (II) (35.8 ng g^{-1}), while the Cerasuola variety from Paceco (Trapani) had the lowest (20.3 ng g^{-1}). The highest concentrations of Pb (II) (67.6 ng g^{-1}) and Cu (II) (53.8 ng g^{-1}) were found in two different samples of the Nocellara del Belice variety. It is interesting to note that a significant percentage of samples (41%) had a Pb (II) concentration of 30-40 ng g^{-1} (Fig. 1).

Sicilian oils from the crop year 2000-2001

The concentrations of the four metals in the 24 oil samples are reported in Table 3 together with variety, location, harvesting method and harvesting date. Zn (II) was also present in the greatest quantity, with respect to the other metals, in the crop year 2000-2001; the mean values were between 335.0 ng g^{-1}

Table 2 - Lead (II), copper (II), cadmium (II) and zinc (II) concentrations (ng g^{-1}) determined in thirty-six samples of Sicilian olive oils (crop year 1999-2000). Each value is the mean of three determinations; each determination was repeated three times. The confidence interval of the mean value corresponds to 99% probability.

Variety	Location	Pb	Cu	Cd	Zn	Harvesting method	Harvesting date*
Nocellara del Belice	Castelvetrano	40.4±0.3	31.7±1.9	n.d.	250.0±3.3	manual	07/10/99
"	"	36.3±0.5	53.8±3.0	n.d.	175.9±2.1	"	04/11/99
"	"	56.6±0.8	34.2±1.0	n.d.	237.8±3.3	"	12/11/99
"	"	53.3±0.3	21.6±0.9	n.d.	222.5±2.1	mechanical	07/10/99
"	"	48.6±0.4	45.7±3.1	n.d.	130.9±1.1	"	04/11/99
"	"	67.6±0.7	27.9±1.6	n.d.	142.0±1.6	"	12/11/99
	M± σ	**50.5±11.3**	**35.8±11.9**	n.d.	**193.2±50.7**		
Biancolilla	Caltabellotta	32.0±0.4	28.3±0.7	n.d.	130.4±1.1	manual	11/10/99
"	"	32.1±0.5	26.2±2.3	n.d.	214.1±3.1	"	05/11/99
"	"	39.2±0.7	43.4±0.5	n.d.	208.9±1.7	"	19/11/99
"	"	29.3±0.2	13.4±2.6	n.d.	101.3±1.3	mechanical	11/10/99
"	"	38.8±0.4	39.8±2.6	n.d.	197.0±4.3	"	05/11/99
	M± σ	**34.3±4.5**	**30.2±11.9**	n.d.	**170.3±51.2**		
Cerasuola	Paceco	31.4±0.8	31.4±1.7	n.d.	136.0±0.9	mechanical	27/10/99
"	"	31.2±0.2	22.1±0.8	n.d.	194.9±2.2	manual	"
"	"	56.0±1.0	19.6±1.3	n.d.	217.5±1.4	mechanical	10/12/99
"	"	41.3±0.2	8.2±0.7	n.d.	154.8±1.1	manual	"
	M± σ	**42.1±10.3**	**20.3±9.6**	n.d.	**175.8±37.4**		
Tonda Iblea	Chiaramonte	26.7±0.1	30.5±1.1	n.d.	314.6±5.5	manual	20/10/99
"	"	21.4±0.2	26.2±1.1	n.d.	355.7±2.6	"	28/10/99
"	"	22.5±0.1	50.5±1.8	n.d.	86.5+0.3	"	03/12/99
	M± σ	**23.5±2.8**	**35.7±12.9**	n.d.	**252.3±145.0**		
Biancolilla	Delia	39.4±0.5	22.1±1.0	n.d.	108.7±2.6	unknown	09/11/99
"	"	32.1±0.5	14.0±0.8	n.d.	117.6±0.9	"	"
"	"	23.6±0.2	42.1±2.3	n.d.	215.6±4.4	"	"
"	"	25.8±0.1	17.1±0.9	n.d.	126.6±1.1	"	"
"	"	22.3±0.3	32.1±1.2	n.d.	132.2±2.2	"	"
"	"	32.0±0.4	10.7±0.8	n.d.	68.7±0.5	"	"
"	"	30.2±0.2	21.8±1.4	n.d.	138.5±1.4	"	12/11/99
"	"	46.8±0.6	22.9±2.0	n.d.	120.4±0.9	"	"
"	"	52.5±0.8	17.3±1.1	n.d.	196.8±2.3	"	"
"	"	31.9±0.3	20.7±1.8	n.d.	190.0±1.1	"	"
"	"	32.5±0.3	34.0±1.9	n.d.	210.1±2.8	"	"
"	"	26.2±0.5	19.9±0.7	n.d.	130.1±1.1	"	"
"	"	32.8±0.4	18.7±1.3	n.d.	88.0±1.1	"	"
"	"	29.5±0.1	18.0±2.1	n.d.	263.1±2.8	"	"
"	"	25.4±0.2	34.3±1.1	n.d.	286.4±1.1	"	"
"	"	33.1±0.7	33.3±1.6	n.d.	384.0±1.1	"	"
"	"	47.3±1.1	30.9±1.7	n.d.	168.6±2.8	"	"
"	"	48.1±0.9	49.1±2.8	n.d.	437.8±3.1	"	"
	M± σ	**34.0±9.1**	**25.5±10.3**	n.d.	**188.0±100.2**		

n.d. = not detectable (<1.2 ng g^{-1}); M±σ = mean ± standard deviation; * day/month/year.

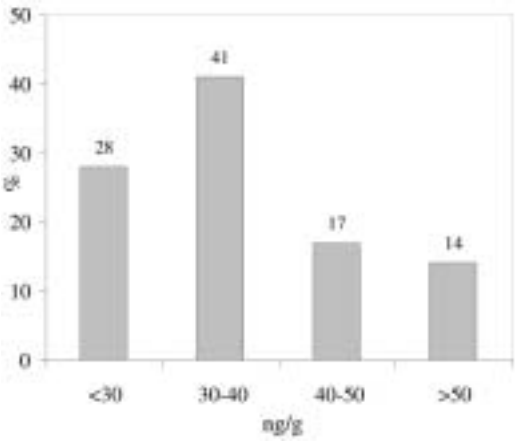


Fig. 1 - Pb concentration groups (%) of thirty-six Sicilian olive oils (crop year 1999-2000).

for the Cerasuola variety and 161.9 ng g^{-1} for the Biancolilla variety. The Cerasuola variety had the highest concentration of Zn (II) (485.9 ng g^{-1}) with Pb (II) and Cu (II) levels less than 50 ng g^{-1}. Cd (II) was not found in any of the samples from this year. The mean Cu (II) concentration was particularly low in the Crastu variety (14.8 ng g^{-1}) from Buonfornello (Palermo) and in the Biancolilla variety (16.7 ng g^{-1}) from Lucca Sicula (Agrigento), while the highest average concentration (36.4 ng g^{-1}) was in the Cerasuola variety. Moreover one Cerasuola oil sample had the highest copper content (55.3 ng g^{-1}). The average Pb (II) concentrations found in the Sicilian samples for the crop year 2000-2001 were almost identical: the highest mean value was calculated for the Cerasuola variety (27.8 ng g^{-1}) and the lowest for the Crastu variety (25.4 ng g^{-1}). The Nocellara del Belice variety had the highest Pb (II) content. It is interesting to note that 79% of the samples from the crop year 2000-2001 had Pb (II) concentrations less than 30 ng g^{-1} (Fig. 2).

Between the 1999-2000 and 2000-2001 crop years, there was a 27% decrease in the average Pb concentration in the Sicilian oils derived from manually collected olives and a 45% decrease in oils obtained from mechanically picked olives. In the second crop year 52% more of the Sicilian samples had lead concentrations less than 30 ng g^{-1}. Moreover the mean Zn (II) level for the Cerasuola variety from the crop year 2000-2001 was significantly higher with respect to the other cultivars from the same year and the previous year.

Apulian oils from the crop year 1999-2000

The concentrations of the four metals in the 22 samples of oil from Apulia are reported in Table 4 together with variety, location, harvesting method and harvesting date. In these samples, the mean Zn (II) concentration was higher than the other metals. The mean value for the Leccino variety from Copertino (Foggia) (590.8 ng g^{-1}) was particularly high, while oils from mixed varieties such as Ogliarola from Foggia and Coratina from Bitetto (Bari) had an average concentration of 285.2 ng g^{-1}. The mean zinc concentrations for the other Apulian varieties were similar to those found in the Sicilian oils, varying from 246.8 ng g^{-1}, in the Cellina variety from Nardò (Lecce) to 142.5 ng g^{-1} in the Ogliarola Garganica variety. A particularly low mean value (61.2 ng g^{-1}), was found in the Carolea variety from Copertino (Foggia). The highest Zn (II) concentration was found in the Leccino variety, (610.1 ng g^{-1}) that had Cu (II) and Pb (II) concentrations that were less than 50 ng g^{-1}.

The average Pb (II) concentrations were similar to those determined for the Sicilian oils; the maximum mean value was 49.6 ng g^{-1} for the Leccino variety and the minimum was 18.0 ng g^{-1} for the Cellina variety. The highest (56.6 ng g^{-1}) and the lowest (15.4 ng g^{-1}) Pb (II) concentrations were found in two different samples of the Leccino variety and Cellina variety, respectively. Most samples of the Apulian oils (32%) had Pb (II) concentrations between 40-50 ng g^{-1} (Fig. 3).

Table 3 - Lead (II), copper (II), cadmium (II) and zinc (II) concentrations (ng g^{-1}) determined in twenty-four samples of Sicilian olive oils (crop year 2000-2001). Each value is the mean of three determinations; each determination was repeated three times. The confidence interval of the mean value corresponds to 99% probability.

Variety	Location	Pb	Cu	Cd	Zn	Harvesting method	Harvesting data*
Cerasuola	Paceco	29.7±0.5	43.3±3.0	n.d.	321.2±3.6	mechanical	17/10/00
"	"	23.8±0.1	26.7±1.5	n.d.	297.4±4.2	manual	"
"	"	25.2±0.4	39.4±4.0	n.d.	254.1±2.6	manual	31/10/00
"	"	27.1±0.2	55.3±2.1	n.d.	379.2±7.1	mechanical	"
"	"	30.4±0.6	14.6±0.7	n.d.	456.3±5.3	manual	10/11/00
"	"	34.4±0.8	17.4±1.8	n.d.	485.9±4.9	mechanical	"
"	"	26.2±0.1	52.8±3.9	n.d.	236.0±3.0	manual	"
"	"	25.4±0.1	41.5±1.3	n.d.	249.6±2.9	mechanical	"
	M± σ	**27.8±3.5**	**36.4±15.3**		**335.0±96.1**		
Biancolilla	Lucca Sicula	20.8±0.1	10.4±0.3	n.d.	152.1±1.1	manual	15/11/00
"	"	22.4±0.5	14.1±0.8	n.d.	157.7±2.0	mechanical	"
"	"	27.5±0.1	17.5±1.9	n.d.	163.8±1.9	manual	"
"	"	34.6±0.8	24.7±0.9	n.d.	174.0±2.3	mechanical	"
	M± σ	**26.3±6.2**	**16.7±6.1**		**161.9±9.4**		
Nocellara del Belice	Castelvetrano	29.1±0.1	27.5±1.1	n.d.	139.3±1.9	manuale	24/11/00
"	"	27.3±0.1	24.1±0.6	n.d.	79.4±0.9	mechanical	"
"	"	17.8±0.4	34.7±1.5	n.d.	118.2±3.0	manual	"
"	"	25.3±0.3	21.9±0.9	n.d.	76.1±0.6	mechanical	"
"	"	24.4±0.1	22.4±0.8	n.d	271.7±3.5	"	05/11/00
"	"	19.2±0.4	23.1±1.2	n.d	134.5±1.9	manual	"
"	"	34.7±0.3	14.5±0.8	n.d	214.2±4.4	"	14/11/00
"	"	44.8±0.9	12.9±0.3	n.d	196.4±2.1	mechanical	"
"	"	22.6±0.1	13.9±0.9	n.d	229.4±1.9	manual	"
"	"	24.6±0.1	16.5±1.1	n.d	229.7±2.1	mechanical	"
	M± σ	**27.0±7.9**	**21.1±6.9**		**168.9±68.3**		
Crastu	Buonfornello	28.6±0.1	16.0±0.8	n.d	172.1±1.5	manual	06/11/00
"	"	22.2±0.5	13.6±0.3	n.d	182.4±1.3	manual	"
	M± σ	**25.4±4.5**	**14.8±1.6**		**177.3±7.3**		

n.d. = not detectable (<1.2 ng g^{-1}); M±σ = mean ± standard deviation; * day/month/year.

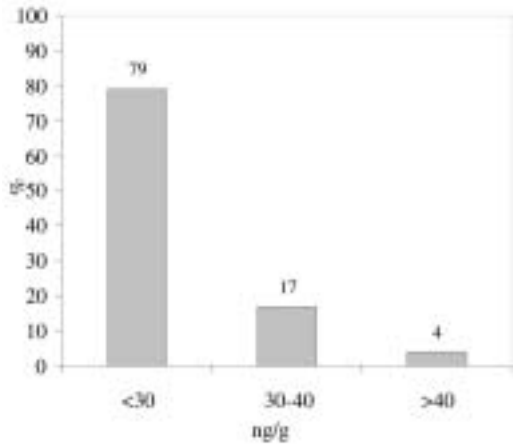


Fig. 2 - Pb concentration groups (%) of twenty-four Sicilian olive oils (crop year 2000-2001).

The Apulian oils from the crop year 1999-2000 had Cu (II) concentrations that were a little lower than those found in the Sicilian oils from the same crop year. The mean values were between 28.7 ng g^{-1} for the Leccino variety and 11.5 ng g^{-1} for the Cellina variety. The highest (37.5 ng g^{-1}) and lowest (8.9 ng g^{-1}) Cu (II) concentrations were found in the samples of the Ogliarola Garganica and Cellina varieties, respectively.

Very low concentrations of cadmium (II), close to the calculated detection limits, were found in a mixed Ogliarola and Coratina sample, 1.6 ng g^{-1} and in a Coratina sample 2.1 ng g^{-1}.

Table 4 - Lead (II), copper (II), cadmium (II) and zinc (II) concentrations (ng g^{-1}) determined in twenty-two samples of Apulian olive oils (crop year 1999-2000). Each value is the mean of three determinations; each determination was repeated three times. The confidence interval of the mean value corresponds to 99% probability.

Variety	Location	Pb	Cu	Cd	Zn	Harvesting method	Harvesting date*
	Foggia	43.3±0.3	26.8±0.9	n.d.	148.7±2.2	unknown	29/10/99
Ogliarola Garganica	"	36.6±0.3	37.5±1.4	n.d.	142.2±1.0	"	12/11/99
"	"	30.3±0.1	20.8±0.3	n.d.	136.6±2.5	"	23/12/99
"		**36.7±6.5**	**28.4±8.5**	n.d.	**142.5±6.0**		
	Bitetto	31.8±0.8	18.9±1.5	n.d.	410.2±6.6	unknown	06/11/99
Ogliarola + Coratina	"	53.6±0.2	13.4±0.3	1.6±0.2	300.9±2.9	"	15/11/99
"	"	41.8±0.3	15.5±0.9	n.d.	144.5±1.1	"	28/11/99
"		**42.4±10.9**	**15.9±2.8**	**0.5±0.1**	**285.2±133.5**		
	Bitetto	40.5±0.9	15.5±0.3	n.d.	163.6±1.3	unknown	07/11/99
Coratina	"	32.5±0.4	11.3±0.4	2.1±0.3	166.6±1.0	"	22/11/99
"		**36.5±5.7**	**13.4±3.0**	**0.7±0.1**	**165.1±2.1**		
"	Copertino	40.5±0.1	16.3±0.6	n.d.	68.5±0.9	unknown	25/11/99
Carolea	"	31.5 ±0.2	14.4±0.3	n.d.	53.9±0.4	"	07/12/99
"		**36.0±6.4**	**15.4±1.4**	n.d.	**61.2±10.3**		
	"	48.0±0.7	29.1±2.1	n.d.	566.7±7.5	unknown	29/11/99
Leccino	"	56.6±0.2	34.1±1.7	n.d.	595.7±6.1	"	09/12/99
"	"	44.2±0.6	23.1±1.1	n.d.	610.1±6.9	"	05/01/00
"		**49.6±6.4**	**28.7±5.5**	n.d.	**590.8±22.1**		
	S. Severo	44.8±0.9	23.6±1.1	n.d.	148.4±1.1	unknown	26/11/99
Paranzana	"	38.2±0.2	32.1±1.3	n.d.	189.3±1.7	"	23/12/99
"	"	55.0±0.5	26.0±1.5	n.d.	133.0±0.9	"	08/01/00
"		**46.0±8.5**	**27.2±4.4**	n.d.	**156.9±29.1**		
	Nardò	21.0±0.3	10.0±0.6	n.d.	176.5±1.9	unknown	28/11/99
Ogliarola Leccese	"	20.6±0.4	17.7±0.9	n.d.	180.8±2.3	"	29/12/99
"	"	26.2±0.1	12.1±1.0	n.d.	221.2±3.1	"	21/01/00
"		**22.6±3.12**	**13.3±4.0**	n.d.	**192.8±24.7**		
	"	15.4±0.1	9.1±0.3	n.d.	187.0±1.4	unknown	28/11/99
Cellina	"	22.4±0.3	8.9±0.3	n.d.	260.2±2.1	"	29/12/99
"	"	16.4±0.2	16.5±1.1	n.d.	293.2±2.7	"	21/01/00
"	"	**18.0±3.8**	**11.5±4.4**	n.d.	**246.8±54.4**		

n.d. = not detectable (<1.2 ng g^{-1}); M±σ = mean ± standard deviation; * day/month/year.

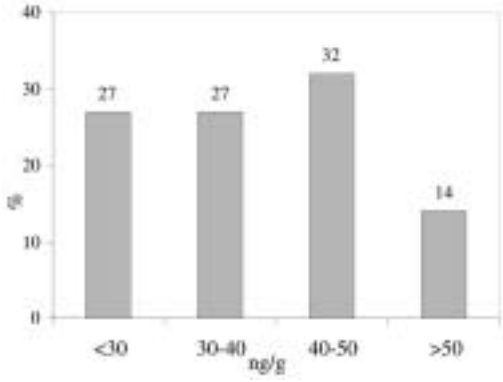


Fig. 3 - Pb concentration groups (%) of twenty-two Apulian olive oils (crop year 1999-2000).

CONCLUSIONS

The proposed method provides a sensitive and convenient procedure for determining copper (II), lead (II), cadmium (II) and zinc (II) levels in olive oils by dPSA. The easy sample preparation, short analysis time and the good sensitivity of the instrument made it possible to analyze a large number of Sicilian and Apulian oil samples.

As a part of a study aimed at improving Sicilian and Apulian oil quality, the Pb (II), Cu (II), Cd (II), and Zn (II) concen-

trations in the olive oils determined by dPSA, were correlated to some aspects of oil production: variety, location, olive picking methods, harvesting and milling date.

To analyze the results, all the samples were grouped on the basis of the crop year, olive variety and place of origin. Cadmium (II) was only present in two oils in very low concentrations. The highest lead (II) and copper (II) concentrations found in the oils were always less than 100 ng g^{-1}. These levels indicate that the oils were of good quality considering the toxicity of lead (II) (U.S. FOOD AND DRUG ADMINISTRATION, 1994) and the ability of copper (II) to catalyse fatty acid chain oxidation, thus favouring rancidity (CODEX ALIMENTARIUS COMMISSION, 1982).

Olive harvesting dates did not significantly influence the metal concentrations in the oils studied.

The possible relationship between harvesting techniques and metal concentrations was examined by analysing oil samples of the same variety, produced from olives collected manually and oils from olives mechanically harvested on the same day. Harvesting techniques did not influence the copper (II) and zinc (II) content in the oils studied, whereas the lead (II) concentration seemed to be significantly higher in Sicilian oils produced from olives picked by mechanical means, in both crop years 1999-2000, and 2000-2001.

The irregularity of the data about copper, lead and zinc concentrations suggests that metal concentrations in oils depend on several factors. These elements may originate from the soil or fertilisers and are transferred into the oily matrix (CERUTTI, 1993). They may be introduced in the production process (DE FELICE *et al.*, 1979), during the processing steps such as bleaching, hardening, refining and deodorizing or by contact with metal processing equipment. Other factors that may influence the presence of metals in oil are availability of water, rainfall, use of irrigation (LA PERA *et al.*, 2001), pesticide treatments (CERUTTI, 1993; PAOLETTI *et al.*, 1999) and pollution (PAOLETTI *et al.*, 1999).

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PAPER

MEAT QUALITY OF THREE CHICKEN GENOTYPES REARED ACCORDING TO THE ORGANIC SYSTEM

QUALITÀ DELLA CARNE DI POLLO DI TRE GENOTIPI ALLEVATI SECONDO IL METODO BIOLOGICO

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ABSTRACT

The meat quality of three poultry genotypes with differing growth rates (fast-growing Ross; medium and slow-growing Kabir and Robusta maculata, respectively) was compared. All the birds were reared according to the organic production system which requires a paddock with grass pasture (4 m^2/bird) and a slaughter age greater than 81 d. The trial was carried out on 100 female chickens per strain. The meat quality was affected by the different degree of maturity of the strains at slaughter age, which was 70% for Ross, 52% for Kabir and 78% for Robusta maculata. Ross and Kabir were slaughtered at 81 d, whereas Robusta maculata, required 120 d to reach a commercial weight (>2 kg). The meat of all the three genotypes showed good qualitative traits. The main differences of the three genotypes

RIASSUNTO

È stata confrontata la qualità della carne di tre genotipi di pollo aventi una diversa capacità di crescita (Ross a crescita rapida, Kabir e Robusta maculata a crescita media e lenta, rispettivamente). I polli sono stati allevati con il metodo biologico che richiede la presenza di un paddock esterno inerbito (4 m^2/capo) ed un'età di macellazione non inferiore ad 81 d. La prova è stata eseguita su 100 femmine di ciascun tipo genetico. La qualità della carne ha risentito del diverso grado di maturità all'età di macellazione che è stato del 70% per il Ross, 52% per il Kabir e 78% per la Robusta maculata. Quest'ultima rispetto agli altri due, macellati a 81 d, ha richiesto 120 d per raggiungere un peso commerciale (>2 kg). Le caratteristiche qualitative della carne di tutti i genotipi sono state buone. Le differenze più rilevanti

- Key words: broilers, meat quality, organic production, strain -

regarded moisture, lipid, pH_u, colour, iron, oxidative stability and overall acceptance. Compared with Kabir and Robusta maculata, Ross meat had more fat, lower pH_u and iron, and was paler. The oxidative stability during display (24-96 h at 4°C) and acceptance were the worst. Kabir chickens, being the least mature strain, had the highest moisture content with a high cooking loss. The slower-growing genotypes showed a good adaptation to the extensive rearing conditions, while the fast-growing genotype showed unbalanced muscle response to the greater activity and the oxidative stability of the meat was reduced.

hanno riguardato il contenuto di acqua, di grassi e di ferro, il pH_u, il colore, la stabilità ossidativa e la gradevolezza. La carne dei polli Ross, rispetto a quella dei Kabir e dei Robusta maculata, è risultata più grassa, con pH e ferro più basso, più pallida, meno gradevole; ha inoltre presentato una minore stabilità ossidativa durante la conservazione (24-96 ore a 4°C). I polli Kabir, stante la loro minor maturità, hanno fornito carni più ricche di acqua e con maggiori perdite di cottura. I genotipi meno produttivi hanno mostrato un buon adattamento alle condizioni estensive di allevamento mentre i polli Ross hanno risposto meno bene alla maggior disponibilità di spazio che ha contribuito a diminuire la stabilità ossidativa della carne.

INTRODUCTION

Organic livestock farming is defined by basic guidelines (EC, 1999) which came into effect in August 2000 and that guarantee high standards for animal welfare and sanitary aspects of the products.

As far as the qualitative characteristics of the carcass and meat are concerned, there is a controversy whether the organic system results in better production than conventional ones due to the numerous factors involved. Among these factors the strain plays an important role. According to the guidelines, more rustic strains are preferred due to their slower growth rate and better adaptation to poorer living conditions. It is also thought (RAUW *et al.*, 1998) that animals genetically selected for high production efficiency are more at risk due to behavioural, physio-biological and immunological problems. For these reasons geneticists have selected strains with the desired traits. One such strain is the Kabir hybrid, selected in Israel, whose positive characteristics are resistance to environmental stress and disease and good adaptation to poor diets (ZVI KATZ, 1995). Many pure breeds also have a slow growth and good rusticity, making them suitable for organic production; another advantage for using them would be to avoid the risk of them going extinct. Robusta maculata, obtained during the 1950s at an experimental station in northern Italy, is rustic and has dual qualities, egg laying (140-160 eggs/year) and meat production (about 4 kg adult body weight).

Few studies exist on the response of these genotypes to the organic production system. LEWIS *et al.* (1997) and FARMER *et al.* (1997) compared data on a slow-growing type (used in France for producing the Label Rouge) and a commercial hybrid (Ross), reared under extensive conditions that can be considered comparable to an organic system. GRASHORN (1999) investigated the meat quality of commercial and slow-growing strains reared in intensive, semi-extensive and extensive systems.

The aim of this paper was to compare the quality of the meat obtained from three different genotypes raised under the organic production system.

MATERIALS AND METHODS

Animals, housing and feeding

Three-hundred female chickens of Ross, Kabir and Robusta maculata strains were reared separately (3 groups of 100 birds each) under brooder lamps for three weeks at the experimental farm of the Animal Production Department (University of Perugia). The environmental temperature ranged from 15° to 25°C and the relative humidity ranged from 65 to 75%. At 21 days-of-age each group of chickens was allocated to its own covered shelter (10 m^2, 0.10 m^2/bird) with straw litter and access to a grass paddock (4 m^2/bird); feeders were available both outdoors and indoors. The trial was carried out from April to July 2001.

The chickens were fed *ad libitum* the same starter (1-21 d) and finisher (22 d to slaughter) diets containing more than 80% certified organic ingredients, bought from a national supplier. The ingredients were ground and mixed in the feed plant of the Animal Production Department. The characteristics of the diets are presented in Table 1.

Ross and Kabir chickens were reared for 81 days, which is the minimum specified according to EC regulations, whereas Robusta maculata was reared for 120 days to reach commercial weight (>2,000 g). The degree of maturity of each strain is expressed as the ratio between the weight at different ages and the adult weight.

Twenty birds per group, each weighing ±10% of the population mean, were

Table 1 - Formulation (%), chemical composition (% d.m.) and energetic value of the diets.

Ingredients		Starter	Finisher
Maize	%	52.0	46.0
Full fat soybean	"	30.5	12.5
Wheat	"	-	20.0
Soybean meal*	"	9.0	14.0
Alfalfa meal	"	2.8	2.8
Fish meal	"	3.0	2.0
Vitamin-mineral premix**	"	1.0	1.0
Dicalcium phosphate		1.0	1.0
Sodium bicarbonate		0.5	0.5
NaCl		0.2	0.2
Chemical composition			
Crude protein	% d.m.	22.65	19.10
Ether extract	"	7.95	4.98
Crude fibre	"	4.67	4.01
Ash	"	5.76	5.59
NDF – Neutral Detergent Fibre	"	10.74	10.11
ADF – Acid Detergent Fibre	"	5.58	4.67
Cellulose	"	4.22	3.56
ADL – Acid Detergent Lignin	"	1.03	1.11
Hemicellulose	"	5.16	5.05
Metabolizable energy	MJ kg^{-1}	12.75	13.03

* from conventional crops.

** Added per kg: Vit. A 11.000 IU; Vit. D_3 2.000 IU; Vit. B_1 2.5 mg; Vit. B_2 4 mg; Vit. B_6 1.25 mg; Vit. B_{12} 0.01 mg; α-tocopheryl acetate 50 mg; Biotin 0.06 mg; Vit. K 2.5 mg; Niacine 15 mg; Folic acid 0.30 mg; Panthotenic acid 10 mg; Choline 600 mg; Mn 60 mg; Fe 50 mg; Zn 15 mg; I 0.5 mg; Co 0.5 mg.

slaughtered in the department processing plant 12 hours after feed withdrawal. Broilers were not transported and were electrically stunned before killing.

Sample collection and analytical determinations

The birds were killed by manual exsanguination, plucked and eviscerated. Immediately before slaughter, blood samples were collected in heparinized vacutainers and centrifuged at 1,500 x g for 10 min at 4°C, in order to measure the antioxidant capacity using the Oxy-adsorbent test produced by Diacron s.r.l. (Italy) (CESARONE *et al.*, 1999).

An aliquot of blood plasma was analysed for α-tocopherol determination. The α-tocopherol was measured according to SCHUEP and RETTENMEIER (1994) by adding 500 µL of distilled water and 1 mL ethanol to 500 µL of sample and then vortexing for 10 sec. Successively, 0.2 mL hexane and butylhydroxytoluene (0.01%) were added and the mixture was carefully shaken and centrifuged. An aliquot of supernatant (0.8 mL) was taken and injected into the HPLC (CM 4000, Milton Roy, Riviera Beach, FL, USA), using a silica column (Beckman, Fullerton, CA, USA). Fluorescence detection was performed with a spectrofluorimeter (excitation and emission wavelength of 292 nm and 330 nm, respectively).

From the refrigerated carcasses (24 hours at 4°C), the *pectoralis major* (breast) and *peroneous longus* (drumstick) muscles were excised and the skin and external fat were removed. Muscle samples (approximately 50 g) were taken immediately for sensory and analytical determinations, while others were taken during storage to quantify the oxidative processes.

Ultimate pH (pH_u) was measured with a Knick digital pHmeter (Broadly Corp., Santa Ana, CA, USA) after homogenization of 1 g of raw muscle for 30 sec in 10 mL of 5 M iodoacetate (KORKEALA *et al.*, 1986).

The water-holding capacity (WHC) was estimated by placing 1 g of whole muscle on tissue paper inside a tube and centrifuging for 4 min at 1,500 x g. The water remaining after centrifugation was quantified by drying the samples at 70°C overnight. WHC was calculated as follows: (weight after centrifugation - weight after drying) / initial weight x 100 (CASTELLINI *et al.*, 1998).

The cooking loss (CL) was measured on samples of about 20 g placed in open aluminium pans and cooked in an electric oven (pre-heated to 200°C) for 15 min to an internal temperature of 80°C. The CL was estimated as the percentage of the weight of the cooked samples, (cooled for 30 min to about 15°C and dried on the surface with a paper towel), with respect to the weight of the raw samples.

Shear force was evaluated on cores (1.25 cm Ø; 2 cm length) obtained from the mid-portions of the roasted samples by cutting them perpendicularly to the direction of the fibre, using an Instron, model 1011, equipped with a Warner-Blatzler meat shear apparatus.

The colour parameters (L*, a*, b*) were measured using a tristimulus analyser (Minolta Chroma meter CR-200), with the CIELAB colour system (1976).

Chemical analyses of diet and meat were done according to AOAC methods (1995).

The extent of lipid oxidation was evaluated after different storage times (24, 48, 72, 96 h) as TBA-RS (Thiobarbituric Acid Reactive Substances) according to the modified method of KE *et al.* (1977). Samples were placed on plastic trays (600 cm^3), over-wrapped with PVC film and displayed at 4°C under continuous cool white fluorescent light (2,300 lux). Ten grams of minced muscles were homogenised for 2 min with 95.7 mL of distilled water and 2.5 mL of 4 N HCl. The mixture was distilled until 50 mL was obtained. Then, 5 mL of the distillate and 5 mL of TBA reagent (15% trichloroacetic acid, 0.375% thiobarbituric acid) were

heated in a boiling water bath for 35 min. After cooling under running tap water for 10 min, the absorbance was measured at 531 nm against a blank of 1 mL double distilled water and 2 mL TBA/TCA solution. TBA-RS values were obtained by multiplying the optical density by 7.843. The amount of TBA-RS was expressed as malondialdehyde equivalents (mg MDA/kg muscle).

The total and haeme iron content in the muscles were determined according to the SCHRICKER *et al.* (1982) and O'BRIEN *et al.* (1992) procedures, respectively.

The fatty acid composition was determined on lipids extracted from muscle samples (about 5 g) in a homogeniser with 20 mL of 2:1 chloroform:methanol (FOLCH *et al.*, 1957), followed by filtration through Whatman No. 1 filter paper. Fatty acids were quantified as methyl esters with a Mega 2 Carlo Erba gas chromatograph, model HRGC (Milano, Italy), using a D-B wax capillary column (25 mm, 30 m long). Their percentages were quantified with Chrom-Card software and the mean value of each fatty acid was used to calculate total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

Sensory analyses

A sensory panel test was conducted on samples of *pectoralis major* muscle (immediately after its removal), roasted without salt or spices. The roasted samples were immediately sliced into eight pieces and randomly offered to eight trained panelists during four successive sessions. The traits assessed were overall acceptability, initial and final tenderness, initial and final juiciness and fibrousness. The 5-point scale proposed by CROSS *et al.* (1986) was used: 1 = very disagreeable, very tough, very dry, very fibrous; 2 = moderately disagreeable, slightly tough, dry, fibrous; 3 = slightly agreeable, tender, slightly juicy, moderately fibrous; 4 = agreeable, very tender, juicy, traces of fibrousness; 5 = very agreeable, extremely tender, very juicy, without fibre.

Statistical analyses

Data were analysed with a linear model (SAS/STAT, 1990 – procedure GLM) including the effect of breed. Significance of differences was evaluated by the multiple t-test.

RESULTS AND DISCUSSION

Live weight and degree of maturity

The genotypes showed very different precocities, as indicated by the large differences in the absolute live weight at a fixed age, and the time needed to attain the same degree of maturity (Fig. 1). e.g. the slow-growing chickens, Kabir and Robusta maculata, needed about 75 d and 82 d, respectively, to reach 50% of the final body weight, whereas the fast-growing Ross chicken needed only 56 d.

Ross chickens had a very fast growth rate even though increased locomotion (low animal density) and reduced nutritional quality of the feed (intake of grass pasture) in the organic rearing system reduced their growth potential. CASTELLINI *et al.* (2002b) showed that birds having access to pasture eat some grass which increases fibre intake and reduces digestibility of other nutrients.

At 81 days Ross birds weighed 2,942 g, which is about 70% of the degree of maturity. At the same age Kabir chickens had a live weight of only 2,031 g (52% of the degree of maturity), whereas Robusta maculata needed 120 d to achieve a marketable weight (>2,000 g) which at this age was 2,185 g or 78% of the degree of maturity.

These productive performances are the result of the selection programs used to obtain the different strains. The Ross hybrid underwent strong selection pressure to reduce the rearing time and

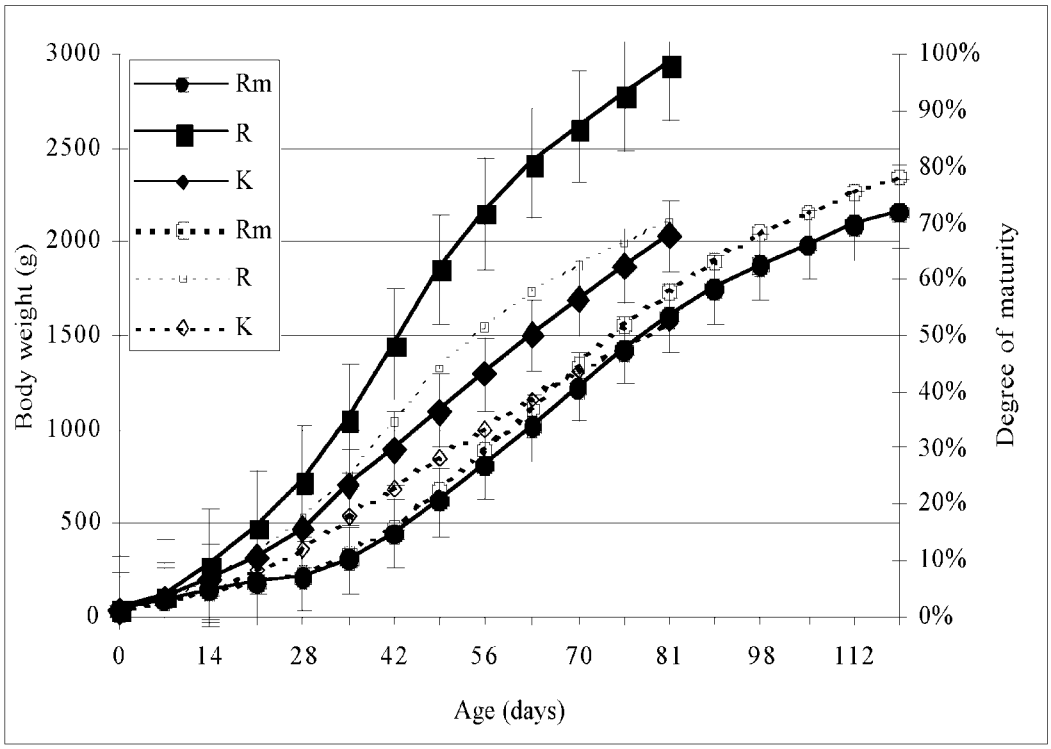


Fig. 1 - Body weights of birds (95% upper and lower limits) and degree of maturity.
Solid line = body weight; dotted line = actual body weight/adult weight. R = Ross; Rm = Robusta maculata; K= Kabir.

therefore the cost of production. Kabir was selected to produce even under poor conditions (environment, rough diet, etc.), and a slower-growing genotype was obtained. Robusta maculata, a slow-growing breed, was not particularly selected for growing performance, but also for having a good aptitude for egg production (ARDUIN *et al.*, 1998).

The feed efficiency was 3.0, 3.3 and 3.9 and the mortality rate was 12%, 9% and 4%, respectively, in Ross, Kabir and Robusta maculata chickens (data not shown).

Chemical and physical characteristics of the breast and drumstick muscles

The different degrees of maturity of the three genotypes accounts in part, for the different characteristics of the two muscles analysed (Tables 2 and 3).

As expected from the low degree of maturity (52%), Kabir chickens had the least mature meat, the lowest protein and the highest moisture content. Presumably, this high water content negatively affected both the water holding capacity and the cooking loss of the meat.

In spite of the differences in age and degree of maturity, the meat of Kabir and Robusta maculata was leaner than that of the fast-growing strain and muscle fibres had similar metabolisms (> pH_u) in agreement with PLAVNIK and HURWITZ (1983). The higher pH_u of Kabir and Robusta maculata chickens did not significantly affect tenderness, in agreement with HULOT and OUHAYOUN (1999).

Table 2 - Chemical-physical characteristics of breast and drumstick

Strain		Ross	Kabir	Robusta maculata	SE
Breast					
Moisture	%	75.73^{a}	77.18^{b}	76.01^{a}	1.82
Protein	"	22.76^{b}	21.51^{a}	22.71^{b}	1.52
Fat	"	0.81c	0.67^{b}	0.49^{a}	0.19
Ash	"	0.70	0.64	0.79	0.74
Gross energy	MJ kg d.m.$^{-1}$	20.98^{b}	20.05^{a}	20.59^{ab}	1.42
pH_u		5.75^{a}	5.84^{b}	5.82^{b}	0.10
Water holding capacity	%	53.49^{b}	46.34^{a}	53.65^{b}	9.23
Cooking loss	"	33.32^{a}	37.07^{b}	33.28^{a}	5.80
Shear force	kg/cm^2	2.69	2.56	2.66	0.96
Drumstick					
Moisture	%	76.89^{a}	78.19^{b}	77.19^{a}	1.48
Protein	"	19.28^{b}	18.34^{a}	19.32^{b}	1.40
Fat	"	2.99^{b}	2.64^{a}	2.49^{a}	0.35
Ash	"	0.84	0.83	1.00	0.83
Gross energy	MJ kg d.m^{-1}	22.31^{c}	21.32^{ab}	21.98^{bc}	1.26
pH_u		6.03^{a}	6.14^{b}	6.14^{b}	0.15
Water holding capacity	%	57.45^{b}	48.68^{a}	57.08^{b}	10.32
Cooking loss	"	34.02^{a}	39.65^{b}	34.23^{a}	6.65
Shear force	kg/cm^2	3.12	2.96	3.07	0.83

Values in the same row followed by different superscript letters differ at P<0.05.

Table 3 - Antioxidant capacity, α-tocopherol, colour, amount of iron in breast and drumstick muscles.

Strain		Ross	Kabir	Robusta maculata	SE
Antioxidant capacity	ηmol HClO/mL^{-1}	528^{A}	750^{B}	725^{B}	84
Serum α-tocopherol	mg L^{-1}	17.2^{a}	18.3^{b}	19.0^{b}	1.7
Breast					
L*		60.03^{c}	51.68^{a}	57.67^{b}	3.76
a*		4.71^{a}	5.75^{b}	5.71^{b}	1.45
b*		5.58^{a}	4.94^{a}	7.69^{b}	2.45
Fe total	mg kg^{-1}	4.15^{A}	6.54^{B}	6.45^{B}	2.56
Fe haeme	"	1.89^{A}	2.94^{B}	2.90^{B}	1.03
Drumstick					
L*		54.63^{bc}	47.13^{a}	52.71^{b}	3.72
a*		5.98^{A}	14.46^{B}	13.86^{B}	8.96
b*		5.29^{a}	4.55^{a}	6.73^{b}	2.05
Fe total	mg kg^{-1}	6.93^{A}	9.62^{B}	9.48^{B}	2.57
Fe haeme	"	3.12^{A}	4.32^{B}	4.26^{B}	1.29

Values in the same row followed by different superscript letters differ at P<0.01 and P<0.05.

It is known, that rapid growth enhances late maturing tissues (i.e. fat) and that a higher body weight is associated with increased glycolytic energy metabolism and with higher acidification (OUHAYOUN, 1998). OUHAYOUN and DALLE ZOTTE (1993) affirmed that the ultimate pH of the muscles is lower in heavy rabbits, but greater acidification does not lead to an increase in cooking loss. We also observed an analogous trend, in the three chicken strains used in this study.

However, the higher pH_u can also be correlated with greater movement which improves oxidative metabolism and increases the number of mitochondria in α–white fibres and hence turns them into α– red fibres (OUHAYOUN and DALLE ZOTTE, 1993).

The similar degree of maturity of Robusta maculata and Ross (78 and 70%, respectively), reduced the genetic differences for many traits, including moisture and protein content, water-holding capacity and cooking loss.

Shear force did not differ between strains and each of them had very tender meat.

The three genotypes showed differences in the colour parameters and iron levels of both muscles (Table 3). The Ross meat was lighter and less red due to the lower pH value. Muscle pH has been shown to affect both the light reflectance and the chemical reactions of myoglobin (RICHARDSON and MEAD, 1999; WARRIS, 2000).

The low level of iron and redness of the Ross meat was similar to that reported by BERRI *et al.* (2001), in lines selected for rapid growth and large breast yield, which had a lower haeme pigment content in the paler, less red breast meat. On the other hand LE BIHAN-DUVAN *et al.* (1998) found high heritability coefficients (h^2) between pH and colour parameters, and after selecting for rapid growth rate (13 generations), obtained less red and yellow meats.

In vivo antioxidant capacity and oxidative processes in *pectoralis major* muscle

The Ross chickens had the lowest antioxidant capacity ($P<0.01$), whereas the other two strains had similar values (Table 3). The alpha-tocopherol level was analogous for the 3 strains. Such a condition affected oxidative processes in fresh and stored breast. In spite of the lower amount of iron, the TBA-RS values (Fig. 2) were always higher ($P<0.01$) in Ross meat, reaching a final value of 4.40 ($P<0.01$), whereas in Kabir and Robusta maculata the final values were 3.35 and 3.10, respectively.

The better oxidative status of these two strains is also due to greater grass ingestion as observed by CASTELLINI *et al.* (2002a) and to the different response of the muscle fibres to activity. The selection for rapid growth and muscle mass (mainly breast) induces a higher proportion of glycolytic fibres (DRANSFIELD and SOSNICKI, 1999). Clearly, these genotypes do not adapt to a greater space allowance and are not suited for continuous movement.

The selection of meat-type animals has caused a dramatic reduction of their locomotory activity, particularly during the finishing period (BIZERAY *et al.*, 2000). At the same time, rearing under intensive housing systems, which provides all the physiological needs (feed, water, temperature), further reduces movement (REITER and BESSEI, 1996). In a previous work, CASTELLINI *et al.* (2002b) observed that Ross, in comparison to Robusta maculata, spent less time walking and preferred to stay indoors rather than outdoors.

The higher oxidative metabolism of slow-growing chickens is magnified under conditions that favour motor activity. This accounts for the higher *in vivo* antioxidant capacity of Kabir and Robusta maculata, because higher oxidative metabolism increases free-radical pro-

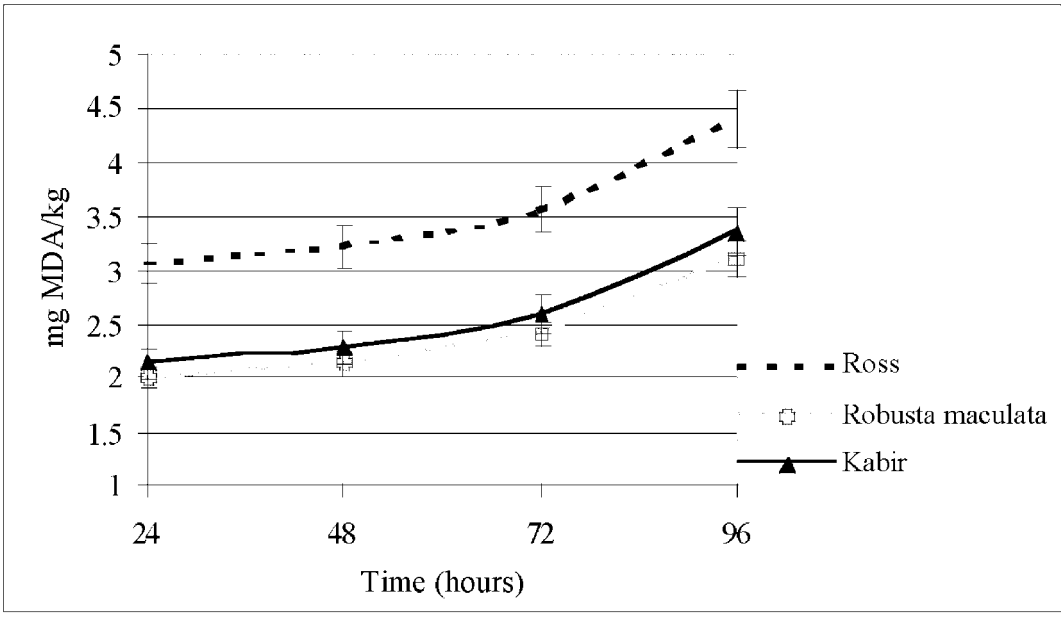


Fig. 2 - TBA-RS in *pectoralis major* during 96 h of display (95% upper and lower limits).

duction and the body develops a more efficient mechanism to control the free radicals (ALESSIO *et al.*, 2000), and to protect myocytes against oxidants (POWERS and LEEUWENBURGH, 1999). In turn, the lower levels of oxidants in Kabir and Robusta maculata muscle reduced the oxidative processes in the meat after 24 h or 96 h of storage.

Compared with the commercial meat broiler, all three genotypes showed higher TBA-RS values (TBA-RS about 1.8: CASTELLINI *et al.*, 2002a) which can be due to higher oxidative metabolism and to the increase of Fe. During storage, cell deterioration frees Fe ions which catalyse lipid peroxidation (STANLEY, 1991).

The above-mentioned trend of oxidative processes reduces the shelf life of meat produced by chickens reared under an organic system, suggesting that it is best to consume the whole carcass instead of its parts or as minced meat. It is known that handling, increase of temperature, light and metal ions, which can be released from cutting blades during processing, increase the free radical production and affect peroxide processes (MORRISSEY *et al.*, 1998).

Fatty acid profile of breast and drumstick muscles

The major fatty acid percentages in breast and drumstick were not affected by strains (Table 4) and the values indicate that suitable dietary strategies are needed to modify the acidic profile of the meat. The data are in agreement with those reported by O'KEEFE *et al.* (1995) and confirm the poor deposition of EPA compared with DHA in chicken (RICHARDSON and MEAD, 1999).

Sensory analyses

The panelists (Fig. 3) showed an overall preference ($P<0.05$) for Robusta maculata and Kabir, in comparison to Ross.

Table 4 - Percentage of major fatty acids in breast and drumstick muscles.

Strain	Ross	Kabir	Robusta maculata	SE
Breast				
∑ SFA	38.05	38.62	38.77	3.12
∑ MUFA	29.41	29.15	29.22	3.25
∑ PUFA	32.54	32.23	32.01	2.95
C20:5(n-3) EPA	0.57	0.53	0.54	0.22
C22:6(n-3) DHA	1.92	1.83	1.89	0.81
∑ (n-3)	5.16	5.11	5.08	0.51
Drumstick				
∑ SFA	36.43	37.01	36.54	2.78
∑ MUFA	31.73	31.96	31.79	3.20
∑ PUFA	31.84	31.03	31.67	2.86
C20:5(n-3) EPA	0.36	0.34	0.35	0.18
C22:6(n3) DHA	1.26	1.20	1.23	0.72
∑ (n-3)	4.81	4.69	4.78	0.45

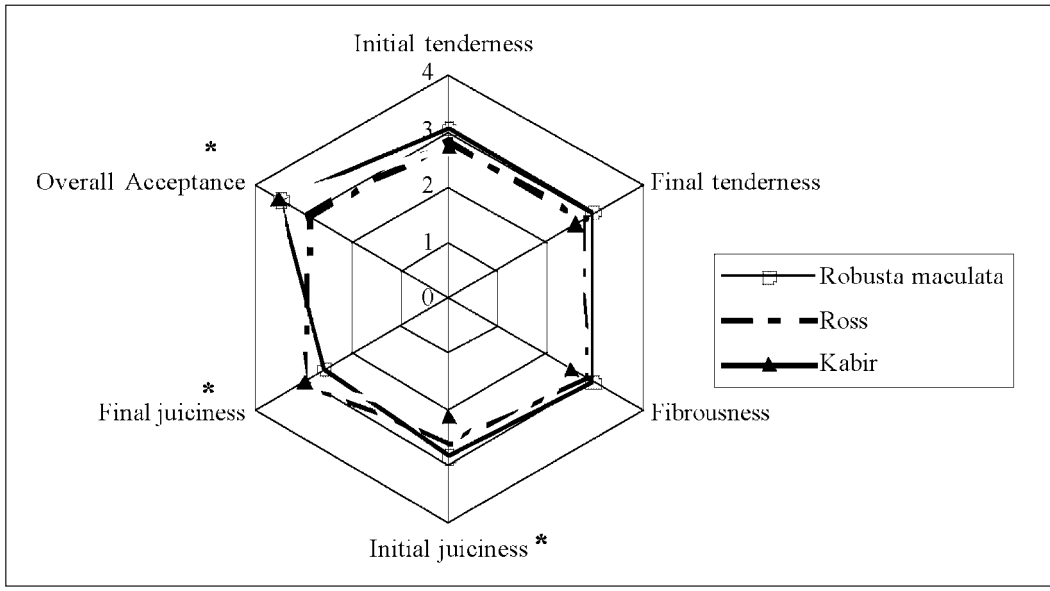


Fig. 3 - Sensory analysis of breast muscle.

Significant differences were also observed for juiciness: Kabir had the lowest value for initial juiciness, presumably due to the high cooking loss that reduced the moisture content; for final juiciness Robusta maculata had the lowest value in relation to the low level of fat in its meat. It is known (CROSS *et al.*, 1978) that initial juiciness is affected by water being freed by mastication, while final juiciness depends on the lipids that promote salivation.

The capacity of a panel to discriminate meat from slow- *vs.* fast-growing geno-

types is largely debated (RICHARDSON and MEAD, 1999). Several authors have reported significant differences but the effect of genotype often interacts with age: e.g. slow-growing chickens require a longer rearing time. FARMER *et al.* (1997), who reported a significant discrimination of meats from various poultry genotypes, included the interaction of age-genotype.

CONCLUSIONS

Robusta maculata and Kabir genotypes reared under an organic production system expressed their natural behavioural patterns, showing good motor activity and good adaptation to poor environmental conditions. Their antioxidant capacity was higher and the oxidative processes of the meat were lower compared with Ross. The sensorial quality of the meat was good.

The growth rate of Robusta maculata was slow; it reached commercial maturity only at 120 d of age while Kabir chickens reached a marketable weight at 81d, but their low degree of maturity negatively affected the moisture and protein contents of the meat and cooking loss.

Ross chickens showed a high growth potential and gave meat with good nutritional quality, except for a low iron content. The mortality rate was high and the oxidative stability of the meat was low, presumably due to less motor activity and a lower intake of grass (containing antioxidants). These factors probably contributed to the lower overall acceptance during the panel test.

Robusta maculata and other slow-growing breeds in danger of extinction should be considered for extensive production in order to assure their preservation. However this strain requires a fattening period that is too long, even if the rearing of both sexes could be more advantageous. It would be advisable to use a cross between females of this breed with males of a heavier genotype, in order to improve the growth rate.

Overall the results show that the environment-genotype interaction is also very important for the qualitative characteristics of the meat; differences between fast- or slow-growing chickens could also be a consequence of different behaviour and muscle metabolism. More detailed study on the muscle response of different genotypes to motor activity is required.

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PAPER

INFLUENCE OF THE TYPE OF DEHYDRATION PROCESS ON THE SENSORY PROPERTIES OF SAFFRON SPICE

INFLUENZA DEL PROCESSO DI DISIDRATAZIONE SULLE PROPRIETÀ ORGANOLETTICHE DELLO ZAFFERANO

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ABSTRACT

Instrumental and sensory quality of saffron samples obtained by different dehydration processes were compared. The chromatic parameters, a* and C*, and the colouring strength (ISO 3632 trade standard) were able to discriminate the dehydration method but not its temperature. Two sensory analyses were carried out: an affective test (102 consumer judges) which stressed the importance of the colour and aroma of hot-air dehydrated samples, and a discriminatory test (15 expert judges),

RIASSUNTO

In questo lavoro sono state confrontate le qualità strumentali e sensoriali di diversi campioni di zafferano ottenuti tramite differenti processi di disidratazione. I parametri cromatici, a* e C*, e la capacità colorante (normativa ISO 3632) permettono di differenziare tra i processi di disidratazione ma non tra le temperature di disidratazione ottenute con aria calda. Sono state eseguite due analisi sensoriali: in una prova (utilizzando 102 consumatori come giudici) è stata sottolineata l'importanza

- Key words: aroma, bitterness, colour, saffron, sensory analysis -

which differentiated samples by their colour, but not by their aroma. Samples dehydrated at room temperature were rated the worst for all of their sensory characteristics.

del colore e dell'aroma nei campioni disidratati mediante aria calda; in una prova discriminatoria (utilizzando 15 esperti come giudici) era sottolineata la differenza tra i campioni in base al loro colore e non in funzione del loro aroma. I campioni disidratati a temperatura ambiente si sono dimostrati i peggiori per tutte le caratteristiche organolettiche.

INTRODUCTION

Saffron (*Crocus sativus* Linnaeus) is an herbaceous and perennial plant. As a bulb, it is capable of surviving the dry summer season by keeping its young shoot underground. Saffron flowers contain a stigma made up of three filaments which, when dried, constitute the spice, known as saffron, widely appreciated throughout history for its flavour, aroma and mainly for its colouring properties. It is currently used in households and the food industry, but there could be novel applications in the future, since a number of compounds isolated from raw extracts have been demonstrated to be active against the development of "in vitro" cancer cells (ESCRIBANO *et al.*, 1996; FERNÁNDEZ and ESCRIBANO, 2000).

The main saffron producing countries are Iran, Spain, Greece, India and Morocco. Several areas of Italy (Toscana, Abbruzo, Sardinia) are also traditional saffron producers (CASTELLANI *et al.*, 1989). In Spain, this crop is of great social and economic importance in areas such as La Mancha (MAPA, 1996) where the first protected designation of origin (PDO), namely "Azafrán de la Mancha", was obtained (DOCM, 1999; OJEC, 2000).

The production of saffron spice is extremely laborious, which explains its high price on the market. There are many factors such as climate, soil conditions, harvesting, removal of the stigma or the dehydration process that may have some influence on saffron quality (PÉREZ, 1995; SÁNCHEZ, 1997). It is believed that the dehydration process has the greatest effect on the final sensory properties (CASTELLANI *et al.*, 1989). In this process, fresh stigma lose at least 80% of their weight (INGRAM, 1969; ALONSO *et al.*, 1998).

In Spain and Italy, the dehydration process is called "toasting", and saffron stigma are dehydrated at high temperatures using different heating sources. First the stigma are spread over a metallic or silk sieve, which is then put on a gas cooker, live coals or an electrical coil (ALONSO *et al.*, 1998; CASTELLANI *et al.*, 1989). Another method consists of spreading the stigma over large areas and drying them at room temperature in the sun-light or in air-ventilated conditions. Such a process is known as "sweating" and is generally done in Iran.

The trade standard that regulates the national and international saffron trade is the ISO 3632 (1993). Parameters which determine saffron quality are microscopic analysis, presence of flower residues, humidity, ash, and, most importantly, its bitterness, aroma (safranal) and colouring strength, all of which are measured by UV-Vis spectrophotometry at 257, 330 and 440 nm, respectively. The main substance responsible for bitterness is picrocrocin, while safranal

gives it its characteristic aroma, and crocins its colour (CORRADI and MICHELLI, 1979).

Up to the present, analytical techniques have been used to determine the main quality parameters, while sensory evaluation (colour, aroma and bitter taste) is considered only vaguely, thus neglecting consumer preference or their capacity to discriminate among samples.

In this paper, the influence of different dehydration methods ("sweated", "toasted" and hot-air) was studied in terms of instrumental parameters (colouring strength, aroma and bitterness as ISO 3632 trade standard and colour by tristimulus colorimetry). A sensory analysis evaluated by affective and discriminatory tests on different saffron samples was also carried out.

MATERIALS AND METHODS

Plant material and dehydration process

Harvesting and removal of the stigma from saffron flowers (*Crocus sativus* L.) took place on the 30th of October, 2001 in Motilla del Palancar (Cuenca, Spain), by the traditional procedures following the trade standard of the PDO "Azafrán de la Mancha" (DOCM, 1999; OJEC, 2000).

The stigma drying process was performed by three different methods: (1) "sweating" at room temperature (DRT): saffron stigma were dehydrated at room temperature for 72 hours reaching a final moisture content of 7.3%; (2) "toasting": stigma were placed on a sieve, over an electric stove at constant temperature of 57°C for 30 min, reaching a final moisture content of 7.7%; and (3) "drying by hot air" (DHA): stigma were put on a cylindrical sample holder (9.2 cm diameter) with a perforated bottom, while air at different temperatures (70°, 90° and 110°C) was passed through at 6 m s^{-1} for 240, 180 and 120 s, respectively. The moisture content of dry stigma was 7.3% (70°C), 5.6% (90°C) and 4.4% (110°C).

Analytical determinations

Ten measurements were made on each saffron sample. The ISO 3632 (1993) trade standard, established that the colouring, aromatic and bitter strength is a measurement of the specific extinction of a 1% aqueous extract of saffron at 440, 330 and 257 nm, respectively, with a 1 cm thick cell.

The apparent colour was also measured by a colorimetric reflection method using a Minolta CR-300 colorimeter (Minolta Camera Co., Ltd., Osaka 541, Japan). The dehydrated saffron stigma were placed in a special attachment of the colorimeter (CR-A50, Minolta), consisting of a cylindrical plate with a glass dish at the top. The mean value of the readings was taken as the overall value for each case. The tristimulus values obtained were used to calculate the CIEL*a*b* chromatic coordinates: L* (luminosity), a* (red-green component) and b* (yellow-blue component), and the chromatic attributes: C* (chroma) and H* (hue angle), with reference to D65 illuminant and an angle of vision of 2° (CIE, 1986).

Sensory evaluation

The test chosen for assessing the degree of satisfaction was a verbal hedonic scale that is included within the affective tests. Judges were 102 consumers who judged the colour, aroma and flavour properties of different saffron samples. A value along a scale of nine points was assigned to each hedonic description (-4 = dislike extremely, 0 = neither like nor dislike, 4 = like extremely) (PERYAM and GIRARDOT, 1952). Each parameter was analysed separately from the others. The colour and aroma measurements were analysed on saffron filaments, while for flavour characteristics, a solution of 250

mg of saffron/L was used. The tasting sessions were carried out in several supermarkets in the city of Albacete (Spain). A stand was placed in a quiet, well illuminated area within the supermarket, away from strong smells and noise. The tasters represented a variety of ages (18-75 years) and living standards (householders, students, retired persons, professionals, etc.). The only prerequisite was that the person chosen was a usual consumer of saffron.

The discriminatory test was carried out by 15 trained judges (ANZALDÚA, 1994) who ranked the samples by red tone intensity, brightness, aroma and bitter taste, without using any scale. Later, a number was assigned to each sample from 1 (least valued) to 5 (most valued) and the scores for all judges were summed up. The colour and aroma measurements were analysed on saffron filaments, while for bitterness, a solution of 250 mg of saffron/ L was tested. This analysis took place in the tasting room of the Escuela Técnica Superior de Ingenieros Agrónomos (ETSIA) in Albacete. The room was equipped with 12 individual boxes, where the samples were provided, together with the questionnaire and a glass of water for rinsing the mouth between samples.

Statistical analysis

Data were analysed using the SPSS 10.0.6 for Windows (SPSS Inc.) statistical program. Significance of differences among means were determined by one way ANOVA, using a Duncan´s test. To interpret the results in the discriminatory assays, the total range table was used (LARMOND, 1977).

RESULTS AND DISCUSSION

Saffron quality is certified by using the international trade standard ISO 3632 (ISO, 1993). The established values of this trade standard mainly refer to its colouring and aromatic strength as well as to its bitterness, as shown in Table 1. The values obtained after submitting saffron to different dehydration processes are also shown in Table 1. The small differences found between sample moisture (7.3-4.4%) did not affect its colouring strength measurements nor its chromatic co-ordinates (ALONSO *et al.*, 1990).

Colouring strength is the most important parameter in all commercial trade operations. In all cases, the samples studied had a colouring strength higher than 190 units and therefore were included in Category I, according to the ISO trade standard. The DRT samples had poorer colouring strength than DHA samples, which also had less than the toasted ones (Table 1). There was a difference of 27 units between samples, which was comparable to the differences between categories (30-40). Therefore, by choosing an appropriate dehydration method, a batch of saffron could be included in a higher category, resulting in important economic benefits due to its high price on the world market.

The aromatic strength (safranal) is a parameter with less importance in determining saffron quality. For this reason it is not used in classifying, even though saffron needs to have an average value between 20 and 50 units. All samples studied had a mean value in this range. DRT samples had the highest aromatic value, while DHA samples and “toasted” ones had lower values, without significant differences among them.

As regards bitterness, all samples were included in Category I, as established by the ISO (1993) trade standard (Table 1). Toasted samples having the highest bitterness value were significantly different from DHA and DRT samples, with DRT samples having the lowest. This means that dehydration temperature and type of process could have a great influence on this quality parameter.

Table 1 - Mean values (± standard deviation) for colouring strength, aromatic strength (safranal) and bitterness of the saffron samples and requirements of the ISO (1993) 3632 trade standard.

Samples	Colouring strength[3]	Aromatic strength[4]	Bitterness[5]
DRT[1]	220.46±1.35[a]	29.63±0.71[b]	83.34±1.05[a]
Toasted	247.96±2.92[c]	26.56±1.72[a]	98.40±5.32[c]
DHA[2] at 70°C	225.51±1.75[b]	27.74±1.09[a]	93.42±7.68[b]
DHA[2] at 90°C	226.44±1.23[b]	27.43±0.13[a]	92.85±1.23[b]
DHA[2] at 110°C	227.41±1.17[b]	27.17±0.75[a]	92.20±1.20[b]
Requirements ISO 3632			
Category I	190	20-50	70
Category II	150	20-50	55
Category III	110	20-50	40
Category IV	80	20-50	30

Values followed by different letters within a column are significantly different at $p<0.05$ (Duncan´s test);
(1) DRT: Dehydrated at Room Temperature;
(2) DHA: Dehydrated with Hot Air;
(3) Expressed as direct reading of the absorbance of crocins at about 440 nm, dry basis;
(4) Expressed as direct reading of the absorbance at about 330 nm, dry basis;
(5) Expressed as direct reading of the absorbance of picrocrocin at about 257 nm, dry basis.

All the parameters were considered objective measurements, as all saffron samples were analysed in the same way, but they may not be in accordance with consumer appreciation. It is difficult to define quality, but in the case of saffron, as it is a very valuable spice due to its colour, aroma and flavour, the sample with the highest attributes will have the best quality, even if it does not match with ISO (1993) ranges.

Colour

Table 2 shows the mean values of the chromatic coordinates (L*, a*, b*) and the chromatic attributes (C*, H*) of the saffron samples. The highest values of luminosity (L*) were found in DHA samples, specially those processed at 70°C, which were significantly different from the rest of the samples, except for those dehydrated at 110°C. Note that samples DRT and DHA at 90°C had the lowest luminosity values. This suggests that saffron luminosity does not depend on temperature, at least within the range of our trials. As regards the red-green component (a*), the highest values were found in DHA samples which were significantly different from the rest (Table 2). Note that drying methods could be differentiated with component a* but presumably dehydration temperatures could not be. The lowest values of the yellow-blue component (b*) were for DRT samples, which differed considerably from the rest. The higher values corresponded to the DHA samples dehydrated at 70° and 100°C. Therefore, the b* component could be useful for discriminating DRT samples from the others, but not so much for differentiating between toasted and DHA samples. The chromatic attribute, chrome (C*), behaved like the a* component. The highest values were once again obtained for the DHA samples, regardless of temperature. The hue angle (H*) seemed to be an ambiguous parameter, not useful for distinguishing among samples.

Colouring strength (Table 1) did not correlate well with colorimetric parameters (Table 2). DHA samples had the highest a* and C* values, while toasted samples had the highest colouring

Table 2 - Mean values (± standard deviation) of the chromatic coordinates (L*, a* and b*) and chromatic attributes (C*, H*) of the saffron samples.

Samples	L*	a*	b*	C*	H*
DRT	24.06±0.81[a]	15.19±1.08[a]	2.95±0.84[a]	15.48±1.18[a]	10.78±2.54[a]
Toasted	24.87±2.03[ab]	16.62±1.25[b]	4.10±2.13[b]	17.20±1.76[b]	13.28±5.62[ab]
DHA at 70°C	25.75±1.00[c]	18.94±0.52[c]	5.38±1.19[c]	19.72±0.34[c]	15.80±3.66[b]
DHA at 90°C	24.78±1.57[ab]	18.48±0.96[c]	4.17±1.65[b]	18.99±1.31[c]	12.39±4.07[a]
DHA at 110°C	25.36±0.80[bc]	18.96±0.60[c]	5.28±0.96[c]	19.70±0.63[c]	15.47±2.69[b]

*DRT: "dehydrated at room temperature"; DHA "dehydrated with hot-air";
Values followed by different letters within a column are significantly different at p<0.05 (Duncan´s test).

strength. Nevertheless, DRT samples had the lowest colouring strength and colorimetric values.

Table 3 shows the mean values of the sensory parameters evaluated to assess the degree of satisfaction using a verbal hedonic scale. Note that when colour was evaluated, the judges preferred DHA samples. If such evaluation is related to instrumental chromatic parameters, the consumer judges would prefer the samples with higher a* and C* values, and the rest of chromatic parameters would not probably have any influence on them (Tables 2 and 3). The differences observed between instrumental measurements of DRT and toasted samples did not seem to concur with the judges' appreciation (Tables 1-3).

Similar results were obtained with the discriminatory assay (Table 4). The DHA samples were evaluated by the judges as the reddest, showing significant differences with toasted and DRT saffron samples. The differences observed between the two latter regarding a* and C* were not detected by the judges, suggesting that colour is the only quality parameter for which the instrumental analytical techniques are better than sensory evaluation, as claimed by ANZALDÚA (1994). The DHA samples were the brightest, according to the judges, as they were significantly different from the toasted and DRT ones. Furthermore, brightness was greatest for the highest dehydration temperature (110°C), although this does not agree with the luminosity coordinate (L*) data reported in Table 2. It is not surprising that DHA samples were assessed as bright and

Table 3 - Mean values of the sensory parameters evaluated by the degree of satisfaction test with a verbal hedonic scale.

Samples	Colour	Aroma	Flavour
DRT	0.43[a]	-0.23[a]	0.00[a]
Toasted	0.48[a]	0.81[b]	0.13[a]
DHA at 70°C	1.38[b]	1.05[b]	0.54[a]
DHA at 90°C	1.78[b]	1.05[b]	0.44[a]
DHA at 110°C	1.77[b]	1.03[b]	0.25[a]

*DRT "dehydrated at room temperature"; DHA "dehydrated with hot-air";
Values followed by different letters within a column are significantly different at p<0.05 (Duncan´s test).

Table 4 - Sum of the scores given for the sensory parameters evaluated by a discriminatory test.

Samples	Colour		Aroma	Bitterness
	Red Tone	Brightness		
DRT	15[a]	17[a]	26[a]	18[a]
Toasted	31[a]	28[a]	59[c]	50[b]
DHA at 70°C	60[b]	57[b]	43[b]	58[b]
DHA at 90°C	59[b]	56[b]	36[b]	47[b]
DHA at 110°C	60[b]	67[c]	61[c]	52[b]

*DRT "dehydrated at room temperature"; DHA "dehydrated with hot-air";
Values followed by different letters within a column are significantly different at p<0.05 (total range tables).

with an intense red colour, since commercial saffron samples produced by a dehydration process at room temperature, as is the case with Iranian samples, usually have a dark and less bright colour (SÁNCHEZ, 1997).

When comparing the judgements of sensory assays with their respective colouring strength, there were important discrepancies (Tables 1, 3 and 4), possibly because saffron colouring strength was not judged properly, since only the external part of the stigma (red part) was evaluated. This reflects how in many occasions saffron consumers buy the most attractive saffron, rather than the one with the highest colouring strength. This may also be due to the fact that saffron is sold in polystyrene boxes, which have a negative influence on saffron quality, as the substances responsible for colour (crocins) photodegrade. Changing package and labelling according to the ISO trade standard could eliminate many of these problems.

Aroma

As regards the sensory aroma parameter, consumer judges preferred DHA and toasted samples, although there were no significant differences. Surprisingly, the DRT samples were the worst rated (Table 3), but they had the highest aromatic strength (Table 1). This suggests that aroma is not the same as aromatic strength. The main aroma substance in saffron is safranal, accounting for 60% of the total aromatic fraction (RÖDEL and PETRIZKA, 1991), but the aromatic strength (safranal) defined by the ISO (1993) trade standard, does not reflect the real aroma of the sample. There are two reasons for this: first, safranal is a non-polar substance which is determined instrumentally at 330 nm in aqueous saffron extracts. Secondly, at 330 nm the cis isomer crocins are also absorbed (TARANTILIS *et al.*, 1995). The majority of the crocins in saffron are trans crocin 4 and trans crocin 3 (ALONSO *et al.*, 2001) and its content is much higher than safranal, so many interferences can be observed when measured. An explanation of the higher aromatic strength of DRT samples may be that the amount of cis isomer crocins in fresh stigma is greater than in stigmas dehydrated at higher temperatures. After analysing 260 saffron samples from the Castilla-La Mancha production area ALONSO *et al.* (2001), found important differences within the tras/cis ratio, as the UV-visible radiation differed when the flowers were picked.

When the expert judges rated sample aroma, the DHA at 110°C and toasted samples had the highest aroma intensity, while the DRT were the worst (Table 4). This data fit well with the safranal generation mechanism, starting from picrocrocin hydrolysis and later dehydration, proposed by some authors (IBORRA *et al.*, 1992; VISVANATH *et al.*, 1990). As HIMENO and SANO demonstrated (1987), this process takes place after a heating process. It is therefore reasonable to assume that DHA and toasted samples may have higher amounts of safranal and that the judges rated such aroma perception.

Everything about safranal and its determination suggests that in future revisions of the ISO 3632 trade standard (1993), a gas chromatographic analysis should be considered. Then, the maximum absorption at 330 nm should be used to obtain a saffron UV-Vis fingerprint, which could also detect possible adulterations (ALONSO *et al.*, 1999).

Bitterness and flavour

As for the flavour parameter, significant differences between the samples were not found in the affective judgement test (Table 3), possibly because the preparation of the test samples, an infusion, was not be suitable. As regards

the discriminatory test, the expert judges found DHA and toasted samples more bitter than the DRT ones (Table 4). These evaluations agree with the values obtained by UV-Vis spectrophotometry (Table 1). According to PFANDER and SCHURTENBERGER, (1982), crocins and picrocrocin are generated through an oxidative degradation process from a common precursor, protocrocin. It seems likely that such reaction could have taken place at high dehydration temperatures, since samples with a higher crocin content (higher colouring strength) also had a higher picrocrocin content (higher bitterness and bitter strength).

CONCLUSIONS

The dehydration process had an important influence on saffron colouring strength. Colour measurement using CIEL*a*b* parameters was useful to discriminate dehydration methods, but it was not related to the colouring strength, as defined by the ISO 3632 (1993) trade standard. Consumer judges preferred saffron dehydrated with hot-air (DHA), and the expert judges emphasised its tone and brightness. Saffron brightness increased with dehydration temperature. At the same time, expert judges also preferred samples on the basis of their aroma. In all cases, saffron dehydrated at room temperature (DRT) was the worst rated as it had less colour, flavour and aroma.

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SHORT COMMUNICATION

EVALUATING CHEDDAR CHEESE PREFERENCE UNDER DIFFERENT LIGHT SOURCES

VALUTAZIONE DELL'ACCETTABILITÀ DEL FORMAGGIO CHEDDAR SOTTOPOSTO A DIFFERENTI SORGENTI LUMINOSE

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ABSTRACT

Orange, white and marble Cheddar cheeses were evaluated under three light sources. The color of the orange Cheddar was more preferred under metal halide (MH) compared to fluorescent light (FL). The reason was the low red light under FL, as determined by evaluating the light reflected from the product using a fiber optic probe connected to a photodiode array. Color preference under incandescent (INC) light was similar to MH since both emit a strong yellow component. The mar-

RIASSUNTO

Il formaggio Cheddar dei tipi arancio, bianco e marmorizzato è stato analizzato quando sottoposto a tre differenti sorgenti luminose. Il colore del Cheddar arancio è stato preferito sotto luce MH (alide di metallo) rispetto alla luce fluorescente (FL). Il motivo è legato al fatto che la luce rossa era più debole in fluorescenza, quando determinata mediante valutazione della luce riflessa dal prodotto usando una sonda a fibre ottiche connesse con il fotodiodo. La preferenza di colore sotto incandescenza

- Key words: acceptability, appearance, Cheddar, cheese, color, lighting -

ble Cheddar was more preferred under MH and INC than under FL light. Overall, a high proportion of panelists preferred to buy the cheeses presented under MH light.

(INC) era simile a quelle con MH, poiché entrambe le lampade emettono una forte componente gialla. Il Cheddar marmorizzato era preferito sotto MH e INC piuttosto che sotto FL. In ogni caso, una elevata percentuale dei valutatori ha preferito il Cheddar presentato sotto luce MH.

INTRODUCTION

The color of food at the retail store has a strong effect on the consumers' buying decision. Overall, the appearance of a food product is not only affected by its true color, but also by the light source, the observer's position, light intensity, etc. (HUTCHINGS, 1999). PANGBORN (1967) indicated that "to a large extent man recognizes, discriminates and selects nutrients with the eye... food colors are identified with previously experienced quality and serve as instant indicators of good or bad, according to the product and its intended use". Cheese is no exception, and various reports have demonstrated that the appearance of cheese is one of the most important factors determining its acceptability (MORGAN, 1933; RIHA and WENDORFF, 1993). Consumers have few if any means of evaluating the texture and flavor of packaged cheese. Therefore, when choosing a product at the store, consumers usually base their decision on visual appearance and past experience.

Food stores allow a relatively large display space for high value cheeses. To attract consumers, they use attractive display cases, signs, and bright light to illuminate the area. Different light sources can be found in and above display cases. They range from overhead fixtures of fluorescent (FL), incandescent (INC) and metal halide (MH), lights to lights positioned inside the display case. FL lightbulbs have relatively low heat output compared to INC and MH, and cause less stress on the refrigeration unit [FL bulbs radiate about one-fifth of the heat generated by INC bulbs (KROPF, 1980)]. Both FL and MH are widely used because of their lower energy requirements compared to INC (BICKFORD and DUNN, 1972; ANONYMOUS, 1991). However, neither FL nor MH are considered a full-spectrum light source. This is especially true for the least expensive, and most popular, Cool White FL bulb, which has a weak emission in the red region. An incorrect color presentation should be of concern to the cheese industry which spends substantial resources to produce and maintain an attractive package and color (e.g. adding annatto to enhance color, vacuum packaging to minimize oxidation and off color/flavor development).

Evaluating consumer's preference of color is an important aspect in developing and marketing cheese. Overall, most published reports indicate the use of commercial color meters which rely on an internal light source (e.g. Xenon light) and are calibrated against a white plate. For example, RIHA and WENDORFF (1993) evaluated the effect of smoked exterior color on the preference of smoked Cheddar and Swiss cheeses, LEMAY *et al.* (1994) investigated the effect of using micro-fluidized milk on the characteristics of Cheddar cheese, HONG *et al.* (1995) studied the effect of packaging and exposure to fluorescent light on the discoloration of annatto-colored Ched-

dar, and RUDAN *et al.* (1998) investigated the effect of fat replacer on the appearance of Mozzarella cheese. However, when consumers examine the color and decide about purchasing the product, they usually do so under less than ideal lighting conditions. Thus, the objective of this study was to investigate the effect of three commonly used light sources on panel color preference and the decision to buy orange, white and marble Cheddar cheeses. In addition, relative luminance data were obtained to explain consumers' choices.

MATERIALS AND METHODS

Three packages each of white, orange and marble Cheddar cheese were purchased from a local supermarket. The cheeses were produced using a national brand label in clear vacuum processed wrappers with a label printed on one side. The ingredients listed on all three cheeses showed: milk solids, bacterial culture, salt, rennet, calcium chloride and may contain color. The chemical composition derived from the nutrition labels was 37.5% moisture, 33.0% fat, 24.5% protein and 1.3% carbohydrates for the white and orange Cheddars, and 46, 19, 30 and 3%, respectively, for the reduced fat (light) marble Cheddar. The packages for each category were selected to obtain products of uniform color (e.g. samples obtained from the bottom of the display cooler which were not exposed to light and did not show any fading or pinking).

A group of the three different cheeses was displayed under three different lights and evaluated by nineteen panelists and two color measuring instruments. Sample pieces of cheese (10x10x2.5 cm) were placed on a table covered with a black cloth and marked as products A, B and C. The black background was used to eliminate any color differences that might show on a colored surface. The light sources included: incandescent (150 W, 120 V, Extended Service, Sylvania, Danvers, MA), fluorescent (34 W, Supersaver Lite White, Sylvania), and metal halide (175 W, 120 V, NL175MH-120-L, Acculite, Kitchener, ON). The height for setting the lights was based on achieving 70-foot candles (ft cd) at the cheese surfaces. A portable light meter (Cole Parmer DLM2, Chicago, IL) was used to set the lights. The level of 70 ft cd (760 lux) was chosen after surveying the light level at a few stores in Guelph, which ranged from 60 to about 100 ft cd (650-900 lux).

In the laboratory, the actual light reflected from each product was measured by a photo diode array detector (Model LDA 2000/S, Sciencetech Inc., London, ON), which was attached to a glass fiber optic probe (4 mm diameter, random fiber arrangement). The system was operated via a computer using the LDA 2000 software supplied by Sciencetech. The samples were scanned (1,500 ms each) at three different locations from 400-700 nm. The tip of the fiber optic was positioned 1 cm from the sample at a 45° angle, while samples were illuminated from above. This was a pragmatic commercial simulation of how the consumer would view the color of the product in the store. The reflected emission spectra was corrected for the spectra response of the photo diode array by a software program written by Sciencetech, and is expressed as relative luminance. A color-meter (Minolta Spectro-photometer CM-1000, Osaka, Japan) was also used (D65 illumination) to evaluate the samples and obtain reflectance curves as well as the CIE L* (lightness), a* (redness) and b* (yellowness) values. This colormeter uses a Xenon light source and was calibrated against a white plate supplied by the manufacturer.

The experiment was set up as a split plot design, with light and day as the main plots and products as sub plots. The products were displayed on two con-

secutive days (refrigerated in between viewing) and evaluated by the same group of nineteen panelists. The panelists included staff and graduate students from the Department of Food Science who were familiar with dairy products, and purchase them on a regular basis. The reason for the two evaluations was to obtain a repeated measure by each panelist. The panelists were asked to provide their color preference for each product (1 = dislike, 10 = like), verbally describe the color, and indicate if they would purchase the product under each light source. Data were analyzed by the Mixed Procedure (SAS, 1997), which uses restrictive maximum likelihood to estimate variance of random effects.

RESULTS AND DISCUSSION

The three Cheddar cheeses appeared differently under the three light sources (Table 1). The orange Cheddar cheese was most preferred under the MH and INC lights. Under MH light, the panelists described the color as bright orange (65% of the panelists) and yellow-orange (35%). Under INC, it was described as deep orange (70%) and orange (30%). Overall, the color of other objects is commonly expressed in one or two words, describing the main color and its shade. This is the result of summarizing all reflected light and expressing them as one color. The actual light reflected from the products presented under INC light and seen by the panelists is shown in Fig. 1. The relative luminance curve shows that the orange Cheddar had a strong reflection in the orange (560-625 nm) and red (625-700 nm) regions. Under MH (Fig. 2), the product also showed a strong reflection in the orange and red regions. The light reflected and the associated peaks are typical of the emission spectra of MH lightbulbs. The emission spectra of the MH light was also evaluated with a white plate and the results (higher peaks at the same locations; graph not presented here) were consistent with other commercial MH sources (ANONYMOUS, 1991). The strong orange peak at 590 nm (Fig. 2) made the product appear brighter orange, as described by some of the panelists. Under FL light, the product was less preferred than under MH (Table 1), but not different than under INC light. The panelists used pale orange (60%) and orange (40%) to describe the color of the cheese presented under FL light. The relative luminance data (Fig. 3) explains the results, showing a red component, in the region of 625-700 nm, reflected back from the product (i.e. the FL light bulb lacks emission in this region). This was also evaluated with a white plate which showed the same typical FL light peaks as reported by others (BICKFORD and DUNN, 1972; ANONYMOUS, 1991). Under FL, the product was described as pale orange by more than half of the panelists, compared to bright orange and yellow-orange under MH light. The appearance of the product was also evaluated with a commercial spectrophotometer that uses a Xenon light source (Fig. 4), and the results were similar to those obtained under INC light. The reason for this resemblance is the fact that INC light is a fairly balanced light source (note, even though it has relatively lower emission in the 400 nm region compared to higher wavelengths, and in fact is considered one of the most balanced commercial

Table 1 - Preference of Cheddar cheese under different light sources (presented under 70 ft cd).

Product	Incandescent	Metal halide	Flourescent
Orange Cheddar	6.52^{ab}	7.71^{a}	5.70^{b}
White Cheddar	6.76^{ab}	7.83^{a}	6.12^{b}
Marble Cheddar	6.82^{a}	7.50^{a}	5.58^{b}

^{a,b} Means, within each row, followed by a different superscript are significantly different ($P<0.05$). Sums of nineteen panelists evaluating the product on two successive days; scale 1 (dislike) - 10 (like).

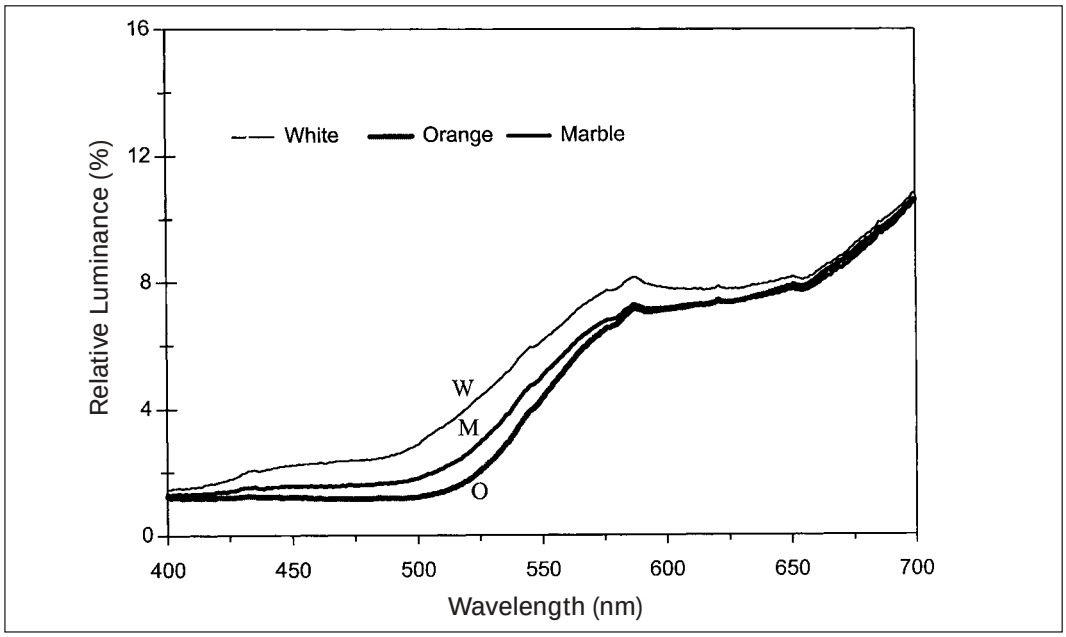


Fig. 1 - Relative luminance of white (W), orange (O) and marble (M) Cheddar cheeses under incandescent light (70 ft cd).

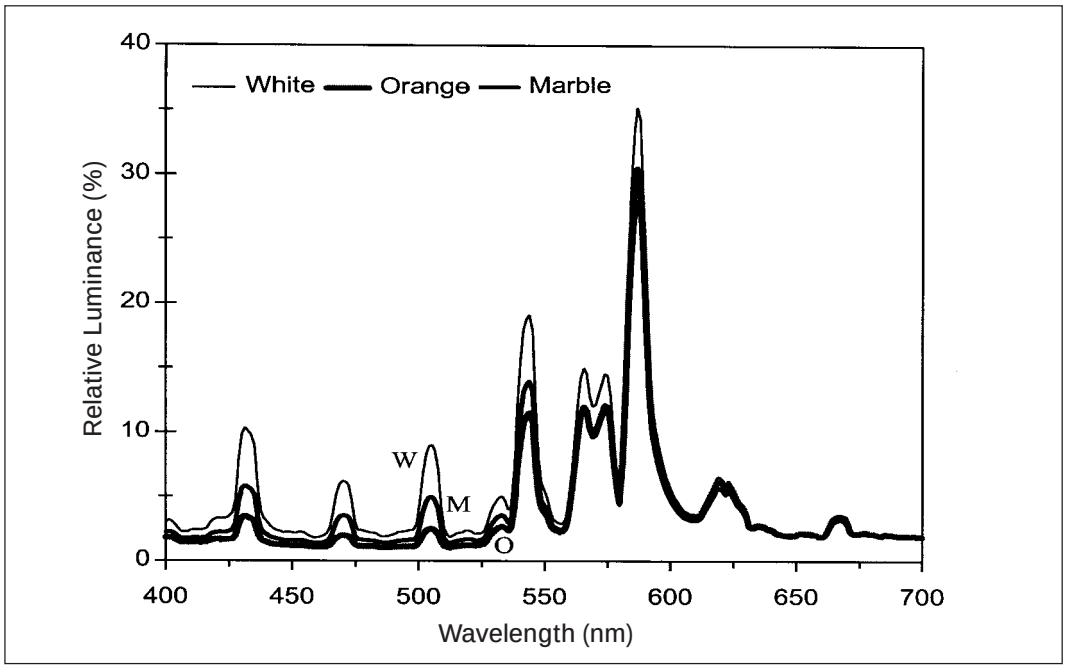


Fig. 2 - Relative luminance of white (W), orange (O) and marble (M) Cheddar cheeses under metal halide light (70 ft cd).

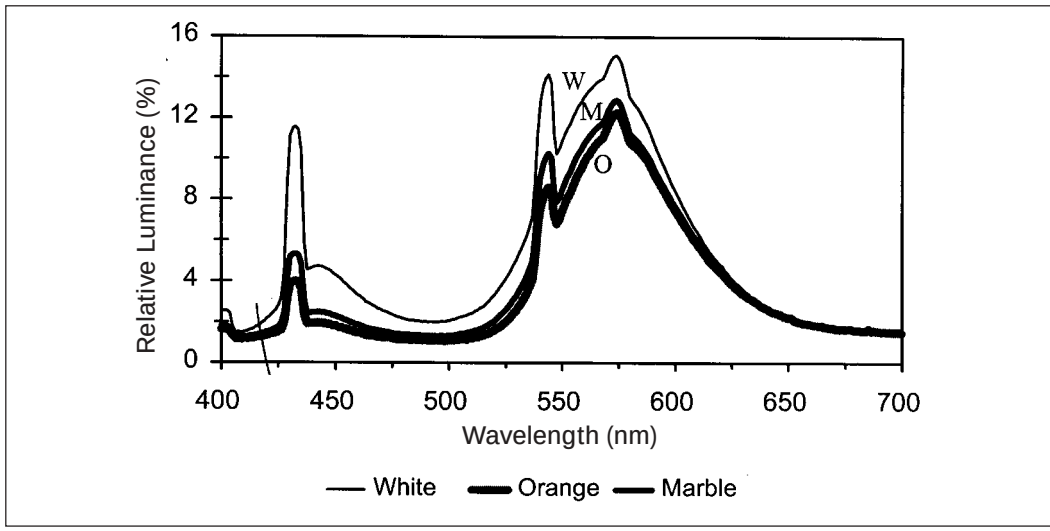


Fig. 3 - Relative luminance of white (W), orange (O) and marble (M) Cheddar cheeses under flourescent light (70 ft cd).

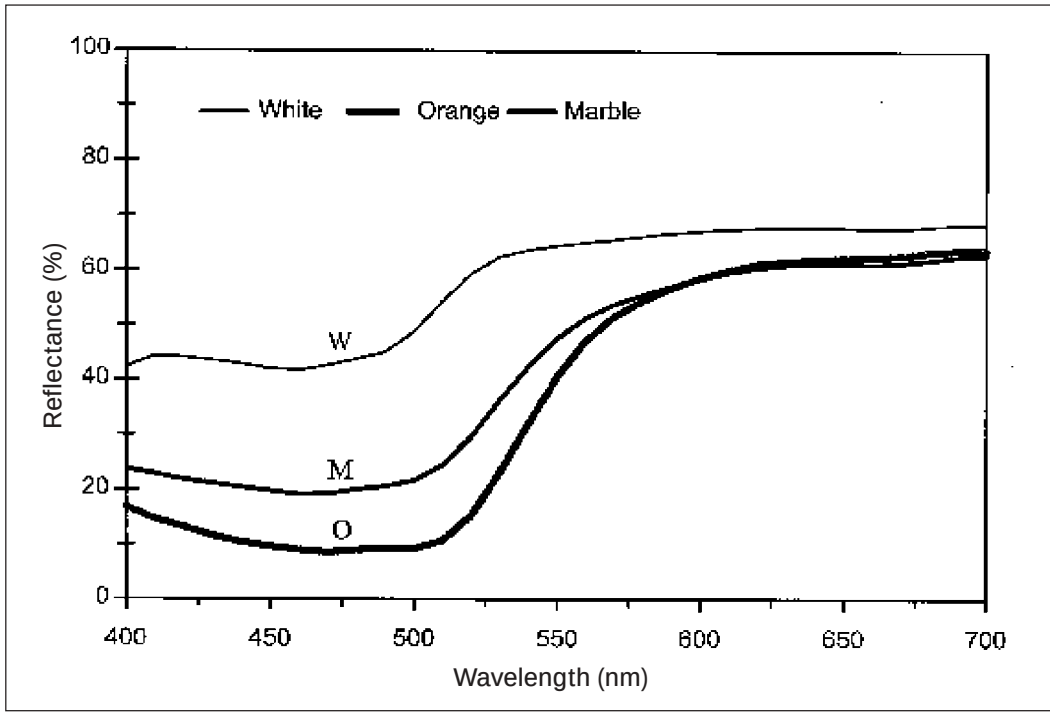


Fig. 4 - Reflectance data of white (W), orange (O) and marble (M) Cheddar cheeses obtained with a commercial spectrophotometer/ color meter.

light sources). The spectrophotometer also provided absolute color values (Table 2). The lightness value (L*) of the orange Cheddar was 64.4, redness (a*) 12.8 and yellowness (b*) 46.4. These values are in the range reported by DRAKE *et al.* (1998) for a full fat Cheddar cheese; L* = 72.5, a* = 14.9 and b* = 34.5. These authors produced the commercial-type product in their laboratory to investigate the effect of fat reduction, and employed a Minolta spectrophotometer (CM2002) to evaluate the product, but did not used a panel to obtain preference scores. The relatively high redness and yellowness values indicate that a food colorant, such as annatto (the most commonly used color in cheese) was added to the product.

The panelists showed the same preference pattern for the white Cheddar cheese presented under the different lights as for the orange Cheddar (Table 1). The reason might have been the presence of a sizable yellow component (b* = 17.8). Color values of a low fat Mozzarella cheese reported by RUDAN *et al.* (1998) were L* = 77, a* = -5.0 and b* = 1.8. White whey protein gels have also been reported to have a high L* value and very low a* and b* values (BARBUT, 1996). Overall, the white Cheddar was lighter than the orange Cheddar as is evident by the L* values (76.7 vs 64.4, respectively; Table 2), the relative luminance curves (Figs. 1-3) and the reflectance curve (Fig. 4), which were the highest for the white Cheddar, meaning the product reflected more light than the other two products. The higher reflectance in the low end of the spectrum resulted in the product appearing whiter than the other products. The panel described the white Cheddar presented under MH as light cream (65%) and white (35%), under INC as cream (60%) and white (40%), and under FL as white (50%) and cream (50%).

For the marble Cheddar, which was composed of a mixture of clearly identifiable white and orange particles (can also be described as islands of orange Cheddar in white Cheddar or vice versa), the preference scores (Table 1) showed higher values when presented under MH and INC. Also in this case, the orange and red components of the orange Cheddar areas showed more vividly under MH and INC. The marble Cheddar showed intermittent L*, a* and b* color values, and relative luminance curves (Table 2, Figs. 1-3). The color values represent average values obtained from evaluating the surface which was composed of a mixture of orange and white sections. In general, it is important that a light source emit light needed to reveal the true color of a product. In the case of a whole fresh chicken (yellow skin), BARBUT (2001) demonstrated that INC was a better light source than FL and MH in bringing out the desirable yellow color; however, for the dark thigh meat presentation, light source was not as important.

Table 2 - Color values (average ± S.D.) of Cheddar cheese expressed as L* (lightness), a* (redness), and b* (yellowness) obtained with a commercial color meter.

Product	L*	a*	b*
Orange Cheddar	64.4±0.6	12.8±0.4	46.4±2.9
White Cheddar	76.7±0.6	0.0±0.5	17.8±0.4
Marble Cheddar	66.9±1.2	5.0±1.6	34.4±5.0

Table 3 - Responses (yes/no) for buying Cheddar cheese under different light sources (70 ft cd).

Product	Incandescent	Metal halide	Flourescent
Orange Cheddar	24/14n.s.	38/0**	26/12*
White Cheddar	30/8**	36/2**	27/11*
Marble Cheddar	28/10**	36/2**	26/12*

* P<0.05, ** P<0.01; n.s. Not significant. Sums of nineteen panelists evaluating the products on two successive days.

The overall buying decision was affected by the light source (Table 3), where a stronger preference to buy all three products was observed under the MH light. Under INC and FL, fewer panelists indicated that they would buy the products, but the overall numbers did not show a negative buying attitude.

In summary, the panel results indicate that when an orange colored Cheddar cheese is presented, a light source emitting red and orange light is important. Both the MH and INC sources were preferred, with the MH light showing higher preference, probably due to its strong orange light emission. The INC light source was not significantly different from the MH in the overall preference scores. In any case, a light source which lacks yellow and/or red components should not be used or used with an overhead supplement emitting orange light. New designs of FL lightbulbs with a better color rendering index (i.e. producing more red light) should also be considered in replacing the commonly used cool-white FL lightbulbs positioned inside display coolers. Alternatively, full spectrum overhead light fixtures (e.g. incandescent) can be added to supply additional red light.

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SHORT COMMUNICATION

KINETIC ANALYSIS OF *SACCHAROMYCES CEREVISIAE* INACTIVATION BY HIGH PRESSURE CARBON DIOXIDE

ANALISI CINETICA DELL'INATTIVAZIONE DI *SACCHAROMYCES CEREVISIAE* MEDIANTE ANIDRIDE CARBONICA AD ALTA PRESSIONE

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ABSTRACT

Carbon dioxide inactivation kinetics of *Saccharomyces cerevisiae* in potato dextrose broth at 100, 75, 50 and 25 atm was evaluated at 50°, 40°, and 30°C. Inactivation rates increased with increasing pressure, temperature and time. The come-up time to reach a pressure exerts an important effect on the survival of *S. cerevisiae*. Yeast inactivation followed first order reaction kinetics, with inactivation rates and decimal reduction times that varied from 0.0795 to 0.5722 min^{-1}, and from 4.0248 to 28.9686 min, respectively. The activation volume change of *S. cerevisiae* pressure inactivation was -526.6, -605.3 and -232.3 cm^3/mol at 50°, 40° and 30°C, respectively. The

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Sono state valutate le cinetiche di inattivazione di *S. cerevisiae* su brodo di destrosio di patata con CO_2 alle pressioni di 100, 75, 50 e 25 atm ed alle temperature di 50°, 40° e 30°C. La velocità di inattivazione aumenta all'aumentare della pressione, della temperatura e del tempo. Il tempo necessario a raggiungere una determinata pressione esercita una influenza importante sulla sopravvivenza di *S. cerevisiae*. L'inattivazione del lievito segue una cinetica di primo ordine con velocità di inattivazione e tempi di riduzione decimale variabili rispettivamente tra 0,0795 e 0,5722 min^{-1} e tra 4,0248 e 28,9686 min. Il cambiamento del volume di attivazione della pressione di inat-

- Key words: High-pressure carbon dioxide, inactivation, kinetics, *Saccharomyces cerevisiae* -

pressure dependence of the yeast inactivation rates can be described by the apparent activation volume (ΔV*) and pressure z_p value.

tivazione di *S. cerevisiae* è pari rispettivamente a -526,6, -605,3 e -232,3 cm^3/mol a 50°, 40° e 30°C. L'effetto pressione sulla velocità di inattivazione è descrivibile mediante il volume di attivazione apparente e mediate un valore di pressione pari a z_p, rivelando, in ambedue i casi, una maggior dipendenza alla pressione dei sistemi ad alta temperatura.

INTRODUCTION

There is currently increased interest in the use of high pressure carbon dioxide (HPCD) as an alternative to heat treatments in food processing. This is due to the reported beneficial aspects on quality attributes and the unique physical and sensory characteristics of pressure-processed foods (BALLESTRA *et al.*, 1996; BARBOSA-CANOVAS *et al.*, 1998; ERKMEN, 2000b; 2001a; ISENSCHMID *et al.*, 1995; LIN *et al.*, 1994). There is also increased interest regarding the effects of HPCD on protein denaturation, lipid phase changes and rupture of biomembranes in biological systems with the consequent inactivation of micro-organisms (BALLESTRA *et al.*, 1996; HAAS *et al.*, 1989; LIN *et al.*, 1994). Resistance of micro-organisms to pressure is extremely variable. SHIMODA *et al.* (2001) reported on first order and D-value inactivation kinetics of *Saccharomyces cerevisiae* with dissolved pressurized CO_2 in a flow system, but there are no reports about the inactivation kinetics of yeast with HPCD in batch-pressurized CO_2 and other kinetic parameters.

Carbon dioxide can be used as an antimicrobial agent because it is an ultrapure, toxicity-free substance which can be easily removed from food products. The antimicrobial activity of pressurized CO_2 is remarkable, and hence, the application of pressurized CO_2 for sterilizing food is expected to minimize the degradation of heat-sensitive substances. The microbial inhibitory activities of pressurized CO_2 have been reported for *Leuconostoc dextranicum* (LIN *et al.*, 1993), yeast cells (LIN *et al.*, 1991; ISENSCHMID *et al.*, 1995), and *Listeria* and *Salmonella* (WEI *et al.*, 1991). In the development of this technique for the food industry, a continuous processor using microbubbles of pasteurized CO_2 was built by SHIMODA *et al.* (1998). This process demonstrated an extremely high performance on the inactivation of microorganisms, but it was impossible to analyze the inactivation process kinetically.

BALLESTRA *et al.* (1996) and ERKMEN (2000b; 2001b) observed two phases in the survival curves. The early stage was characterized by a slow rate of decrease in the number of micro-organisms which increased sharply at the later stage. To increase microbial safety and assure microbial stability of foods processed by HPCD, the pressure treatment must ensure a satisfactory reduction in the initial microbial counts, thus kinetic analysis and information about the pressure dependence of microbial inactivation rates are needed.

The purpose of this research was to investigate the HPCD inactivation kinetics of *S. cerevisiae* in potato dextrose broth at various temperatures and pressures. Inactivation was described by the values of k (inactivation rate), D (time at a specific pressure required to reduce the viable population by 90%), ΔV* (activa-

tion volume of the reaction), and z_p (the pressure increment, needed to reduce the D-value by a factor of 10).

MATERIALS AND METHODS

Culture preparation

Saccharomyces cerevisiae ATCC 9743 was obtained from the American Type Culture Collection (Rockville, MD). The strain was activated in potato dextrose broth (PDB; Difco, Detroit) and stock culture was prepared on potato dextrose agar (PDA, Difco, Detroit) slants and incubated at 30°C for 48 h for growth. The stock culture was maintained at 4°C. The cultures for experiments were subcultured twice from the stock culture using 10 mL of PDB. The working culture was prepared by adding 20 mL of 48 h-culture into 300 mL PDB (pH=3.8) in a 500 mL flask. The final number of *S. cerevisiae* in the working culture ranged from $1.62x10^6$ to $5.13x10^6$ colony-forming units (cfu)/mL. The culture used in all experiments was freshly prepared every day using the same procedure and stored in a refrigerator at 4°C.

Pressure treatments

Ten milliliters of working culture were added to two loosely capped (metallic cap) sterile test tubes (14x110 mm), and then subjected to CO_2 pressure as described by ERKMEN (2000b). The experiments were performed under 100, 75, 50 and 25 atm CO_2 pressures at 50°, 40°, and 30°C. The control tube containing 10 mL of *S. cerevisiae* PDB culture was incubated under atmospheric pressure in a water bath. Each treatment was replicated three times. Viable *S. cerevisiae* counts (initial and after HPCD treatment) were determined by surface plating from appropriate dilutions (or nondilution) on PDA. Two plates were used for each dilution and incubated at 30°C for 48 h, after which all characteristic colonies were counted (ERKMEN, 2000a). The average number of colonies from duplicate plates was then recorded for each sample. The number of survivors is expressed as log N/N_0 (N: cfu after treatment and N_0: initial cfu).

Data analysis

Pressure inactivation of micro-organisms was assumed to follow the first order rate kinetics expressed as:

$$\ln (N/N_0) = -kt \qquad \text{Eqn [1]}$$

Where N = number of surviving micro-organisms after a pressure treatment for time t (min), N_0 = initial number of micro-organisms and k = reaction rate constant (min^{-1}). The death velocity constants or inactivation rates (k) were calculated from the slope of the survival curves following a traditional kinetic analysis. The survival curve of *S. cerevisiae* under HPCD at 100, 75 and 50 atm at 40°C is shown as an example in Fig. 1. The treatment time at any given pressure that will destroy 90% of the existing microbial population, i.e. resulting in one decimal reduction in the surviving population, is referred to as the decimal reduction time or D-value. This is obtained as the negative slope of the log (N/N_0) vs. time curve and is therefore related to k: D = 2.303/k. D values for yeast destruction were obtained from their respective k values using the above relationship.

The pressure z_p value of the process is defined as the pressure increment needed to reduce the D-value by a factor of ten. This is analogous to treatment of thermal death time (TDT) data of micro-organisms widely used in thermobacteriology and thermal process calculations. The z_p value was calculated from the regression of log (D) vs. pressure data as the negative slope.

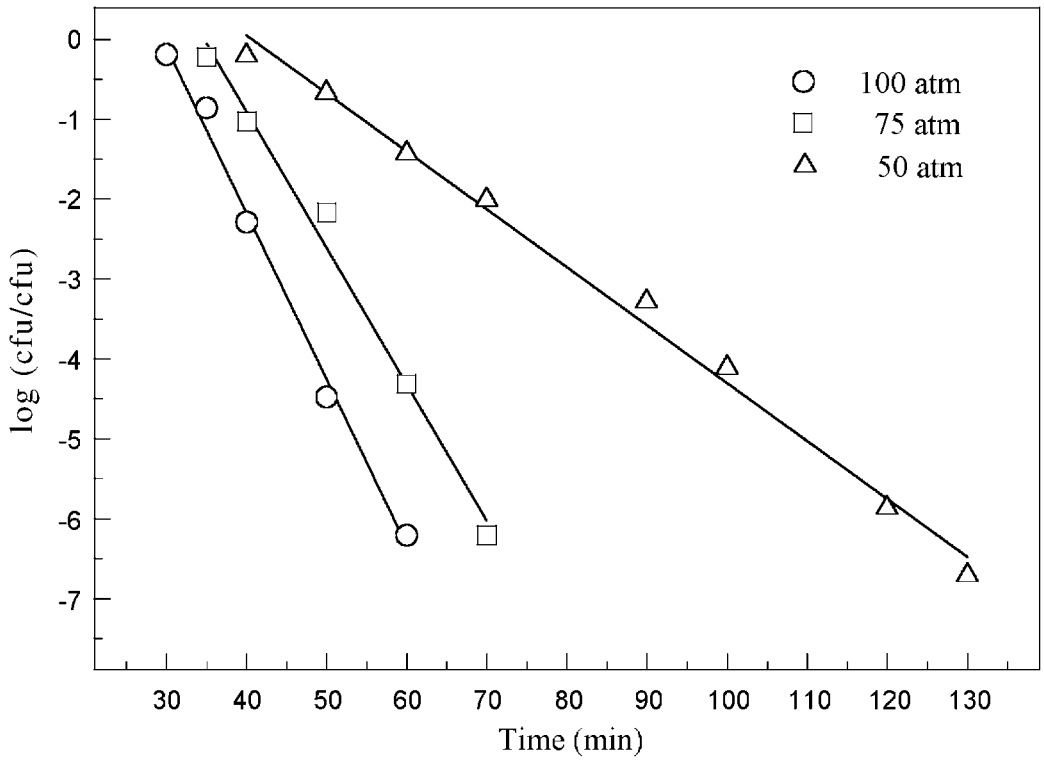


Fig. 1 - First order pressure inactivation kinetics of *S. cerevisiae* in PDB as a function of CO_2 pressure at 40°C.

Using the Arrhenius concept, the pressure sensitivity of the rate constant k is obtained as the volume change of inactivation (ΔV^*) based on the equation shown below:

$$\Delta V^* = -RT \left(\frac{\delta \ln k}{\delta P} \right)_T \qquad \text{Eqn [2]}$$

where P is the pressure (MPa), T is the absolute temperature (K), R is the gas constant (8.314×10^{-6} m^3 MPa K^{-1} mol^{-1}) and ΔV^* is the volume change of activation (m^3 mol^{-1}).

Analyses of variance were performed on data obtained at different stages of HPCD treatment by means of a computer program, Statgraphics 2.0 (Stsc., Inc., Rockville, MD).

RESULTS AND DISCUSSION

Two phases were observed in the survival curves of *S. cerevisiae* with pressurized CO_2. At each pressure, the early stage was characterized by a slow rate of inactivation, which increased sharply afterward.

Effect of HPCD on *S. cerevisiae* in the first phase

Table 1 presents the percent reduction of *S. cerevisiae* in the first phase of the survival curve (come-up time to reach the second phase of the survival curve). The pressure come-up time to the second phase of the survival curve exerts an important effect on the survival fraction. *S. cerevisiae* counts decreased as

pressure increased, about 24.2, 23.2, 15.4 and 11.3% reductions were observed at 100, 75, 50 and 25 atm HPCD treatments, respectively, at 50°C.

Table 1 - Reduction (%) of the initial *S. cerevisiae* population under HPCD in come-up time[a] to the second phase of the survival curve.

Temperature (°C)	Pressure (atm)	Come-up time (min)	% Reduction
50	100	10	24.2
	75	15	23.2
	50	30	15.4
	25	60	11.3
40	100	30	23.2
	75	35	24.1
	50	40	20.6
	25	180	10.4
30	100	50	12.4
	75	60	14.4
	50	100	10.7
	25	240	7.8

[a] Come-up time (first phase of survival curve) indicates the time required to reach the second phase of the survival curve.

Effect of HPCD on *S. cerevisiae* in the second phase

S. cerevisiae decreased linearly with time (Fig. 1). *S. cerevisiae* cells were inactivated more rapidly with increasing pressure. There was no reduction in the number of *S. cerevisiae* in PDB culture at 40°C incubated under normal atmospheric pressure.

The death rate constants (k) or specific inactivation rates were calculated from the slope of the survival curves from a traditional kinetic analysis. The calculated inactivation rates were greater at higher pressure and temperature than at low pressure and temperature (Table 2). Comparable k values were obtained in pressure treatments at 100 (0.5722 min^{-1}), 75 (0.4874 min^{-1}), 50 (0.2531 min^{-1}) and 25 atm (0.1336 min^{-1}) at 50°C, indicating that high-pressure treatment significantly ($P<0.05$) affected the yeast response.

In order to compare the effectiveness of pressure treatments and to optimize the process conditions, the D-values can be used to compare the resistance of

Table 2 - Effect of HPCD on the inactivation rates (k) and decimal reduction times (D) of *S. cerevisiae* in PDB[a].

Temp. (°C)	Pressure (atm)	k (min^{-1})		D (min)		
		$\bar{x}$[b]	s_x	$\bar{x}$[b]	s_x	r^2
50	100	0.5722	0.086	4.0248	2.8730	0.98
	75	0.4874	0.0120	4.7251	0.5300	0.99
	50	0.2531	0.0132	9.0992	4.7092	0.98
	25	0.1336	0.0032	17.2380	5.7290	0.99
40	100	0.4861	0.0328	4.7377	1.0900	0.99
	75	0.3841	0.0215	5.9958	2.0542	0.99
	50	0.1657	0.0014	13.8986	1.9842	0.99
	25	0.0881	0.0006	26.1407	3.8745	0.99
30	100	0.1706	0.009	13.4914	1.5128	0.99
	75	0.1439	0.0132	16.0042	2.7865	0.98
	50	0.1204	0.0328	19.1279	3.2348	0.99
	25	0.0795	0.0023	28.9686	2.1083	0.99

[a] $\bar{x}$ = means and s_x = standard deviations of three determinations, r^2 = regression coefficient.
[b] There are significant differences ($P<0.05$) between different treatments.

micro-organisms. A decimal reduction time (D-value) can be defined as the time needed to reduce 90% of the initial population and can be calculated as D = 2.303/k. There were significant differences ($P<0.05$) among D-values at constant pressure between temperatures (Table 2). The inactivation rate of *S. cerevisiae* increased as the temperature increased.

Fig. 2 presents the fit of the experimental data to Eqn [2]. The positive slope obtained from the plots of pressure inactivation rates (ln k) vs. pressure, and therefore a negative activation volume, indicates that a decrease in volume is related to the *S. cerevisiae* inactivation process. The calculated activation volumes and z_p-values are reported in Table 3. The z-values differed with treatment temperatures. The apparent ΔV^*, calculated considering the activation process of *S. cerevisiae* inactivation as one step, indicates the volume variation between the "activated complex" and the initial states of the bacterial pressure inactivation "reaction". The volume change of activation for *S. cerevisiae* pressure inactivation was -526.6, -605.3 and -232.3 cm^3/mol at 50°, 40° and 30°C, respectively. A negative activation volume represents a reaction favored by increased pressure, so a reaction with a higher absolute ΔV^* value indicates that increments in pressure can accelerate the response on *S. cerevisiae* inactivation rate. It is very difficult to give a precise physical meaning to the activation volumes found. The calculated apparent ΔV^* may be considered as the sum of several changes such as chemical, conformational, intra- and intermolecular

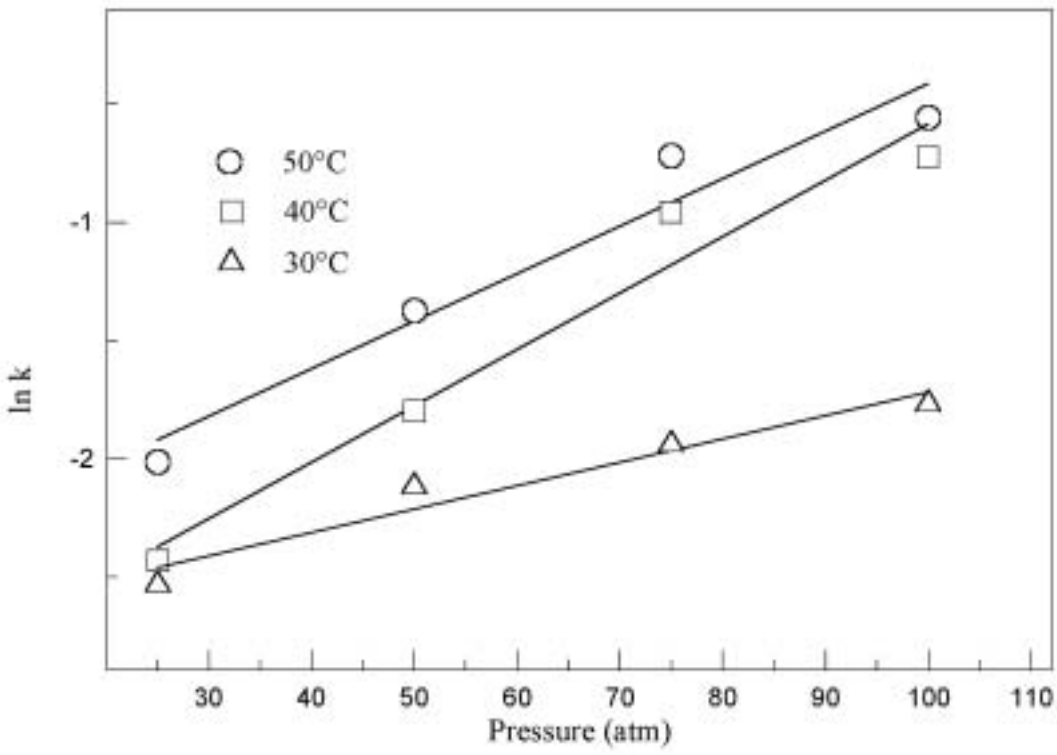


Fig. 2 - Effect of HPCD on the inactivation rate (k) of *S. cerevisiae* in PDB as a function of temperature.

Table 3 - Effect of HPCD on the activation volume (ΔV^*) and z_p values of *S. cerevisiae* in nutrient broth at pressure range 25-100 atm[a].

Temperature	V* (cm^3/mol)		z_p (atm)		
(°C)	$\bar{x}^b$	s_x	$\bar{x}^b$	s_x	r^2
50	-526.6	12.86	114.1	6.24	0.99
40	-605.3	13.63	96.4	2.46	0.99
30	-232.3	20.95	234.2	4.41	0.98

[a] $\bar{x}$ = means and s_x = standard deviations of three determinations, r^2 = regression coefficient.
[b] There are significant differences (P<0.05) between different treatments.

interactions (PALOU *et al.*, 1997). The changes can also occur at different levels and places in the microbial cell and account for the bacterial inactivation. The calculation of an apparent ΔV^* can be useful to describe the pressure and temperature dependence of microbial inactivation rates.

A "pressure z_p value" can be defined as the pressure increment needed to reduce the D value by a factor of 10, and can be calculated as the slope in a plot of log D vs. pressure. The z_p value for *S. cerevisiae* inactivation was about 2.05 times lower at 50°C than that obtained at 30°C (Table 3). The temperature, 30°C, is just below the critical temperature of CO_2. This might explain the low sensitivity of CO_2 pressure at 30°C. These values showed that *S. cerevisiae* was more temperature-sensitive at 50°C. The z_p values were significantly different (P<0.05) among temperatures, indicating the sensitivity to pressure changes was not similar among temperatures. Although it is generally true that a high z_p value corresponds to higher resistance, this does not mean that this is always the case.

The mean pH in PDB (initial 3.80) ranged from 3.79 to 3.75 after treatment of PDB (initial pH 3.8) with HPCD. Further study is needed not only on the effect of pressure and temperature on the inactivation of micro-organisms but also on the effects of other parameters like composition of media or foods (such as pH, peptides, amino acids, sugar and vitamin content), initial yeast count, and pH on the inactivation rates (ERKMEN, 2000b; HAAS *et al.*, 1989; LIN *et al.*, 1994). D values at constant temperature and pressure indicated that pressure and temperature had an effect on the survival of *S. cerevisiae*. This may indicate that processing parameters need to be modified to compensate for temperature and pressure. As a result, HPCD seems to be suitable for inactivating *S. cerevisiae*. The inactivation of yeast at the second stage of the survival curves follows first order kinetics for pressure inactivation.

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SHORT COMMUNICATION

FATTY ACID COMPOSITION OF MEDLAR (*MESPILUS GERMANICA* L.) FRUIT AT DIFFERENT STAGES OF DEVELOPMENT

COMPOSIZIONE IN ACIDI GRASSI DEL FRUTTO DEL NESPOLO (*MESPILUS GERMANICA* L.) A DIVERSI STADI DI SVILUPPO

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ABSTRACT

Changes in the fatty acid composition at different stages of development in the mesocarp of medlar (*Mespilus germanica* L.) fruit was studied by gas liquid chromatography (GLC). Twenty different fatty acids were identified, 10 of which were saturated and 10 unsaturated. The total fatty acid content of the fruit changed throughout development, reaching a maximum value of 6,121 μg/g dry wt by mid-August and thereafter declining to a value of 2,583 μg/g dry wt in the fully ripe fruit (October). Li-

RIASSUNTO

I cambiamenti di composizione del mesocarpo del Nespolo (*Mespilus germanica* L.) a diversi stadi di sviluppo sono stati esaminati mediante gascromatografia (GLC). Sono stati identificati 20 acidi grassi di cui 10 saturi e 10 insaturi. Il contenuto totale in acidi grassi del frutto cambia nel corso del suo sviluppo, raggiungendo un valore massimo di 6.121 μg/g di peso secco a metà agosto per poi diminuire a valori di 2.583 μg/g di peso secco a piena maturazione (Ottobre). L'acido linoleico

- Key words: Development, fatty acid composition, medlar (*Mespilus germanica* L.), ripening -

noleic acid (C18:2n-6) contributed most to the percent composition of the total fatty acids in the lipid extract at all five stages of development, contributing 51% of the total fatty acid content (1,291.7 μg/g dry wt) in fruit harvested in October. Even in the ripest fruit, α-linolenic (18:3n-3) acid contributed 13.5% to the total fatty acid content at 359.9 μg/g dry wt. The combined levels of both linoleic acid and linolenic acid contributed ~65% of the total fatty acids at 1,652 μg/g dry wt.

(C18:2n-6) è il più rappresentativo fra gli acidi grassi in tutti e cinque gli stadi di sviluppo, raggiungendo il 51% del contenuto totale (1.291,7 μg/g di peso secco) nei frutti raccolti in ottobre. Anche nei frutti più maturi, l'acido α-linolenico (18:3n-3) contribuisce per il 13,5% del contenuto totale con valori di 359,9 μg/g di peso secco. I valori combinati di acido linoleico e linolenico rappresentano il 65% del contenuto totale in acidi grassi (1.652 μg/g) di peso secco).

INTRODUCTION

Mespilus germanica L. (medlar) is a small, deciduous, spiny shrub of the *Rosaceae* family that is native to Europe and Asia. The brown apple-shaped fruits of medlar are subglobose or pyriform and crowned by foliaceous sepals, ranging from 1.5-3 cm in diameter (BROWICZ, 1972). Several well-known varieties of medlars in Europe and Asia are Dutsch (big fruit), Common (medium fruit), Royal (small fruit), Nottingham (small tasty fruit) and Stoneless. Medlar fruit is widely consumed by the people in Turkey, especially in northeast Anatolia where most of the medlar varieties are native, but several European cultivars (commonly Dutsch) have also been introduced into the region. Medlar fruits are usually eaten when they are soft and very ripe. The fruit can be harvested directly from the plant in late autumn when overly ripe or it can be harvested at physiological ripeness and then stored in straw until over-ripened. These well known traditions are practiced even today. The very ripe fruits have a sweet, slightly acidic taste. Jams and jellies can be made from the fruit and a surprisingly long list of recipes can be found (BIGNAMI and TSIPOURIDIS, 1998).

The astringency of the fruit was well-known in ancient times and ripe medlar pulp was a popular remedy against enteritis. In the twenties, modern medicine recognized its curative properties for treating constipation, kidney and bladder stones and it is also used as a diuretic (BIGNAMI and TSIPOURIDIS, 1998; BAYTOP, 1999).

Lipids are partly responsible for the physical and chemical properties of food and those that are of major nutritional interest are the fatty acid esters. Many lipid properties in food are explained in terms of their fatty acid composition (BLANCO-GOMIS *et al.*, 2001). Fruit usually contains minor amounts of lipids which are considered precursors of various volatile compounds that contribute to the development of characteristic aromas and flavors (GHOLAP and BANDYOPADHYAY, 1980) and the nutritional value of the fruit (SUPRAN, 1978).

Plant physiologists and food analysts have become interested in the nature and chemical composition of medlar during maturation and ripening. ROMERO-RODRIGUEZ *et al.* (2000) described some of the physical (weight, colour, firmness), physicochemical (pH, soluble solids) and chemical (moisture, soluble sugars, starch, etc.) changes that occur during maturation of Spanish medlars and De

PASCUAL *et al.* (2000) analyzed flavanols in medlar fruit. More recently, GLEW *et al.* (2002) reported the mineral composition of medlar during fruit development. Other than these studies, data on the composition and nutritional value of medlars are scarce, and to our knowledge no data is available in the literature about changes in the fatty acid composition during development and maturation of medlar fruit.

This paper reports the changes in fatty acid composition throughout fruit development and maturation. The information may be useful in understanding the source of the flavors and the nutritional content of medlar. In addition, the optimum harvest times for the maximum or minimum fatty acid composition are also reported.

MATERIALS AND METHODS

Reagents and standards

All reagents were of analytical grade. The calibration and internal standards of the fatty acids were purchased from Nu-Check (GLC-68, Elysian, MN, USA), and the solvents (hexane, chloroform and methanol) were purchased from Burdick and Jackson (Muskegon, MI) and EM Science (Gibbstown, NJ, USA). Hexane was UV grade and other solvents were distilled-in-glass quality. The water used for the analyses was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Fruit sampling

Medlar (*Mespilus germanica* L., wild type) fruit was harvested in 1999 in Trabzon, Turkey. Fruits were harvested in the mid-morning from different parts of several trees located on the grounds of the campus of Karadeniz Technical University and neighboring areas. The blossoms were considered to be in full bloom on May 8, 1999. Five samplings were made from late spring, when the fruit was dark green, to late autumn when the ripe fruit was brown [39, 66 102, 131 and 154 days after full bloom (DAF)]. One kg of medlar fruit was gathered in triplicate at each sampling time. The harvested fruit was stored at -80°C and freeze-dried. After lyophilization, the hard, dried fruits were broken up with a steel hammer and then ground to a fine powder using a stainless steel mill.

Extraction

Samples of finely ground powder of medlar mesocarp (1 g) in triplicate were weighed and extracted with chloroform: methanol (2:1, v/v) (CHAMBERLAIN *et al.*, 1993) and a saline solution (NaCl, 0.9%) was added at a rate of 20% of the extraction volume. The mixture was shaken and centrifuged (IEC HN-SII Benchtop Centrifuge, International Equipment Company, Needham Heights, MA, USA) at 1,000 rpm for 5 min to allow phase development. The bottom (organic) layer was collected and filtered. The total extracted lipid material was recovered after the solvent was removed in a stream of nitrogen. The samples were dissolved in anhydrous chloroform. Sixteen micrograms of triheptadecanoin (containing 3 molecules of heptadecanoic acid; 17:0) and a 0.1 mL aliquot of the lipid sample were transferred to a 15 mL teflon-lined screw tube. Fatty acid methyl esters were obtained using 14% (w/v) boron trifluoride (BF_3) in methanol (MORRISON and SMITH, 1964). After removing the solvent by nitrogen gas, the sample was mixed with 0.5 mL of the BF_3 reagent and placed in a warm bath at 100°C for 30 min. After cooling, saline (NaCl, 0.9%) and hexane were added and the fatty acid methyl esters were extracted into the hexane. A mixture of known fatty acid methyl ester standards was used to calibrate the gas chromatograph and to identify the fatty acid methyl ester peaks.

Gas chromatography

Aliquots (1-2 μL) of the hexane solution containing the fatty acid methyl esters were analyzed using a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a fused-silica capillary column (Omegawax; 30 m x 0.32 mm I.D., Supelco, Bellefonte, PA) and a flame-ionization detector. The injector temperature was set at 200°C, detector at 230°C, oven at 120°C initially, then 120°-205°C at 4°C/min, and then held at 205°C for 18 min. The carrier gas was helium (99.999%) and the flow rate was approximately 50 mL/sec. Electronic pressure control in the constant flow mode was used. The fatty acids are reported as the average of three determinations conducted on three independent assays. The internal standard (heptadecanoic acid, 17:0) and a calibration mixture of fatty acid standards (GLC-68, Nu-Check, Elysian, MN, USA) were used to identify and quantify the fatty acids in the various lipid extracts.

RESULTS AND DISCUSSION

Time of harvest, stages of fruit maturity, fruit weight and diameter are shown in Table 1. Gas chromatographic analysis of the fatty acids in the total lipid fraction of the medlar fruit revealed the presence of 20 different fatty acids, 10 of which were saturated and 10 unsaturated (Table 2). Palmitic acid (C16:0), stearic acid (C18:0), linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3) were the principal fatty acids. The total fatty acid content (sum of the individual fatty acids) of the fruit changed throughout development, reaching a maximum value of 6,121 μg/g dry wt by mid-August (102 DAF) and declining thereafter to 2,583 μg/g dry wt in the fully ripe fruit at 154 DAF.

Linoleic acid (C18:2n-6), which is an essential nutrient for humans, contributed most to the total fatty acid content in the lipid extract. Linoleic acid made up 48% of the fatty acids in the fruit that was harvested in August and September. In the ripest fruit at 154 DAF (harvested October 8), this acid contributed 51.0% of the total fatty acid content with 1,291.7 μg/g dry wt. The second most abundant unsaturated fatty acid was a-linolenic acid (C18:3n-3), which contributed only 13.5% of the total fatty acid content with 359.9 μg/g dry wt at 154 DAF (Fig. 1, Table 2). However the highest content of α-linolenic acid was recorded at the earliest stage of development at 39 DAF when the medlar fruit was unripe and light green.

The palmitic acid content was the lowest between June and August, from 16.2% to 9% (Fig. 1), it reached the highest level at 102 DAF (546 μg/g dry wt, Table 2). The sum of the oleic acids (C18:1n-5, C18:1n-7 and C18:1n-9) varied greatly during development, increas-

Table 1 - Harvest dates and stages of fruit maturity as days after full bloom (DAF), and fruit weight and diameter of medlar (*Mespilus germanica* L.).

Harvest number	Harvest date	DAF	Fruit colour, state of fruit maturity	Fruit Weight (g)	Fruit Diameter (cm)
1.	15 June 1999	39	Unripe, light green	2.9±0.1	0.7±0.1
2.	12 July 1999	66	Unripe, greenish-light brown	5.1±0.1	1.4±0.2
3.	17 Aug 1999	102	Unripe, fully light brown	6.6±0.1	2±0.1
4.	15 Sep 1999	131	Half ripe, fully brown	8.8±0.1	2.5±0.2
5.	8 Oct 1999	154	Ripe, brown	7.5±0.2	2.9±0.2

Table 2 - Total amounts of fatty acids (μg/g dry wt), percent of unsaturated (%US), saturated (%S) and unsaturated/saturated (U/S) ratio during fruit development and maturation of medlar[x].

	DAF (days after full bloom)				
Fatty acids	39	66	102	131	154
C10:0	6.2±0.2 a	9±0.9 a	6.5±1.3 a	7.7±0.7 a	6.7±0.4 a
C12:0	7.3±0.1 a	8±1.9 a	7.9±0.7 a	4.7±0.0 a	6.9±2.9 a
C13:0	11.7±1.9 a	13.2±0.9 a	12.3±0.0 a	7.1±0.0 a	7.7±2.2 a
C14:0	13.1±0.1 c	13.2±0.5 c	12.1±1.2 bc	10.3±0.2 ab	9.6±0.4 a
C14:1	11.7±0.3 a	14.7±0.3 b	11.7±0.5 b	5.7±0.4 a	7.4±1.9 a
C15:0	7.7±0.7 b	4.5±0.2 a	2.8±0.7 a	3.2±0.4 a	3.6±0.1 a
C16:0	368.4±14.4 a	417.7±8.8 ab	546±41.1 c	470.7±1.5 bc	420±9.6 ab
C16:1	10.3±0.3 b	10.1±0.3 b	10.4±0.4 b	10.5±0.4 b	8.9±0.2 a
C18:0	89.9±0.6 a	92.2±2.8 b	123.4±2.0 c	70±4.5 a	68.0±3.1 a
C18:1n-9	268.3±3.9 b	458.9±15.4 d	1,132.2±27.7 e	332.5±2.9 c	250.6±1.7 a
C18:1n-7	20.1±0.5 a	24.1±0.6 b	31.9±1.2 c	25.0±0.4 b	24.1±0.6 b
C18:1n-5	3.0±0.5 b	7.1±0.0 c	2.4±0.0 a	N.D.	N.D.
C18:2n-6	821.6±6.9 a	1,395.7±47.2 bc	3,668.3±65.4 d	1,475.2±16.6 c	1,291.7±7.7 b
C18.3n-3	503.1±6.7 c	534.2±2.8 d	339.2±1.9 a	495.9±2.9 c	359.9±3.2 b
C20:0	46.4±0.9 b	53.2±2 c	88.2±1.1 d	47.1±0.2 b	36.6±1.05 a
C20:1n-9	4.8±0.1 a	22.0±1.6 c	18±1.2 b	4.7±0.2 a	4.2±0.2 a
C20:1n-7	10.5±0.4 ab	9.1±0.6 a	12.3±0.4 c	11.1±0.3 bc	9.4±0.8 a
C22:0	59.4±0.4 d	54.0±0.4 c	55.6±1.4 cd	45.3±1.4 b	39.7±1 a
C22:1n-9	3.3±0.2 a	3.8±0.1 a	4.6±0.1 b	3.5±0.2 a	3.3±0.0 a
C24:0	22.4±0.0 a	26.2±0.0 bc	35.6±0.2 d	28.1±0.9 c	24.6±0.6 b
TFAT[y]	2,289±18	3,171±72	6,121±92	3,058±24	2,583±5 a
%US	72.7±0.2 a	78.2±0.2 c	85.3±0.0 d	77.2±0.2 c	76.2±0.3 b
% S	27.5±0.2 d	22.1±0.2 b	14.7±0.1 a	22.9±0.2 b	23.9±0.3 c
U/S	2.7±0.0 a	3.6±0.0 c	5.8±0.0 d	3.4±0.01 bc	3.2±0.1 b
Other acids[z]	215±1.6 c	241±3.0 d	278±0.6 e	189±0.2 b	169±2.3 a

[x] Each value is the mean ±SD of triplicate extractions and determinations.
[y] TFAT; Total fat is given as the sum of individual absolute amount of fatty acids identified in the fruit. Values in the same row for each stage having the same letter are not significantly different at P = 0.05.
[z] The other acids category is the sum of C10:0, C12:0, C13:0, C14:0, C14:1, C15:0, C16:1, C20:0, C20:1n-7, C20:1n-9, C22:0, C22:1n-9 and C24:0.

ing from a low value of 291 μg/g dry wt (12.8%) in June to 1,167 μg/g dry wt (18.8%) in August and then declining to 275 μg/g dry wt (10.4%) at 154 DAF (in October 8) (Fig. 1, Table 2).

The medlar fruit contained very small amounts (0.1-2%) of C10-15, C16:1 and C20-24 fatty acids. These amounts were summed and are reported as "other acids" in Fig. 1. The total amount of "other acids" was 215 μg/g dry wt at 39 DAF, and the level rose to 278 μg/g dry wt at 102 DAF, similar to the increase in the major fatty acids, and then leveled off at its minimum content at 154 DAF (169 μg/g dry wt) (Table 2). The unsaturated/saturated ratio of the fatty acids changed significantly (P = 0.05) throughout development, reaching a maximum value of 5.8 in the August fruit (102 DAF), and the values were between 3.4 and 3.2 in half ripe and ripe fruits, respectively (Table 2). The unsaturated/saturated ratio, as well as the percent of

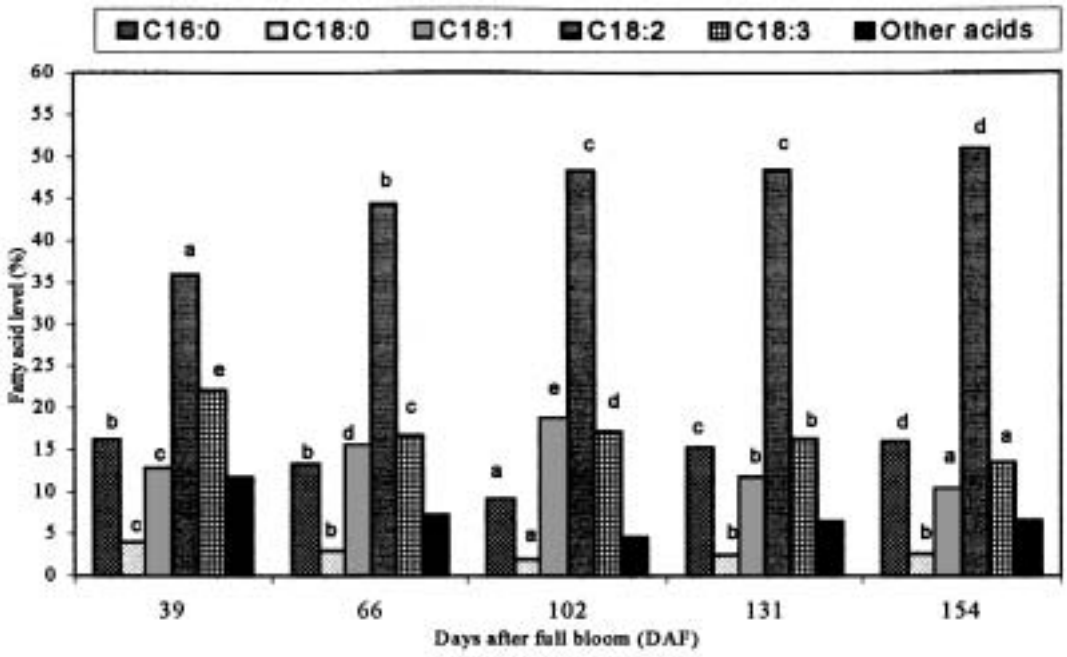


Fig. 1 - Percentage composition of the most relevant major fatty acids of medlar (*M. germanica*) during fruit development (detection limit was <0.01. The other acids category is the sum of C10:0, C12:0, C13:0, C14:0, C14:1, C15:0, C16:1, C20:0, C20:1n-7, C20:1n-9, C22:0, C22:1n-9 and C24:0. Values are the mean ±SD of triplicate extractions and determinations. Values with the same letter on the column of each individual acid are not significantly (P = 0.05) different.

unsaturated and saturated fatty acids increased gradually reaching the maximum (85 and 14.7%, respectively) at 102 DAF, and then remaining low through 131 and 154 DAFs, at 76.2 and 23.9%, respectively, (Table 2).

Fruit species have characteristic fatty acid compositions and profiles during development and ripening. In general, the C16:0 (palmitic acid), C16:1 (palmitoleic acid), C18:1 (oleic acid), C18:2 (linoleic acid) and C18:3 (linolenic acid) are the most predominant and abundant fatty acids in fruit during development, maturation and ripening, but generally, the levels and proportions of these acids depend on the type of fruit. For example, C16:1 (~18-31%) was found to be the most abundant in ripening mango fruit (BANDYOPADHYAY and GHOLAP, 1973), C18:1 (~60-79%) during the development and maturation of avocado fruit (RATOVOHERY *et al.*, 1988; MARTINEZ-NIETO and MORENO-ROMERO, 1995), and 18:2 (~28-34%) during maturation of sweet orange cultivars (NORDBY and NAGY, 1979). In small fruits, such as blueberries, the level of C18:2 (~40-45%) was the most predominant fatty acid in the fruits of highbush bluberry varieties (Coville, Darrow, Herbert, Jersey and Bluetta) (WANG *et al.*, 1990). In cherry laurel fruit, a member of the *Rosaceae* family like medlar, C18:2 was also the predominant fatty acid during fruit maturation. It made up ~53% (2,046 mg/100g dry wt) of the total fatty acid composition (AYAZ and KADIOGLU, 2000). In contrast to the above fruits, in peach (cv. Redhaven) C16:0 (~30-35%) was the most abundant free fatty acid during fruit development and ripening, while the level of C18:2 was ~25% in the ripest peach fruit (IZZO *et al.*, 1995).

To sum up, the content of the major fatty acids (C16:0, C18:0, C18:1, C18:2, C18:3) increased continually from 39 until 102 DAF. Only the level of C18:3 continued to increase until 131 DAF. The increase in the fatty acid content until 102 DAF is expected, because from 39 to 102 DAF the increasing quantities of fatty acids indicate intense metabolic activity and a rapid rate of lipid synthesis in the mesocarp cells. This phase is characterized by rapid cell expansion, with a consequent increase in the cell volume of the mesocarp and the formation of large intercellular spaces. From 39 to 102 DAF, the size of the medlar fruit increased rapidly with a continuous increase in dry weight (Table 1). The decrease in fatty acid content in September and October (131 and 154 DAFs) was due to an accumulation of carbohydrates rather than lipids in the fruit. During this period fructose (22-35 mg/100g dw) and glucose (13-23 mg/100g dw) levels increase continually (ROMERO-RODRIGUEZ *et al.*, 2000).

Research in human nutrition and health has shown a relationship between diet and the increasing frequency of lifestyle diseases among the people in industrialized countries (SKJERVOLD, 1993). Nutritional deficiency is mainly due to the low content of n-3 (Omega-3) fatty acids in the diet and HORROBIN (1993) and INNIS (1996) have shown the dietary importance of these essential fatty acids and their relationship to human health. The polyunsaturated fatty acids, linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3), are essential to the human diet but cannot be synthesized (SINCLAIR, 1990; INNIS, 1996). Medlar fruit contains these essential fatty acids, and the combined levels of linoleic acid and linolenic acid make up ~65% of the total fatty acid content (Fig. 1) at 1,652 μg/g dry wt (Table 2). Even though the levels of these two essential fatty acids are not as high as those in other well-known sources such as olive oil, soya oil, hazelnut, walnut, peanut, etc., consuming medlar during its rapid ripening period would supply some of the Omega-3 fatty acid requirements in the diet. The information obtained on the fatty acid composition in medlar fruit throughout its development and maturation is of interest to consumers and food technologists, as well as to plant breeders for selecting *M. germanica* with improved nutritional quality.

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OCCURRENCE OF OCHRATOXIN A IN CEREALS FROM PUGLIA (ITALY)

PRESENZA DI OCRATOSSINA A IN CEREALI DELLA PUGLIA

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ABSTRACT

The presence of Ochratoxin A in farm-stored cereal grains from the Puglia region was surveyed. Two hundred ten cereal samples (70 durum wheat, 70 maize, and 70 barley) were analysed using liquid chromatography after extraction and clean-up of the samples with solid phase micro columns and fluorescence detection. Ochratoxin A was found in 19 samples of maize, 8 of barley and 6 of wheat, with a positive incidence of 15.7% and a mean contamination of 1.6±1.4 ng/g. Seventeen cereal samples (8.1%) had Ochratoxin A levels in the LOD range (0.05 ng/g) -0.9 ng/g, 15 samples (7.1%) in the range of 1.0-4.9 ng/g, and only 1 maize sample (0.5%) had a val-

RIASSUNTO

È stata condotta una indagine sulla presenza di Ocratossina A in campioni di cereali prodotti in diverse aziende della Puglia. La determinazione di Ocratossina A è stata effettuata mediante cromatografia liquida, previa estrazione e purificazione dei campioni con microcolonne in fase solida. Sono stati esaminati 210 campioni (70 di grano duro, 70 di orzo e 70 di mais). La presenza di Ocratossina A è stata riscontrata in 19 campioni di mais, 8 di orzo e 6 di grano, con una incidenza risultante del 15,7% ed un livello medio di contaminazione di 1,6±1,4 ng/g. Diciassette campioni di cereali (8.1%) avevano livelli di Ocratossina A nell'intervallo LOD (0,05 ng/g) -0,9 ng/g, 15 campioni (7,1%) nell'intervallo 1,0-4,9 ng/

- Key words: barley; crops; durum wheat; HPLC; maize; mycotoxin -

ue (5.2 ng/g) higher than the maximum limit of tolerance of Ochratoxin A in cereals (5.0 ng/g) as fixed by the European Community.

g e solamente un campione di mais (0.5%) presentava un valore (5,2 ng/g) superiore a 5,0 ng/g, che rappresenta il valore massimo ammissibile per l'Ocratossina A nei cereali fissato dal Regolamento CE 472/2002.

INTRODUCTION

Ochratoxin A (OA), a secondary metabolite with teratogenic, hepatotoxic, carcinogenic and nephrotoxic activity in many animal species, including human beings (HOHLER, 1998), is mainly produced by *Aspergillus ochraceus* and *Penicillium viridicatum*. Being ubiquitous, these moulds can easily contaminate foodstuffs. OA occurs predominantly in cereal grains, cereal products, cocoa, spices, oilseeds, coffee beans and legumes. Moreover, OA has been identified in tissues of animals fed contaminated feed.

The International Agency for Research on Cancer (IARC, 1993) has classified OA as Group 2B, a possible human carcinogen. In several areas of Eastern Europe, where chronic exposure to OA occurs, involvement of this mycotoxin in the cancer aetiology of the urinary system, and in kidney pathologies typical of Balkan endemic nephropathy (BEN) has been suspected. Studies on the correlation between OA and BEN (JURJEVIC *et al.*, 1992; VRABCHEVA *et al.*, 2000; PUNTARIC *et al.*, 2001) have shown higher OA contamination level in cereals from endemic areas with respect to cereals from non-endemic areas.

The European Union's Scientific Committee for Human Feeding deemed it necessary to take prudential measures aimed at reducing the degree of exposure to OA to below the daily level of 5 ng/kg b.w.. For this reason, the maximum admissible levels of OA have been recently fixed by EC Regulation 472/2002 (E.C.R., 2002), although restricted to few foodstuffs such as cereals, processed cereals and raisins. For cereals (rice and buckwheat included) the maximum limit is 5.0 ng/g, for raisins it is 10 ng/g, and for processed cereals and cereals destined for direct human consumption it is 3.0 ng/g. At present, in Italy, the legal limit for OA in other foodstuffs such as coffee beans, cocoa, spices, wine, grape-juice and beer is 3.0 ng/g, according to the Italian Ministerial Circular of June 1999 (C. M., 1999).

The present survey aims at contributing to the knowledge of the overall status of OA contamination levels in some cereals produced in Puglia with respect to the regulatory standards of the European Community. This is in compliance with the requirements of the National Health Service to the official laboratories for severe and accurate control of this very dangerous contaminant.

MATERIALS AND METHODS

Samples

The cereals examined (70 durum wheat, 70 barley and 70 maize samples) were collected between the harvest of 1999 and the following harvest of 2000 by sampling cereals produced and stored on farms chosen from among the major cereal producers in the province of Foggia. The 210 farms were grouped in 6 large areas surrounding the cities of Foggia (20, 20, 13), Lucera (10, 20, 20), San Severo (10, 10, 7), Cerignola (10, 10, 10), Manfredonia (10, 5, 10), and Trinitapoli (10, 5, 10).

Samples were drawn from three different levels of the cereal storage bins and combined to have a 1.5-2 kg bulk sample, from which a 500 g sub-sample was drawn, according to the Italian Ministerial Decree of 23 December 2000 (D.M., 2000), the EC Directive 53/98 (D.C.E., 1998), and, specifically for OA, the EC Regulation 472/2002 (E.C.R., 2002).

All samples were analysed at the "Istituto Zooprofilattico Sperimentale della Puglia e Basilicata", according to the methods specified by the Italian Ministry of Health (D.P.R., 1997) aimed at consumer protection. Just before analysis, each sample was ground with a Cemotec 1090 mill (Tecator, Helsing Borg, Sweden) to a particle size of <1 mm, thoroughly homogenised, and a 25 g portion was taken for analysis.

The determination of OA in the ground cereal samples was carried out using the procedure described by NESHEIM *et al.* (1992). Briefly, OA was extracted with chloroform-aqueous phosphoric acid, and isolated by liquid-liquid partitioning into an aqueous bicarbonate solution. The extract was transferred to a C18 solid-phase extraction (SPE) column, and OA was eluted with ethyl acetate-methanol-acetic acid. OA was identified by reversed-phase Liquid Chromatography (LC) and quantified by fluorescence detection.

Reagents

Chloroform, ethyl acetate, benzene, methanol, water were HPLC grade; acetic acid, sodium bicarbonate and phosphoric acid were ACS grade (J.T. Baker, Koeln, Germany). Celite 545 was obtained from Merck (Darmstad, Germany) and SPE-Isolute-C18 microcolumns from Intern. Sorbent Techn. (Mid Glamorgan, U.K.). Standard OA was purchased from Sigma (Sigma Chemical Co., St Louis, Missouri, USA). All the standard solutions of OA were prepared from a standard stock solution (0.24 mg OA in 10 mL benzene-acetic acid mixture (99+1)) just before use. The standard stock solution was checked for the concentration of OA as in AOAC method 973.37C (AOAC, 1990).

Apparatus

The chromatographic system was a Waters Model 600E multi-solvent delivery system (Waters, Milford, MA, USA) equipped with a Waters Model U6K universal injector. A Waters Model 579 fluorescence detector and a Waters model 5200 integrator-recorder (Milford, MA, USA) were used for OA detection and quantification.

Chromatographic conditions

The LC column was a Supelcosil C-18 reversed-phase packing, 4.6x250 mm, 5 µm film thickness (Supelco, Bellefonte, PA, USA). The mobile phase was a water-acetonitile-acetic acid (99+99+2) mixture. The flow rate was 0.8 mL/min, the injection loop was 20 µL and the temperature was 30°C. The fluorescence detector was regulated at 330 nm for excitation and at 470 nm for emission wavelengths.

Analytical quality assurance

For internal quality control, the linearity of the response, y (peak area counts) versus concentration c (ng/g) plot was tested using four standard solutions (0.5, 1.5, 3.5, 9.0 ng/mL) of OA in benzene-acetic acid, each injected in quadruplicate. The linearity over this concentration range was satisfactory (r^2=0.989). The precision of the method was checked on a maize sample using a series of 10 replicates. For a mean OA concentration value of 4.8 ng/g found, a standard deviation of 0.4 ng/g and a coefficient of variation of 8.3% were obtained.

The accuracy was evaluated through recovery assays at three fortification levels (1.0, 2.0, 4.0 ng/g) of OA in uncon-

Table 1 - Frequency of distribution of ochratoxin A in cereal grains from Puglia.

Cereal	Samples tested n.	Positive n (%)	Frequency distribution of samples				Contamination	
			<LOD[a] n (%)	LOD -0.9 ng/g n (%)	1.0-4.9 ng/g n (%)	≥5.0 ng/g n (%)	Range ng/g	Mean±σ ng/g
Durum wheat	70	6 (8.6)	64 (91.4)	5 (7.1)	1 (1.4)	-	0.4-1.4	0.8±0.3
Maize	70	19 (27.1)	51 (72.8)	10 (14.3)	8 (11.4)	1 (1.4)	0.2-5.2	1.7±1.6
Barley	70	8 (11.4)	62 (88.6)	2 (2.8)	6 (8.6)	-	0.7-3.9	2.1±1.1
Total	210	33 (15.7)	177 (84.3)	17 (8.1)	15 (7.1)	1 (0.5)	0.2-5.2	1.6±1.4

[a)] LOD=0.05 ng/g.

taminated maize samples. Recoveries, were respectively, 76, 83 and 91%. The detection limit of the method was 0.05 ng/g of OA at a signal-to-noise ratio of 3.

Confirmation of OA by methyl ester derivative formation was deemed unnecessary since the original method (NESHEIM *et al.*, 1992) was thoroughly validated by the authors and by 11 collaborators independently using this procedure on the same matrices (corn and barley) investigated in this paper.

RESULTS AND CONCLUSIONS

Table 1 reports the results on the OA levels in the 210 cereal samples analysed. OA was detected in 15.7% of the samples, with contamination levels ranging from 0.2 to 5.2 ng/g with a mean of 1.6±1.4 ng/g. Only one maize sample (0.5%) had an OA content (5.2 ng/g) above the maximum limit of tolerance for OA in cereals (5.0 ng/g).

The highest frequency of contamination was observed in the 70 maize samples, with 19 positive cases (27.1%) ranging from 0.2 to 5.2 ng/g and a mean concentration of 1.7±1.6 ng/g. For the 70 durum wheat and 70 barley samples, the positive cases were 6 (8.6%) and 8 (11.4%), respectively, with OA contamination levels ranging from 0.4 to 1.4 ng/g and 0.7 to 3.9 ng/g, respectively. Wheat was the least contaminated cereal with OA ranging from 0.4 to 1.4 ng/g and the lowest mean concentration of 0.8±0.35 ng/g. Barley had the highest mean level of OA contamination with 2.1±1.1 ng/g.

Table 2 reports the distribution of the

Table 2 - Distribution of the OA positive cereal samples in different areas of Puglia.

Origin	Product								
	Wheat			Maize			Barley		
	Samples tested n.	Positive n (%)	Average (ng/g)	N. tested samples	Positive n (%)	Average (ng/g)	N. tested samples	Positive n (%)	Average (ng/g)
Foggia	20	-		20	6 (30)	1.6 ±1.7	13	2 (15)	0.7±0.1
Lucera	10	-		20	2 (10)	0.6±0.1	20	-	
S. Severo	10	3 (30)	0.6±0.2	10	3 (30)	2.0±0.6	7	2 (28)	2.4±0.1
Cerignola	10	-		10	2 (20)	0.6±0.2	10	1 (10)	1.3
Manfredonia	10	3 (30)	0.9±0.5	5	3 (60)	0.9±0.5	10	1 (10)	2.2
Trinitapoli	10	-		5	3 (60)	4.0±0.2	10	2 (20)	3.4±0.7

OA positive samples, in the areas where the sampled cereals were produced. OA contamination in wheat was found in two of the six areas, each with 3 positive cases out of 10 tested samples with mean concentrations of 0.6±0.2 ng/g and 0.9±0.5 ng/g, respectively. OA contaminated maize samples were found in all six areas. The maize samples with the highest OA values, ranging from 0.2 ng/g to 5.2 ng/g and with an average of 4.0±0.2 ng/g, were found in the Trinitapoli area. OA contamination in barley was found in all the localities except for Lucera, with incidences ranging from 10 to 28%. Also for barley samples, the highest values of OA were found in the Trinitapoli area with a mean of 3.4±0.7 ng/g. OA contamination in wheat appeared to be limited to only two areas, while for maize and barley it was ubiquitously distributed throughout the Foggia province.

Probably due to the lack of systematic research programs, there are very few studies on the presence of OA in cereals in Italy. In a study on mycotoxin contamination in Italy MIRAGLIA *et al.* (1998), determined the OA concentration in some cereals and cereal products collected from 1990 to 1996. They found percentages of OA positive samples ranging from 20 to 100%, and mean concentrations from 1.8 to 5.3 ng/g. In a study on the use of various clean-up procedures for the analysis of OA in cereals, SOLFRIZZO *et al.* (1998) reported the presence of OA in 21 out of 69 rye samples collected from different regions of Italy, with a mean concentration of 13.4 ng/g (see Table 3). The frequencies of positive samples and the OA mean concentrations reported by these authors were general-

Table 3 - Occurrence of ochratoxin A in cereals and cereal products in different countries as found in the literature.

Reference	Year	Sample origin	No of tested samples (No of positive samples)	Positivity %	Range ng/g
BACHA *et al.*	1988	Tunisia	100 (70)	70	180-320
GOLINSKI *et al.*	1988	Poland	688 (144)	21	3.06 (average)
FUKAL	1990	Czechoslovakia	100 (8)	8	>20
KORDIC *et al.*	1990	Serbia	344 (20)	5.8	0.3-2.5
RANFFT *et al.*	1990	Germany	100 (18)	18	>0.75
MAFF-UK	1994	UK	611 (2)	0.3	1-15
JURIC *et al.*	1994	Yugoslavia	155 (42)	36.9	10-240
MAFF-UK	1995	UK	450 (39)	8.7	1-33
MAFF-UK	1996	UK	67(37)	(55.2)	0.2-7
MAFF-UK	1997	UK	152 (7)	4.6	0.2-6.4
MIRAGLIA *et al.*	1998	Italy	178 (70)	39.0	1.8-5.3
SCUDAMORE *et al.*	1998	UK	67 (2)	3.0	2.0 (average)
SOLFRIZZO *et al.*	1998	Italy	69 (21)	30.4	13.4 (average)
MAFF-UK	1999 a	UK	300 (63)	21	0.2-17.8
TRUCKSESS *et al.*	1999	USA	486 (67)	13.8	0.03-5.0
			486 (5)	1.0	>5.0
MAFF-UK	1999 b	France	97(11)	11.3	0.1-1.4
		Argentina	37 (1)	2.7	0.1-0.3
		Europe	5 (2)	40.0	0.1-1.5
PUNTARIC *et al.*	2001	Croatia-Slovenia	143 (91)	63.6	0.02-160
VRABCHEVA *et al.*	2000	Bulgaria	20 (7) a	35	1.5 (average)
			74 (27) b	36.5	9.9 (average)
WOLFF	2000	Germany	2374 (33)	1.4	>3

ly higher than those found in the present study. The discrepancy in the results may be partly ascribed to the modest number of samples and/or to the matrix difference of the cereals examined.

Table 3 summarizes the literature data on the occurrence of OA in cereals and processed cereals since 1988. Except for the cases of OA occurrence in Tunisia (BACHA *et al.*, 1988), Yugoslavia (JURIC *et al.*, 1994), Bulgaria (VRABCHEVA *et al.*, 2000) and Croatia and Slovenia (PUNTARIC *et al.*, 2001) where the high incidence of positive cases and contamination level reflect the endemic condition of exposure to OA, the results of the present survey are comparable with those reported by the other authors.

In conclusion, the very low percentage (0.5%) of cereal samples with an OA concentration above the EU legal limit is a reassuring finding on the overall status of the OA contamination level in the cereals produced in Puglia. But, the presence in some cereal samples of OA levels close to the tolerable limit indicates that the surveillance of OA in cereals and other matrices should be continued, in compliance with the EU programs on food quality assurance.

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MANSHOLT PRIZE AWARDED TO SLOW FOOD MOVEMENT

On November 7, in Brussels, Commission President Romano Prodi presented the 2002 Mansholt Prize to Slow Food, a world-wide movement committed to promoting the diversity of local and regional quality food. The bi-annual € 25,000 prize is named after Sicco Mansholt (1908-1995), one of modern Europe's founding fathers and its foremost agricultural leader. The prize seeks to stimulate inspiring, integrating and innovative ideas in the field of sustainable agriculture and a fair trade system.

The Slow Food Movement is the first recipient of the Mansholt Prize. Founded in Italy in 1986, the movement now has 74,000 members in 50 countries. It is headquartered in Bra in the Italian province of Piemonte. One of Slow Food's major projects is The Ark of Taste, a massive effort to rescue an increasingly endangered biodiversity. The aim is to identify and catalogue products, dishes and animals that are in danger of disappearing. Another important project is the Slow Food Award for the Defense of Biodiversity, which was first presented in 2000.

IAFP 2003 CALL FOR ABSTRACTS

The International Association for Food Protection is now accepting abstracts for IAFP 2003 to be held in New Orleans, Louisiana on August 10-13, 2003.

The deadline for submissions is January 6, 2003. IAFP accepts abstracts online or via E-mail.

Abstract submission, general information and instructions, Speaker Reference Guide, Policy for Commercialism and information on the Developing Scientist Awards Competition are available at our Web site, www.foodprotection.org.

Poster or oral presentation formats are available. Abstracts are limited to 250 words and must report the results of original research pertinent to the subject matter. Papers may also report subject matter of an educational and/or nontechnical nature. Abstracts submitted for IAFP 2003 will be evaluated for acceptance by the Program Committee. Information in the abstract data must not have been previously published in a copyrighted journal.

The Annual Meeting has become the leading meeting concerned with the protection of the world-wide food supply. Over 1,400 top industry, academic and government food safety professionals attend each meeting.

Questions regarding abstract submission should be directed to Bev Corron, IAFP Public Relations, at bcorron@foodprotection.org or call 515.276.3344; 800.369.6337.

FOOD PROTECTION TRENDS TO DEBUT IN JANUARY

Beginning January 1, 2003, Dairy, Food and Environmental Sanitation will change its name to Food Protection Trends. The journal also incorporates the by-line "Science and News from the International Association for Food Protection".

Food Protection Trends is the primary vehicle for communication to Association members. The journal introduces a new look although the content remains the same. Each issue contains refereed

articles on applied research, applications of current technology and general interest subjects for food safety professionals. Regular features include industry and association news, an industry-related products section and a calendar of meetings, seminars and workshops. Updates of government regulations and sanitary design are also featured.

All IAFP Members receive Food Protection Trends, which is read by over 9,000 individuals around the world.

6TH INTERNATIONAL SYMPOSIUM ON SUPERCRITICAL FLUIDS
April 28-30 2003
Versailles (France)

The program will comprise three plenary lectures (45 min), oral communications (20 min) and posters sessions. An exhibition of supercritical fluid Service and Equipment (process, analytical, control) will be organized.

Supercritical fluid processes open promising opportunities to manufacture new products from natural or synthetic sources, to "design" new materials with specific properties or to improve incal materials or to propose new waste management routes.

The purpose of this meeting is to reflect recent progresses in areas of great scientific interest as well as industrial developments.

Sessions will be organized according to all topics related to Supercritical fluids, both on fundamental aspects and on applications in various industries (food, pharmaceuticals, nutraceuticals, cosmetics, electronics, textile, fine chemistry, waste management,...) including industrial scale-up and economical evaluation.

Secretary: Françoise Brionne - ISASF - ENSIC, 1, rue Grandville - BP 451 - F 54001 Nancy - France; e-mail: brionne@ensic.inpl-nancy.fr - Fax: +33 (0)3 83350811. Phone: +33 (0)3 83175003.

UPCOMING SCIENTIFIC MEETINGS

2003 – 16th International Symposium, Exhibit and Workshops on reparative/ Process Chromatography, lon Exchange, Adsorption/Desorption Processes & Related Separation Techniques. Westin St. Francis Hotel, San Francisco, CA, USA. Chairman: Dr. Georges Guiochon (www.prepsymposium.org). June 29-July 2, 2003.

- ISPPP-2003: 23rd International Symposium on the Separation of Proteins, Peptides, and Polynucleotides. Delray Beach/West Palm Beach, Florida, USA. Co-chairs: Dr. Joseph J. DeStefano and Dr. Mark Schure (www.isppp.org). November 9-12, 2003.

2004 – PREP-2004: 17th International Symposium, Exhibit and Workshops on Preparative/Process Chromatography, lon Exchange, Adsorption/Desorption Processes & Related Separation Techniques. Chairman: Dr. Georges Guiochon (www.prepsymposium.org). May, 2004.

- HPLC-2004: 28th International Symposium & Exhibit on High Performance Liquid Phase Separations & Related Techniques. Philadelphia Convention Center, Philadelphia, Pennsylvania, USA. Chairman: Dr. Mark R. Schure (www.hplcsymposium.org). June 12-18, 2004.

- CHIRALITY - 2004: 16th International Symposium on Chirality (ISCD). Kimmel Center for University Life at New York University, New York City, New York, USA. Chair: Prof. James Canary; Co-chairs: Prof. Nina Berova & Dr. Chris Welch (www.nyu.edu/ chirality). July 11-14, 2004.

2005 – PREP-2005: 18th International Symposium, Exhibit and Workshops on Preparative/Process Chromatography, lon Exchange, Adsorption/Desorption Processes & Related Separation Techniques. Loews Hotel, Philadelphia, PA, USA Chairman: Dr. Georges Guiochon (www.prepsymposium.org). May 8-11, 2005.

BOOKS

NEW GUIDE TO PESTICIDE REGULATIONS

A new review from CCFRA explains the regulatory framework surrounding pesticides, which is an important part of food and environment safety and quality assurance systems.

The regulations relating to pesticide registration and setting of Maximum Residue Levels (MRLs) can be confusing - not least because there are several sets of regulation and standards that need to be addressed (e.g. UK, EU, Codex), some of which are being harmonised. An overview of pesticide legislative controls and surveillance (CCFRA Review No. 33) explains the legislative controls of pesticide use in the UK and describes the surveillance exercises carried out in support of the regulations.

It includes 2 appendices that summarise typical surveillance data from the UK and results of studies into the effects of processing on pesticide residue levels.

The major sections cover the harmonisation of European pesticide registration – including pesticide approval, off-label approval and pesticides approved for use in organic agriculture – as well as a summary of Codex, EU and UK maximum residue levels and their interrelation.

A third section provides an overview of UK, EU and US pesticide residue surveillance programmes. The Review will be of interest to anyone involved in food-related pesticide issues.

For further details contact Mrs Sue Hocking +44(0)1386 842225 or e-mail: pubs@campden.co.uk.

FUNCTIONAL FOODS NEW REPORT FROM CCFRA

A new report from CCFRA will help companies to target the marketing of new functional foods by understanding consumer awareness of and attitudes towards these products. The emergence of the market for functional foods reflects a change in attitude amongst some groups within the population, away from "balanced" diet for "adequate" nutrition towards optimising diet to maximise health and reduce risk of disease. This market has grown significantly in recent years, but there has been little research into what UK consumers think of these products or even into the level of awareness of their existence.

Consumer awareness of and attitudes towards functional foods (CCFRA Report 162) provides a factual analysis of consumer awareness of and attitudes towards functional foods and their perceived health benefits. It describes the approaches and the findings obtained. In addition to attitudes to the foods themselves, the study explored more general attitudes to food in relation to health and also looked at reasons for buying or not buying particular food types – including the influence of sources of information, such as media coverage.

For further details contact Mrs Sue Hocking +44(0)1386 842225 or e-mail: pubs@campden.co.uk.

CCFRA PUBLISHES FOOD SAFETY GUIDES FOR LIVESTOCK FARMERS

A new set of case studies from CCFRA will help livestock farmers with HACCP

(Hazard Analysis Critical Control Points). A HACCP approach can be used by farmers to assure the safety of the food materials they produce, and there is increasing commercial and legislative pressure to do so. Major manufacturers and retailers now expect farmers and growers to adopt HACCP to help assure product safety.

The new case studies, which cover pig rearing, dairying, poultry meat and eggs, and farmed fish, supplement CCFRA's general guide to HACCP in livestock operations (HACCP in Agriculture: Livestock Production) published last year with the first case study which looks at beef cattle. The guide and five case studies can be used independently or in any combination. The supplements illustrate what HACCP is, how it works and how it can be approached for the first time.

Based on extensive experience in the use of HACCP in the production of food materials, the guide and new case studies are based on the internationally agreed principles of HACCP and are fully compatible with HACCP systems used by food processors and manufacturers and with CCFRA's HACCP Documentation Software.

For further details contact Mrs Sue Hocking +44(0)1386 842225 or e-mail: pubs@campden.co.uk.

IDF Publications

FRESH PERSPECTIVES ON BIOACTIVE DAIRY FOODS

Proceedings of the Nutrition and Health Conference
IDF World Dairy Summit 2001 Auckland, New Zealand

The Farming Conference in Auckland examined farming in the context of profit, efficiency and lifestyle. It compared and contrasted seasonal, pasture-based and high-input dairy farming and their impact on profit, milk output and quality of life.

135 pp. - English only
Bulletin N. 375/2002 - 97 Euro

THE IMPLICATIONS OF POLICY CHANGES FOR THE WORLD DAIRY INDUSTRY

Proceedings of the Policy, Economics and Marketing Conference
IDF World Dairy Summit 2001 Auckland, New Zealand

The Policy, Economics and Marketing Conference in Auckland highlighted current and future developments in the main milk-producing regions of the world. It summarized the market situation for dairy products, examined national and international policies in the areas of farm income, the environment, food safety and trade and how changes to these policies may impact on the world dairy industry. Finally, it covered progress in marketing and communications with consumers of dairy products.

103 pp. - English only
Bulletin N. 376/2002 - 95 Euro

HEALTH BENEFITS AND SAFETY EVALUATION OF CERTAIN FOOD COMPONENTS

Food micro-organisms – health benefits, safety evaluation and strains with documented history of use in foods

G. Mogensen, S. Salminen, J. O'Brien, A. Ouwehand, W. Holzapfel, C. Shortt, R. Fondén, G.D. Miller, D. Donohue, M. Playne, R. Crittenden and B. Bianchi Salvadori

This scientific summary reviews the latest research related to health benefits and safety evaluation of lactic acid bacteria (LAB) and introduces an inventory of food micro-organism species with a history of safe use in foods.

6 pp. - English only

INVENTORY OF MICRO -ORGANISMS WITH A DOCUMENTED HISTORY OF USE IN FOOD

G. Mogensen, S. Salminen, J. O'Brien, A. Ouwehand, W. Holzapfel, C. Shortt, R. Fondén, G.D. Miller, D. Donohue, M. Playne, R. Crittenden and B. Bianchi Salvadori

In order to classify traditionally used micro-organisms (starter cultures) as safe food ingredients, the IDF in collaboration with EFFCA (European Food and Fed Cultures Association) has compiled an inventory of micro-organisms with a documented history of use in food. -The inventory is contained in full in this paper.

10 pp. - English only

TRANS FATTY ACIDS

Y. Soustre, B. Laurent, J. Schrezenmeir, M. Pfeuffer, G. Miller and P. Parodi

The expression "trans fatty acids" encompasses a large number of substances having highly heterogeneous roles. Several experimental and epidemiological studies suggest that trans fatty acids (mainly elaidic acid) would seem to have negative effects on plasma cholesterol levels and the incidence of cardiovascular disease, as described in this paper.

12 pp. - English only

MILK IIPIDS IN DIET AND HEALTH - MEDIUM CHAIN FATTY ACIDS (MCFA)

M. Pfeuffer and J. Schrezenmeir

Due to its unique absorption and metabolism characteristics, medium chain triglyceride (MCT) preparations have been used therapeutically since the 1950s, mostly for parenteral nutrition and in the treatment of long-chain triglyceride (LCT) malabsorption, or as concentrated source of energy for preterm infants. This paper looks at the potential role of MCFA/MCT on health and performance in an everyday setting and in modulating Western life style health risks.

11 pp. - English only
Total: 42 pp. Bulletin N. 377/2002 - 45 Euro

WORLD DAIRY SITUATION 2002

Results of annual survey presented by the chair of the Standing Committee "Dairy Policies and Economics" at the IDF World Dairy Summit, October 2002.

The 2002 edition of the World Dairy Situation report includes the customary collation of figures and comments from IDF National Committees and other sources where such Committees do not exist. Contributions from the ZMP which, on behalf of IDF, continued its networking with other institutions working on the economy of the world dairy sector have significantly added to the publication. Milk production, industrial milk processing, trade in dairy products, development of demand and consumption, prices for 2001 are presented, along with some dairy product information and forecasts for 2002. Country reports provided by experts from Member States, describing developments and sometimes subjective prognosis, are pre-

sented. The WDS Forum section comprises three papers: Prospects for world markets, critical analysis of the concept of producer subsidy equivalent, school milk: recent developments and emerging issues

66 pp. - English only
Bulletin N. 378/2002 - 70 Euro

NEW PROCESSING TECHNOLOGIES FOR THE FUTURE

Proceedings of the Emerging Technologies Conference

IDF World Dairy Summit 2001 Auckland, New Zealand

The Emerging technologies Conference in Auckland examined new processing technologies for dairy products. The benefits to be derived from the technologies covered were in the areas of product flavour, texture, nutritional and health value, convenience, safety and consumer confidence. A wide range of new technologies was presented at this conference.

74 pp. - English only
Bulletin N. 374/2002 - 80 Euro

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