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

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

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

DIETARY INTAKE OF DDT GROUP RESIDUES FROM SEAFOOD IN ITALY

ASSUNZIONE ATTRAVERSO LA DIETA E RESIDUI DI PESTICIDI ORGANOCLORURATI IN PRODOTTI ITTICI ITALIANI

M.M. STORELLI, A. STORELLI and G.O. MARCOTRIGIANO*
Dipartimento Farmaco-Biologico, Facoltà di Medicina Veterinaria,
Università di Bari, Strada Prov.le per Casamassima km 3,
70010 Valenzano, Ba, Italy
* Corresponding author: Tel. +39 080 5443867, Fax +39 080 5443863,
E-mail: g.o.marcotrigiano@veterinaria.uniba.it

ABSTRACT

Contamination by DDT group pesticides (p,p'-DDE, p,p'-DDT, o,p'-DDT, p,p'-DDD, o,p'-DDD = DDTs) was determined in fish and bivalve molluscs. Mean DDT concentrations ranged from 0.24-6.63 μg/kg w. wt. Higher values were found in fish (0.24-6.63 μg/kg w. wt) than in bivalve molluscs (0.46-1.43 μg/kg w. wt). In both types of seafood p,p'-DDE was the prevalent compound. Among the various zones of Italy (Northern, Central, Southern and the Islands) a difference in dietary patterns exists. In northern Italy there is a lower consumption of seafood than in the Central, South and Islands, where fish is a relevant part of the diet. Therefore, organochlorine pesticide intake and, con-

RIASSUNTO

Il livello di contaminazione da pesticidi organoclorurati (p,p'-DDE, p,p'-DDT, o,p'-DDT, p,p'-DDD, o,p'-DDD= DDTs) è stato determinato in alcune specie di pesci e molluschi bivalvi. Il range di concentrazione medio di DDTs era 0.24-6.63 μg/kg peso umido. I valori più alti sono stati riscontrati nei pesci (0.24-6.63 μg/kg p. u.) rispetto ai molluschi (0.46-1.43 μg/kg p. u.). In entrambi il p,p'-DDE era il composto prevalente fra i pesticidi organoclorurati. È stata riscontrata una differenza nella dieta nelle diverse zone dell'Italia (Nord, Centro, Sud ed Isole). Il Nord Italia registra un minore consumo di pesce rispetto ai prodotti carnei, mentre al Centro e nel Sud ed Isole il pesce occupa una posizione

- Key words: DDT group pesticides, estimated daily intake, fish, Italy, mussels -

sequently, health risk may differ, in relation to place of residence and the kind of food consumed. On this basis, the estimated daily intakes (EDIs) of DDTs from different seafoods were determined. The intakes of the evaluated pesticides were less than 1% of acceptable daily intakes.

rivelante nella dieta. Pertanto, l'assunzione di pesticidi organoclorurati e di conseguenza, il rischio per la salute può differire tra la popolazione, in relazione alla luogo di residenza ed al tipo di cibo consumato. Su questa base sono state determinate le stime di assunzione giornaliera (EDIs) per i pesticidi organoclorurati in differenti prodotti ittici. L'assunzione stimata di pesticidi era al disotto dell'1% dell'ADIs.

INTRODUCTION

Organochlorine compounds are a class of widespread organic contaminants ubiquitous in the environment. These compounds, highly stable and resistant to biological degradation, enter the food chain, accumulating at particularly high levels in adipose tissues. They are of great concern because of their toxic effects on wildlife and human health. It has been found that >80% of the total intake of organochlorine pesticide residues by nonoccupationally exposed human beings is accumulated through the food chain (DOONG and LEE, 1999; MARTINEZ *et al.*, 1997; TROTTER and DICKERSON, 1993; BENTABOL and JODRAL, 1995). NAKAGAWA *et al.*, (1995) reported that the estimated daily intakes (EDIs) of total DDT in Japan, Canada, and the United States were 1.42, 6.38, and 1.3 μg/day, respectively. DOONG (1999) found a daily intake of 0.54 μg/day in Taiwan, while CHEN and GAO (1993) reported a very high value of 20.47 μg/day in China. In Italy the use of DDT was prohibited by the Ministerial Decree of 11.10.1978 (G.U., 1978). Despite the ban and the restrictions on the use of persistent organochlorine pesticides (POCs), some countries are still using these substances for agricultural and public health purpose because of their low cost and versatility against various insects. This continued use results in high exposure to organochlorine pesticides in the areas near the sources of pollution as well as in zones further away because of long-range atmospheric transport. The main food sources of POCs are fish and meat (CHEN and GAO, 1993).

The population of Italy is exposed to different diets, because of Italy's geographical position, mainly outstretched towards the sea, except for the northern zone boardered by the Alps. In Northern Italy there is a low consumption of seafood with respect to meat products, while in Central and Southern Italy and the Islands, seafood products occupy a relevant part of the diet (ISTAT, 1998). Therefore, organochlorine pesticide intake and, consequently, health risk may differ with respect to a person's residence and the kind of food consumed. This study was carried out to estimate the amount of residues of DDT group pesticides in seafood products. In addition, estimated daily intakes (EDIs) of these pesticides from seafood from different Italian regions were evaluated to assess possible health risks.

MATERIALS AND METHODS

Most of the fish products sold in the different Italian markets comes from the Adriatic Sea (STORELLI *et al.*, 1998); therefore the fish and bivalve molluscs ana-

lysed in the present work were caught in this sea zone (Chioggia-S. Maria di Leuca). In total, 2575 specimens of six different fish species [613 *Mullus barbatus* (striped mullet), 232 *Mugil cephalus* (sea mullet), 253 *Aspritrigla lucerna* (red gurnard), 263 *Trigla lucerna* (yellow gurnard), 922 *Merluccius merluccius* (hake) and 292 *Sarpa salpa* (goldline)], and 180 specimens of three different species of wild bivalve molluscs [*Modiolus barbatus* (bearded horse mussel), *Tapes decussatus* (carpet shell), *Cardium tuberculatum* (knotted cockle)] were analysed. Fish muscle tissue and the whole body of the bivalve molluscs were removed, and kept in a deep freezer at -20°C until chemical analysis.

Twenty grams of the homogenised samples were ground with anhydrous sodium sulphate (Pestanal grade from Riedel de Haen, Seelze, Germany), and the mixture was blended three times with 100 mL of petroleum ether (40°-60°C SupraSolv Merck, Darmstadt, Germany). The combined extracts were concentrated and subsamples were taken in order to determine the fat content of the tissue by a gravimetric method (ERNEY, 1983). An aliquot of the remaining extract was cleaned-up with a Florisil SPE cartridge (6 mL, 1.0 g size, 100-120 mesh, Supelco). Sodium sulfate (ca 1.0 cm) was added to the Florisil SPE cartridge, and the cartridge was washed with 6 mL petroleum ether at a flow rate of 5 mL/min. The organochlorine pesticides were eluted with 12 mL petroleum ether-ethyl ether (95+5) at a rate of 2 mL/min. The eluates were concentrated to ca 1-2 mL on a rotary evaporator and then transferred to 10 mL glass tubes with small amounts of hexane. The solvent in the glass tubes was evaporated almost completely under a gentle stream of nitrogen, and the residues were redissolved in 1 mL hexane and analyzed with a gas chromatograph equipped with an electron capture detector (GC-ECD). The concentrations of organochlorine pesticides in the extracts were monitored by GC-ECD using an SPB-608 fused-silica capillary column (30 m length x 0.25 mm i.d. x 0.25 μm film thickness). One-half microliter of each sample was injected into the gas chromatograph (Carlo Erba HR 5300 Mega Series equipped with a ^{63}Ni electron capture detector ECD-400) for separation and determination of organochlorine pesticides. Temperature was programmed according to the following sequence: injection at 50°C; oven temperature steady for the first 1 min and then increased from 50° to 180°C at a rate of 15°C/min; oven maintained at a steady temperature for 1 min and then increased from 180° to 220°C at a rate of 4°C/min; oven maintained at a steady temperature for 20 min and then increased from 220° to 275°C at a rate of 5°C/min; from this point until the end of the analytical run, the column remained isothermal at a temperature of 275°C; injection mode, splitless; detector temperature, 310°C; carrier gas, nitrogen; column flow rate, 1 mL/min; make up gas, nitrogen at 60 mL/min).

GC peaks were identified by comparison with the external standard peak areas. Analytical standards (p,p'-DDE, p,p'-DDT, o,p'-DDT, p,p'-DDD, o,p'-DDD) were purchased from Supelco (Bellefonte, PA). The concentrations were 200 μg/mL. The samples resulting below the limit of detection were not included in the mean. Recoveries were determined by adding specific amounts of POC standards to empty samples before extraction. Two sets of three samples (plus blank) were fortified with POC standards at three levels of concentration and the recoveries were within 80-110%. Blank values ranged from 0.1 to 10 pg. The detection limit of the method was 0.10 ng/g on a wet basis for the pesticides. Quantification was done within the linear range of the detector. Residues in 10% of the samples were confirmed by gas chromatography-mass spectrometry (Fisons MD 800).

RESULTS AND DISCUSSION

Table 1 shows the range, mean values (µg/kg w.w.) and standard deviations of DDT group pesticide concentrations measured in the different species examined.

Of the different fish species the greatest accumulation of total DDT was found in sea mullets followed by striped mullet, hake, yellow gurnard, red gurnard and goldline. The percentage distribution pattern of DDT metabolites was comparable in sea and striped mullet. In both species, p,p'-DDE was the prevalent compound (75.4% in sea mullet; 70.2% in striped mullet), followed by p,p'-DDT (15.2% in sea mullet; 12.9% in striped mullet), p,p'-DDD (9.5% in sea mullet; 8.6% in striped mullet), o,p'-DDD (4.8% in striped mullet), and o,p'-DDT (3.7% in striped mullet). In sea mullet o,p'-DDT, and o,p'-DDD were below the instrumental limit of detection. As regards hake, the major contributor to DDT percentage was p,p'-DDE (71.1%), followed by p,p'-DDT (10%), p,p'-DDD (8.8%) o,p'-DDT (5.5%) and o,p'-DDD (4.8%). Red and yellow gurnard, species belonging to the same family, showed a different percentage distribution of DDT compounds. The only DDT metabolite detected in red gurnard was p,p'-DDE, while in yellow gurnard the percentage distribution of these compounds was: p,p'-DDE (74.9%)>p,p'-DDD (8.2%) >o,p'-DDT (7.5%) >p,p'-DDT (5.6%) >o,p'-DDD (3.7%). In goldline, only p,p'-DDE was found.

In bivalve mollusc species the highest mean values of total DDT were found in bearded horse mussel, followed by knot-

Table 1 - Range and mean + standard deviation of DDT group residues (µg/kg w.w.) in different species of fish and mollusc bivalves and the p,p'-DDE/p,p'-DDT ratio.

Samples	p,p'-DDE	o,p'-DDT	p,p'-DDT	o,p'-DDD	p,p'-DDD	DDTs	p,p'-DDE / p,p'-DDT
St. mullet n. 613	0.20-6.20 2.62±0.33	ND-1.10 0.14±0.27	ND-1.10 0.48±0.26	ND-1.40 0.18±0.31	ND-1.90 0.32±0.43	0.20-11.7 4.73±1.44	5.4
Sea mullet n. 232	1.70-13.0 5.00±3.47	ND	ND-1.50 1.01±1.57	ND	ND-1.80 0.63±0.56	1.70-16.3 6.63±4.51	4.9
R. gurnard n. 253	0.60-4.80 2.29±1.26	ND	ND	ND	ND	0.60-4.80 2.29±1.26	-
Y. gurnard n. 263	0.20-5.30 2.00±1.50	ND-0.40 0.20±0.12	ND-0.50 0.15±0.20	ND-0.20 0.10±0.07	ND-0.70 0.22±0.24	0.20-6.90 2.67±2.00	13.3
Hake n. 922	0.30-9.60 1.92±1.74	ND-0.30 0.15±0.06	ND-1.10 0.27±0.20	ND-0.50 0.13±0.08	ND-0.80 0.24±0.17	0.70-11.4 2.70±2.15	7.1
Goldline n. 292	0.10-0.90 0.24±0.26	ND	ND	ND	ND	0.10-0.90 0.24±0.26	-
B. h. mussel n. 180	0.30-1.70 0.88±0.41	ND	ND-0.70 0.21±0.22	ND-0.50 0.14±0.15	ND-0.60 0.20±0.19	0.30-3.60 1.43±0.83	4.2
C. shell n. 180	0.10-0.60 0.31±0.15	ND	0.10-0.30 0.15±0.07	ND	ND	0.10-0.90 0.46±0.26	2.0
K. cockle n. 180	0.10-3.50 0.63±0.98	ND-0.20 0.15±0.06	ND-0.30 0.20±0.06	ND	ND	0.10-4.00 0.98±1.13	3.1

ND = Not Detected.

ted cockle and carpet shell. In bearded horse mussel p,p'-DDE was the highest (61.5%), followed by p,p'-DDT (14.7%), p,p'-DDD (13.9%) and o,p'-DDD (9.7%). In knotted cockle, the major contributor to DDT percentage was p,p'-DDE (64.3%), followed by p,p'-DDT (20.4%) and o,p'-DDT (15.3%), while the others were absent. In carpet shell, p,p'-DDE was the prevalent compound (67.4%), followed by p,p'-DDT (32.6%), while the other metabolites were below the instrumental limit of detection.

Among the different seafood products the highest mean values were found in fish with respect to bivalve molluscs. Fish exhibit a low metabolism for organochlorines (MUIR *et al.*, 1990), and they concentrate pollutants in their tissues directly through the diet, as well as from water. Bioconcentration in fish depends either on the concentration of chemical compounds in the environment or on the physiological and biochemical processes which occur in the different organisms in relation to the species (BARRON, 1990). Consequently, species which occupy different habitats in the same ecosystem and have different feeding behaviour may manifest a different pollutant load in their tissues. Mullets were chosen as environmental bioindicators by the UNEP/FAO (1981) and for use in monitoring programmes (PORTE and ALBAIGES, 1993; MC DOWELL *et al.*, 1989) because they are species which tend to concentrate contaminants to a greater degree than other species. In fact sea mullet, an omnivorous scavenger that eats detritus and invertebrates and striped mullet, a carnivorous bottom feeder, showed the highest levels of total DDT, while goldline, a herbivorous species, showed the lowest values.

Among the mussels, filtering species and ideal bioindicator organisms of the sources of pollution, the highest levels were detected in bearded horse mussel. This species, among those chosen for this study, belongs to the genus *Mytilus*, which is capable of filtering larger amount of water (about 6-8 L/h) than the other two species (about 3-4 L/h) (RENON and MALANDRA, 1991), and is thus exposed to greater accumulation.

Table 2 shows the consumption of fish and bivalve molluscs (g/day per person) and the estimated daily intake in the different zones of Italy. The lowest seafood consumption of about 27.6 g per day per person was recorded in Northern Italy, in central Italy a mean consumption of 38.9 g, per day per person was recorded, while Southern Italy and the Islands had the highest consumption 45.3 g (ISTAT, 1998). On the basis of this data, as well as the mean total DDT found in fish (3 μg/kg w. wt), the estimated daily intake was calculated. The values determined, although very low (Northern Italy = 0.08 μg/day, Central Italy = 0.11 μg/day, Southern and the Islands = 0.14 μg/day), show EDIs roughly 1.5 to 1.75 times higher in Central and Southern Italy and the Islands than in Northern Italy. For bivalve molluscs the consumption also varied in relation to the different zones. Northern Italy had a very low consumption equal to 5.6 g per person; in Central Italy the consumption was 18.5 g, while in Southern Italy and the Islands consumption was 25.2 g. On the basis of this data and considering the mean value of total DDT found (0.86 μg/kg w. wt), an estimated daily intake of 0.005 μg/day was calculated for Northern Italy, while Central and Southern Ita-

Table 2 - Consumption of fish and bivalve molluscs (g/daily per person) in different zones of Italy and estimated daily intake (EDI μg/day) for DDTs in seafood.

	Consumption	EDI
FISH		
Northern Italy	27.6	0.08
Central Italy	38.9	0.11
Southern and the Islands	45.3	0.14
BIVALVE MOLLUSCS		
Northern Italy	5.6	0.005
Central Italy	18.5	0.01
Southern and the Islands	25.2	0.02

ly and the Islands had values of 0.01 and 0.02 μg/day, respectively, which are about 2-4 times higher that the North. The low values of EDIs found, less than 1% of the acceptable daily intake for total DDT (1,200 μg), clearly reflect the restrictions concerning the use of DDTs. The high values of the p,p'-DDE/p,p'-DDT ratios (Table 1), used to determine whether recent exposure to DDT exists, indicate an earlier use of DDT rather than recent exposure. Therefore, the intake of organochlorine pesticides (DDTs) found compared with the acceptable daily intakes (ADIs) proposed by the Joint Food and Agricultural Organisation/World Health Organisation Meeting on Pesticide Residues (IPCS, 1990), shows that consumption of the seafood analysed does not pose a risk to human health based on total DDT content. Nevertheless, considering that the calculated estimated daily intake does not include the contribution from other foods and from other seafood and considering the hazard of these pollutants, it is opportune to constantly monitor food products, as suggested by the U.S. Food and Drug Administration (FDA) that has the regulatory responsibility to enforce pesticide tolerance in foods and routinely monitor the food supply for residues of these chemicals (U.S. FOOD and DRUG ADMINISTRATION, 1991; 1994).

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PAPER

COLORIMETRIC DETERMINATION OF PHOSPHORUS IN MILK BY FLOW INJECTION ANALYSIS USING A THERMAL/UV INDUCED DIGESTION

DETERMINAZIONE COLORIMETRICA DEL FOSFORO NEL LATTE MEDIANTE ANALISI AD INIEZIONE DI FLUSSO E DIGESTIONE TERMICA INDOTTA DA RADIAZIONE UV

M.J. REIS LIMA*, S.M.V. FERNANDES and A.O.S.S. RANGEL**
Escola Superior de Biotecnologia, Universidade Católica Portuguesa, R. Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
*Permanent address: Escola Superior Agrária de Viseu, Instituto Superior Politécnico de Viseu, Campus Politécnico, Repeses, 3504-510 Viseu, Portugal
**Corresponding author: e-mail: rangel@esb.ucp.pt

ABSTRACT

Two flow injection systems for the determination of total phosphorus in milk were developed: one for the determination of phosphorus using off-line digestion of milk and another with in-line digestion. The first procedure involves a classical Kjeldahl digestion of milk prior to introducing it into the flow system; the second uses acid, heat and UV-catalysed peroxodisulphate oxidation to convert in-line all forms of phosphorus compounds into orthophosphate, allowing a considerable amount of time to be saved. Concentrations of phosphorus in milk within

RIASSUNTO

Sono stati sviluppati dei sistemi ad iniezione di flusso (FIA) per la determinazione del fosforo totale nel latte. Nel primo la determinazione del fosforo avviene dopo che la digestione del latte è stata condotta a parte (off-line), mediante la classica reazione di Kjeldahl; nel secondo la digestione viene condotta direttamente nel sistema di flusso (in-line), trasformando tutti i composti di fosforo in ortofosfato mediante reazione a caldo con acido, catalizzata da radiazione UV, ed ossidazione con perossidisolfato. Quest'ultimo metodo consente un risparmio di tempo significa-

- Key words: Flow-injection, in-line UV/thermal digestion, milk, phosphorus determination, spectrophotometry -

the range of 706 to 984 mg P/L were found and FIA results were in good agreement with those provided by the reference method.

tivo. Le concentrazioni di fosforo trovate nel latte sono risultate comprese tra 706 a 984 mg di P/L. I risultati sono in buon accordo con quelli ottenuti applicando il metodo di riferimento.

INTRODUCTION

Milk is an exceptionally nutritious food primarily because it has a large number of components such as proteins, sugars, vitamins, fats, gases and minerals. However, this complex matrix poses a major problem to the analytical chemist.

The objective of this work was to develop an automatic method to determine the total phosphorus present in milk, a mineral essential to the human diet (PULLIAINEN and WALLIN, 1994). All food contains phosphorus in a minor or major quantity; ultra-pasteurised milk (UHT) contains from 65 to 100 mg of phosphorus per 100 g, in a Ca/P ratio of 1.4.

The spectrophotometric determination of phosphorus involves two main steps: (a) conversion of all forms of phosphorus into orthophosphates by hydrolysis and oxidation; (b) colorimetric measurement using the phosphomolybdenum blue reaction.

Flow injection systems have already been developed for the determination of phosphorus in beer, water and wastewater. They have been applied only to the colorimetric reaction (WOO and MAHER, 1995) using off-line sample pretreatment, or providing automation of the whole process (BENSON et al., 1996; FERNANDES et al., 2000; WILLIAMS et al., 1993). In none of these studies, however, was total phosphorus determined in milk samples. Therefore, it would be desirable to develop a system in which the design and conditions of the manifold would be able to handle the conversion of all forms of phosphorus into orthophosphate, in a complex matrix like milk.

In this work two flow injection analysis (FIA) systems are proposed for the determination of phosphorus in milk; the first one automates the colorimetric phosphomolybdenum blue reaction, being the digestion procedure performed off-line; the second one provides UV-photooxidation/thermal in-line digestion with oxidising and hydrolysing reagents to convert all forms of phosphorus compounds into orthophosphate and the subsequent colorimetric reaction, with the advantage of requiring minimum sample pre-treatment (dilution).

MATERIALS AND METHODS

Reagents and solutions

All chemicals were of analytical reagent grade. Deionised water with a specific conductance of less than 0.1 μS/cm was used throughout. The material used in the experiments was washed with water only and rinsed with 0.1 M H_2SO_4, as detergents highly interfere in these determinations as reported by PULLIAINEN and WALLIN (1994).

A 2 M H_2SO_4 solution was prepared by proper dilution of a concentrated sulphuric acid solution (d=1.98, 98%); a 6 g/L potassium peroxodisulphate solution was obtained by dissolving 3 g of the solid in 500 mL of water.

The phosphorus stock solution (1000 mg P/L) was prepared weekly by dissolving 4.3909 g (weighed in a Sartorius analytical balance) of potassium dihydrogen phosphate per liter of water. An intermediate solution (100 mg P/L) was

prepared daily by rigorous dilution of 10 mL of the stock solution to 100 mL. This solution was used to prepare the working standard solutions used in both manifolds: for the off-line digestion procedure, standards were prepared in the range of 8 to 25 mg P/L in a 0.75 M H_2SO_4 solution; for the in-line digestion method, standards of 6 to 25 mg P/L in water were used.

The colour reagent, a 9.4 g/L ammonium molybdate solution, was obtained by dissolving 5 g of ammonium heptamolybdate-tetrahydrate in 18 mL of concentrated sulphuric acid and diluted to 500 mL. This solution was 0.65 M in sulphuric acid and was prepared weekly.

The stannous chloride solution was obtained using 0.1 g of stannous chloride dihydrate and 1 g of hydrazinium sulphate (which improves the stability of the solution) dissolved in 14 mL of concentrated sulphuric acid; the volume was brought up to 500 mL with water. This solution, prepared every week, was 0.17 g/L in stannous chloride, 0.5 M in sulphuric acid and 2 g/L in hydrazinium sulphate.

For the reference procedure, the colour reagent was prepared by dissolving 6.25 g of sodium molybdate in a 5 M sulphuric acid solution. In a separate vessel, 0.15 g of hydrazinium sulphate was dissolved in 100 mL of water. Then, 25 mL of the molybdate solution was added to 10 mL of the hydrazinium solution and the volume was made up to 100 mL; this procedure was performed daily.

For the preparation of a 10 mg/L intermediate standard solution, 10 mL of the 100 mg/L stock solution prepared for the flow injection procedure were taken and diluted to 100 mL. This solution was used to prepare the working standard solutions from 0.2 to 2 mg P/L.

Instrumentation

In the flow injection systems the solutions were propelled by Gilson Minipuls 3 (Villiers-le-Bel, France) peristaltic pumps and Gilson PVC propelling tubes. The tubing was made of PTFE [Omnifit (Cambridge, UK), 0.8 mm i.d.] and Gilson end fitting connectors and Y-shaped confluences were used to link the different parts of the manifold.

The thermal digestion unit consisted of a 4 m long Omnifit PTFE helically coiled tubing (0.8 mm i.d.) submerged in a Julabo VC (Seelbach, Germany) thermostatic bath with temperature set at 90°C.

The UV digestion system consisted of a 4 m long Omnifit PTFE tubing (0.8 mm i.d.) helically coiled around a 15 W UV tube (Philips, Eindhoven, Holland). An aluminium foil covered this system to prevent the operator from being continuously exposed to this radiation.

An ATI Unicam (Cambridge, UK) 5625 UV/Vis spectrophotometer equipped with a Hellma (Müllheim/Baden, Germany) 178.713-QS flow cell (inner optical volume 18 μL) and connected to a Kipp and Zonen (Delft, Holland) BD chart recorder was used as detection system.

Samples were injected in the manifold using a Rheodyne (Cotati, CA, USA) type 5020 six-port rotary injection valve.

A Bandelin Sonorex (Berlin, Germany) RK 100 ultrasonic bath was used for degassing samples.

Reference method

The colorimetric procedure recommended by the Manuel Suisse des Denrées Alimentaires (MSDA, 1973) was used as the reference method. For preparing the sample, 2 mL of milk, 5 mL of concentrated sulphuric acid and 1 g of a catalyser (mixture of 500 g of anhydrous sodium sulphate, 8 g of copper sulphate and 8 g of selenium) were put into Kjeldahl tubes. This solution was mineralised at 400°C for about 3 h and allowed to cool. Then, 30 mL of water were added and the volume was brought to 100 mL.

From this solution, 2 mL were put in

50 mL volumetric flasks and 25 mL of water and 20 mL of the colour reagent were added. This solution was boiled in a water bath for about 10 min. After cooling, the volume was brought to 50 mL with water and the absorbance measured at 710 nm. The phosphorus content of the samples was then determined from a previously established calibration curve.

Sample preparation

The digestion procedure described above for the reference method was used for preparing the samples to inject in the manifold using off-line digestion.

For the in-line digestion procedure, 2 mL of milk were diluted to 100 mL in a volumetric flask and then degassed in an ultrasonic bath for ten minutes.

Flow injection procedures

The two flow injection systems are presented in Fig. 1. The flow injection procedure with off-line digestion (Fig. 1b) consisted in the mixture of the stannous chloride reducing reagent (R_4) and the molybdate ammonium solution (R_5) in the first confluence (d). The sample (30 µL of digested milk) was injected in the water carrier stream (R_3), merged at confluence (c) with the previously formed colour reagent and allowed to react in L_5 coil. Total phosphorus was then determined spectrophotometrically at 710 nm.

Fig. 1a shows the flow injection procedure with in-line digestion. The sample (S) was continuously mixed in confluence (a) with a 2 M H_2SO_4 stream (R_1) and then flowed through the thermostatic bath (L_1). This solution then merged with a potas-

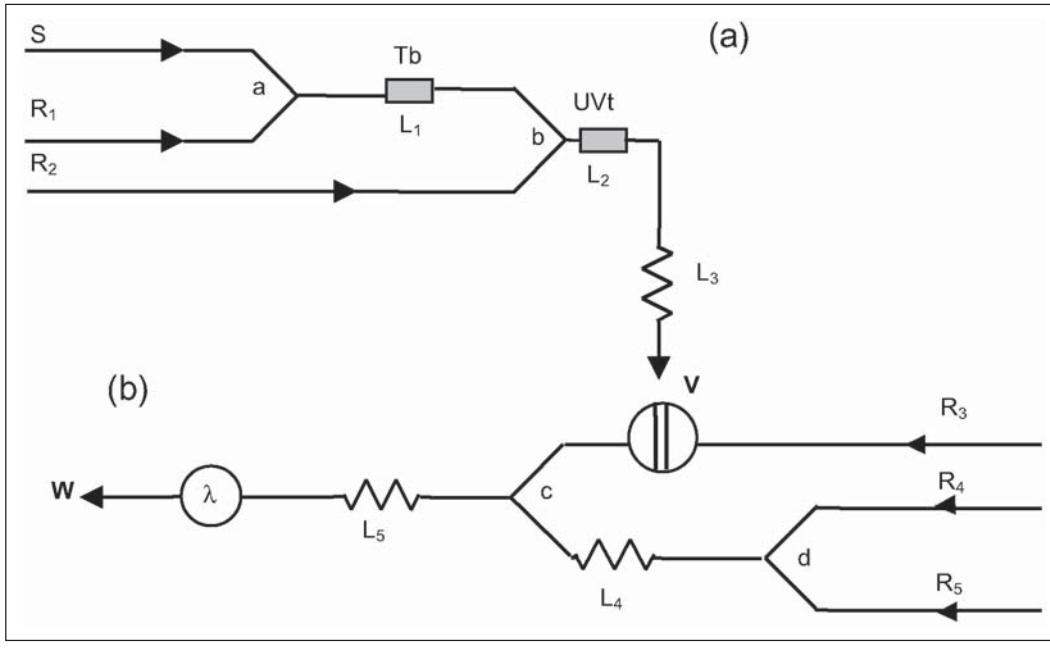


Fig. 1 - FIA manifold used for the determination of phosphorus in milk.
(a) - Digestion unit; (b) - Spectrophotometric determination unit; S - standards or milk samples; R_i - reagent solutions (R_1 = 2 M H_2SO_4; R_2 = 6 g/L potassium peroxodisulphate; R_3 = deionized water; R_4 = 0.17 g/L stannous chloride; R_5 = 9.4 g/L ammonium molybdate); L_i - tube lengths, in cm (L_1 = 400; L_2 = 400; L_3 = 100; L_4 = 200; L_5 = 200); V - injection volume (30 µL); W - waste; a, b, c and d: confluence points; UVt - ultra violet radiation tube; Tb - thermostatic bath (90°C); λ - UV/Vis spectrophotometer.

sium peroxodisulphate solution (R_2) and flowed through the coil (L_2) wrapped around the UV tube. After this point, an aliquot (30 µL) of the digested sample was injected in the manifold described above for the off-line procedure.

RESULTS AND DISCUSSION

Manifold optimisation tests were made by varying each parameter while setting the remainder.

Study of the flow injection system using off-line digestion

The system parameters were studied in order to achieve a compromise between sensitivity of the analytical measurements, repeatability and sampling rate. Since the phosphomolybdenum blue reaction has been widely studied in flow injection systems (LIMA and RANGEL, 1990), the concentrations of the colour and reducing reagents were based on literature values.

The flow rates in channels R_3, R_4 and R_5 were made equal and set at 1.1 mL/min. The length of the coil L_5 was set at 200 cm since it provided the best sensitivity (FERNANDES et al., 2000).

The loop of the injection volume was tested from 25 to 45 µL. It was set at 30 µL as higher injection volumes decreased sensitivity due to the increasing amount of H^+ of the injected plug. Smaller volumes also produced a decrease in sensitivity.

Since the acidity of the medium strongly influences the development of the colorimetric reaction (LIMA and RANGEL, 1990), working standard solutions were prepared with different levels of acidity (from 0.4 to 1.1 M in H_2SO_4) and injected in the manifold. In fact, increasing the acidity of the injected standards strongly decreased the sensitivity of the system (Fig. 2). Considering that the digested samples injected into the system might have different acidities, this parameter was assessed by titration with NaOH. It was found that all digested samples had an acidity of around 0.75 M, expressed in H_2SO_4. This way, the acidity of the standard solutions was matched to that of the injected samples, by preparing flow injection standards in a 0.75 M H_2SO_4 solution.

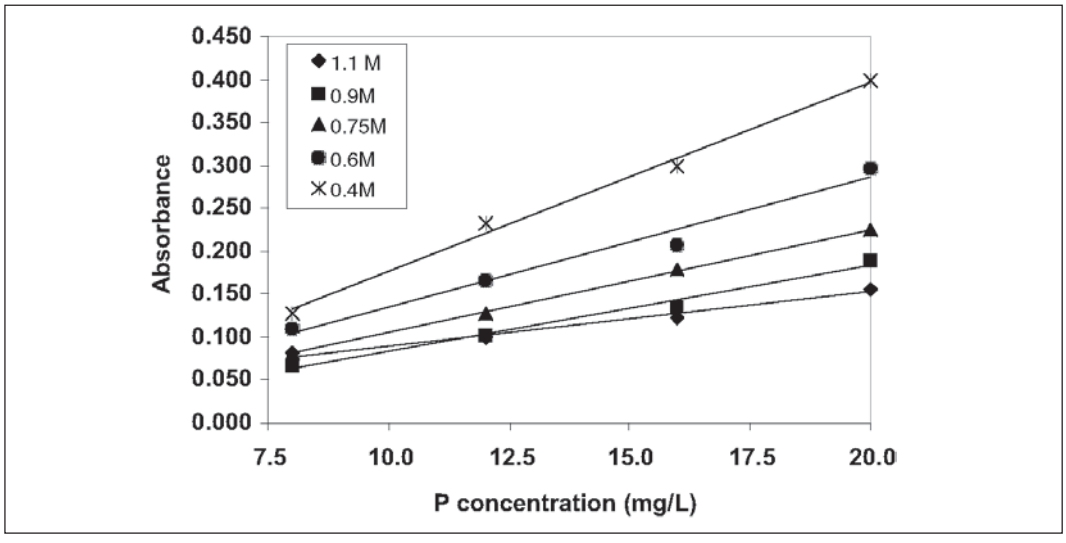


Fig. 2 - Influence of the acidity (expressed in H_2SO_4 molarity) of the injected solutions on the colorimetric determination of phosphorus.

Under the optimised conditions, a sampling rate of 30 samples per hour was achieved with this FIA system. The limit of detection was calculated according to IUPAC (1976) recommendations, corresponding to 3 times the standard deviation of ten consecutive injections of the blank solution (0.75 M H_2SO_4) and was found to be 2 mg/L.

Flow injection system using in-line digestion

For the development of this manifold, the spectrophotometric determination was accomplished by using the system previously described for off-line digestion (Fig. 1b). This way, the only parameters studied were those related to the in-line digestion procedure, seeking to obtain results comparable to the ones achieved with the reference digestion method.

The length of tube L_1 was tested up to 4 m. This length was chosen as it allowed a higher conversion percentage of the phosphorus compounds, because of the increased contact time of the milk with the acid and heat. Tube L_2 was fixed at 4 m, as reported by FERNANDES et al. (2000).

Since one of the factors affecting the rate of hydrolytic degradation of ring and chain phosphates in solution is pH (Williams et al., 1993), the H_2SO_4 concentration was also studied. As reported by FERNANDES et al. (2000), a 2 M H_2SO_4 concentration was enough to convert all forms of phosphorus into orthophosphates. Higher concentrations caused double peaks due to refractive index gradients without increasing the conversion percentage.

Concerning the potassium peroxidisulphate solution, it was tested in the range of 6 to 15 g/L. More than 6 g/L led to a decrease in the sensitivity of the spectrophotometric determination and did not improve the conversion of phosphorus compounds. Under the optimised conditions, the detection limit of this methodology was 4 mg P/L, calculated according to the IUPAC (1976) recommendations.

Analysis of milk samples

Determinations of total phosphorus in milk samples were carried out using both FIA procedures (C_f) and the reference method (C_r). Each standard or milk digestate was injected three times (Fig. 3). Several commercially available types were analysed, including whole milk, reduced fat milk, skimmed milk and different special brand milks. Results are shown in Tables 1 and 2 for off-line and in-line digestion FIA manifolds, respectively.

In order to assess the quality of the FIA results, a relation of type $C_f=C_0+SC_r$ was established, where: C_0=-40 (±180), S=1.04 (±0.20) and r=0.974 for the off-line digestion procedure, and C_0=9 (±112), S=0.973 (±0.154) and r=0.969 for the in-line digestion procedure. The confidence limits of the intercept and slope obtained with a 95% level of significance are in parentheses (MILLER and MILLER, 1993) for 8 and 12 degrees of freedom, respectively. These values show that there was no evidence of systematic differences between the results obtained using the two methodologies. The maxi-

Table 1 - Results for the determination of phosphorus in milk by the off-line digestion FIA system and the reference method.

Samples	Ref. Method (mg/L)	FIA/off-line digestion (mg/L)	Rel. deviation (%)
1	888	864	-2.7
2	983	978	-0.51
3	985	984	-0.10
4	871	845	-3.0
5	858	859	+0.12
6	905	926	+2.3
7	824	813	-1.3
8	914	923	+0.98
9	969	955	-1.4
10	847	841	-0.71

mum relative deviation, calculated as $[(C_f-C_r)/C_r] \times 100$, was 6.2%.

To assess the repeatability of the FIA procedures, the relative standard deviations were calculated from ten consecutive determinations of milk samples. The results were: 0.30, 0.45 and 0.54% for the off-line digestion procedure, with concentrations of 816, 825 and 834 mg P/L respectively, and 0.42 and 0.66% for the in-line digestion procedure, with concentrations of 753 and 668 mg P/L, respectively.

CONCLUSIONS

It can be concluded that both FIA systems are advantageous alternatives to the reference method, featuring all the advantages of automated methods. For the first time a flow injection in-line UV/thermal digestion procedure was successfully applied to the digestion of a complex matrix like milk, yielding results comparable to those of conventional digestion methods and having good precision. This system presents the advan-

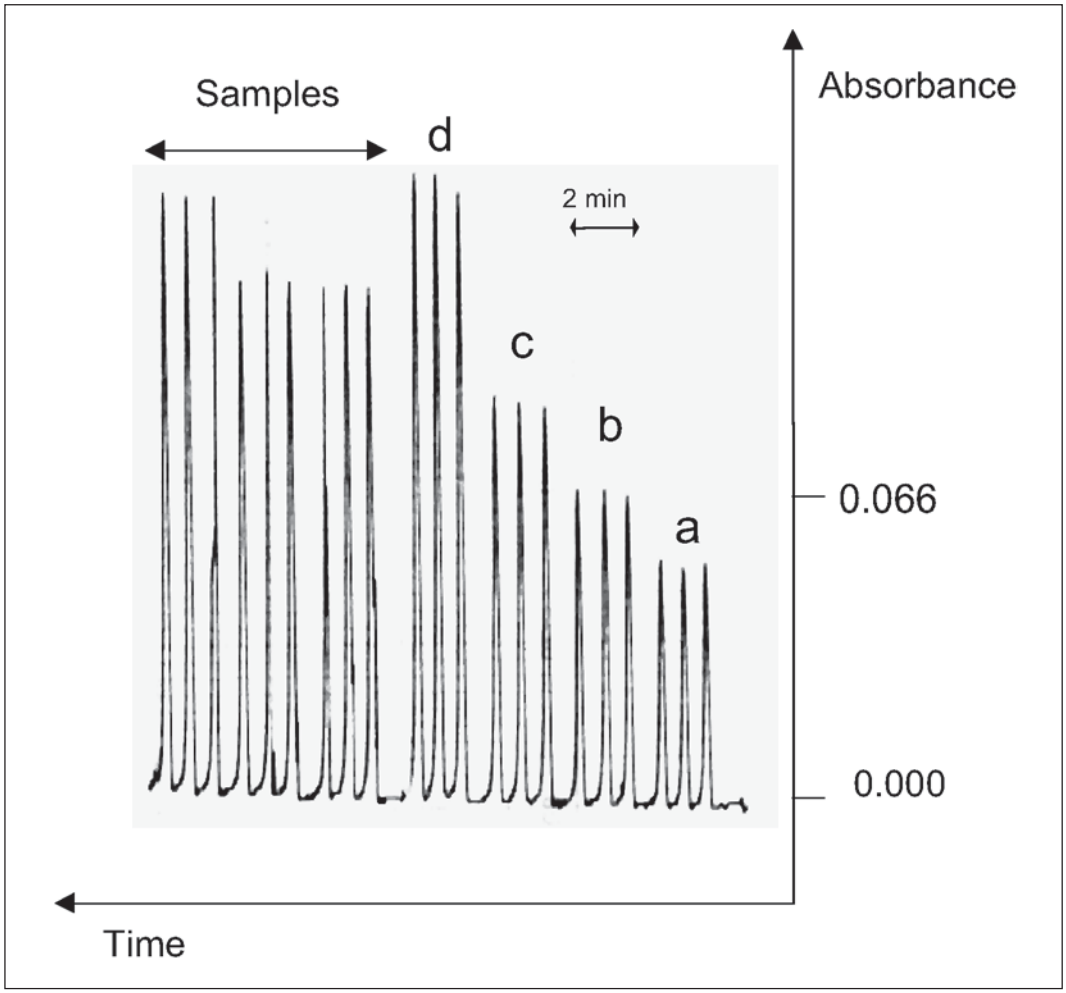


Fig. 3 - FIA recording obtained for determination of total phosphorus in milk with the in-line digestion manifold, corresponding to injections of samples and standards: 6 (a), 8 (b), 10 (c) and 15 (d) mg/L.

Table 2 - Results for the determination of phosphorus in milk by the FIA system using in-line digestion and the reference method.

Samples	Ref. Method (mg/L)	FIA/in-line digestion (mg/L)	Rel. deviation (%)
1	764	753	-1.4
2	659	679	+3.0
3	643	673	+4.7
4	578	542	-6.2
5	792	752	-5.1
6	720	706	-1.9
7	858	844	-1.6
8	712	698	-2.0
9	665	647	-2.7
10	674	667	-1.0
11	669	655	-2.1
12	692	665	-3.9
13	796	806	+1.3
14	823	806	-2.1

tage of lower power requirements in relation to the use of microwave digestion previously reported for other matrices (WILLIAMS et al., 1993), making this proposed approach more suitable for on-line monitoring applications. Making a determination in a complex matrix such as milk, the system saves considerable time and provides good quality results with sampling rates of about 10 determinations per hour.

Although with the off-line digestion FIA methodology smaller relative deviations (FIA vs reference method) and higher determination rates (about 30 per hour) were achieved than with the in-line digestion manifold, in the latter the entire procedure (from digestion to determination) was concluded in about six minutes, compared to more than three hours required for preparing the sample in the former.

The results obtained in this work with the UV/thermal digestion method indicate that it could be applicable to the total phosphorus determination in other complex matrices as well as to the determination of other compounds in milk, like total nitrogen.

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PAPER

ANTIOXIDANT ACTIVITY OF OREGANO (*ORIGANUM VULGARE* L.) LEAVES

VERIFICA DELL'ATTIVITÀ ANTIOSSIDANTE DI FOGLIE DI ORIGANO (*ORIGANUM VULGARE* L.)

A. BENDINI*, T. GALLINA TOSCHI and G. LERCKER
Dipartimento di Scienze degli Alimenti, Università di Bologna,
Via Ravennate 1020, 47023 Cesena (FC), Italy
*Corresponding author: Tel. +39 0547 636121, Fax +39 0547 382348,
e-mail: abendini@foodsci.unibo.it

ABSTRACT

Oregano leaves (*Origanum vulgare* L.) were extracted with ethanol, diethyl ether, *n*-hexane:2-propanol 4:1 (v/v) and *n*-pentane. The highest yield was obtained with ethanol and the ethanolic extract showed the strongest inhibitory effect on lipid oxidation when added (amounts from 0.02 to 5% w/w) to a model lipid system (made up of refined bleached peanut oil), subjected to forced dynamic oxidation, using the Oxidative Stability Instrument (OSI). The hydrolyzed ethanolic extract showed a more powerful antioxidant activity than the untreated extract. Total polyphenols in

RIASSUNTO

Le foglie di origano (*Origanum vulgare L.*) sono state estratte con etanolo, etere dietilico, *n*-esano:isopropanolo 4:1 (v/v) e *n*-pentano. La resa più elevata è stata ottenuta utilizzando l'etanolo e l'estratto etanolico ha mostrato anche il più forte effetto inibitorio nei confronti dell'ossidazione lipidica, quando addizionato (in quantità variabili dallo 0,02 al 5% p/p) ad un sistema lipidico modello (costituito da olio di arachide raffinato e decolorato), sottoposto ad ossidazione dinamica forzata, mediante l'Oxidative Stability Instrument (OSI). L'estratto etanolico idrolizzato ha mo-

- Key words: Antioxidant activity, lipid model system, oregano extracts, oxidative stability instrument, polyphenols -

this extract, expressed as gallic acid, were quantified by spectrophotometric analysis at 280 nm. The ethanolic extract was also analyzed by TLC (thin layer chromatography) and showed the presence of flavones, flavanones and dihydroflavonols, whether glycosilated or not.

strato una maggiore attività antiossidante rispetto all'estratto non trattato. I polifenoli totali di questo estratto, espressi come acido gallico, sono stati quantificati attraverso analisi spettrofotometrica, a 280 nm. L'estratto etanolico è stato anche analizzato in TLC (cromatografia su strato sottile) e ha mostrato la presenza di flavoni, flavanoni e diidroflavonoli, sia liberi che glicosilati.

INTRODUCTION

In recent years, there has been increasing interest in the possibility of using vegetable extracts as antioxidants. The food and cosmetic industries have attempted to select vegetable extracts to replace synthetic phenolic antioxidants, such as BHA and BHT (CERUTTI, 1999; GIESE, 1996; HAMAMA and NAWAR, 1991). Furthermore, the possible use of natural antioxidants for the prevention of some human diseases has stimulated increasing interest in this field. In fact, it is well known that arteriosclerosis, ischaemia events, human cancer and inflammatory diseases are related to significant exposure of cells to oxidative stress (NAMIKI, 1990; PENG *et al.*, 1992; VINSON *et al.*, 1995; PORRINI and TESTOLIN, 1997).

Many vegetable extracts have been studied in relation to their antioxidant activity (LINDBERG and BERTELSEN, 1995; GALLINA TOSCHI *et al.*, 2000). Notably the Labiatae family is well-known for its antioxidative properties, and especially rosemary and sage have been reported to have strong effectiveness (CUVELIER *et al.*, 1990, 1994, 1996; SCHWARZ and TERNES, 1992a, b; SCHWARZ, *et al.*, 1992; CHEN *et al.*, 1992; PIZZOCARO *et al.*, 1994; RICHHEIMER *et al.*, 1996; VARELTZIS *et al.*, 1997). Oregano, thyme and marjoram extracts have also shown a pronounced effect in stabilizing lipids against autoxidation (KIKUZAKI and NAKATANI, 1989; ECONOMOU *et al.*, 1991; CHEVOLLEAU *et al.*, 1992; VEKIARI *et al.*, 1993a, b).

This study was carried out with the aim of selecting the best extraction conditions for oregano leaves, often used in foods for its organoleptic properties, to obtain greater antioxidant power.

MATERIALS AND METHODS

Solvents and reagents were analytical or HPLC grade, from Carlo Erba (Milan, Italy) and Prolabo (Paris, France). Flavonoid standards (99% pure), gallic acid and BHT (99% pure) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Oregano leaves (*Origanum vulgare* L.) were obtained from the region of Calabria (Italy).

Preparation of oregano extracts

Oregano leaves (50 g) were extracted separately with 300 mL of four different solvents: ethanol, diethyl ether (not stabilized with BHT), *n*-hexane:2-propanol 4:1 (v/v) and *n*-pentane. Extractions were conducted at the boiling temperature of each single solvent, in a Soxhlet apparatus, for four hours. After each

extraction, the solvent was dried under vacuum with a Rotavapor (60°, 30°, 50° and 30°C, respectively); the solid was weighed and brought to a final volume of 25 mL with the same solvent.

Acid hydrolysis of oregano extracts

A certain volume of the solutions obtained from the extraction with four different solvents, containing amounts from 1 to 50 mg of oregano extract (calculated on the basis of the extraction yield) were dried under gentle nitrogen flow and then hydrolyzed using 1.5 mL of 1M HCl and kept in a boiling water bath for 1 h (HOLLMAN *et al.*, 1996; LU and FOO, 1997). The hydrolysis products were extracted four times with 0.5 mL of diethyl ether (not stabilized with BHT); the solvent was then dried, and the solid dissolved in 0.15 mL of the same solvent.

Preparation of the lipid model system

A refined peanut oil, from the market, was used as the model lipid system. Ten percent of bleaching earth (previously activated at 110°C for 18 h) was added and the oil was kept for 15 min at 80°C in a Rotavapor, under weak vacuum (BENDINI *et al.* 2001).

Antioxidant activity measurement

The antioxidant activity of the different oregano extracts was tested on the refined bleached peanut oil by subjecting it to forced dynamic oxidation. An eight-channel Oxidative Stability Instrument (OSI) (Omnion Inc., Decatur, Illinois) was used. Five grams (±0.1 g) of peanut oil were weighed in each glass tube, the temperature was set at 98°C and the stream of air bubbled through the oil was 120 mL/min. The OSI measures the changes in conductivity due to the formation of ionic volatile organic acids (mainly formic acid) which are collected in a polycarbonate tube containing deionized water. The OSI time, obtained under standard conditions of temperature and air bubbling (JEBE *et al.*, 1993), corresponds to the change in slope of the volatile organic acid production, which is considered to be the end of the induction period of an oil.

Total polyphenol determination

The quantity of total polyphenols in the oregano ethanolic extract, expressed as weight percent (w/w), was determined by spectrophotometric analysis at 280 nm; the calibration curve was built with standard solutions of gallic acid, as reported by the official method (MARGHERI and FALCIERI, 1972).

Thin-layer chromatographic analysis (TLC) of oregano extracts

TLC plates, precoated with silica gel (20x20 cm, Merck, Darmstadt, Germany) were used to separate the components of the ethanolic oregano extract. The developing system used was toluene/acetone/formic acid (30:60:10, v/v/v).

The following spray reagents were used to identify the chemical compounds: (a) atomization with 5% aluminum chloride in water (w/v) (a yellow color indicates the presence of flavonoids) (VEKIARI *et al.*, 1993a), (b) exposure to ammonia fumes (observation under UV light shows a dark color that persists after exposure to ammonia fumes, if the compounds are flavones, dihydroflavonols or flavanones) (VEKIARI *et al.*, 1993a), (c) vaporization with sulphochromic mixture and carbonization at 140°C (organic compounds appear as brown color), (d) atomization with Folin-Ciacolteau reagent (phosphotungstic and phosphomolybdic acids) and, after three minutes, with 20% sodium carbonate in water (w/v) (polyphenol compounds appeared as blue color) (MARGHERI and FALCIERI, 1972).

RESULTS AND DISCUSSION

The oregano extraction using four different solvents gave yields of: ethanol 8.3% (w/w), *n*-hexane:2-propanol 4:1 6.6% (w/w), diethyl ether 5% (w/w:) and *n*-pentane 3.9% (w/w). The highest yield was obtained with ethanol. Methanol was not used, even if it has been reported to be the best solvent for phenols (MØLLER *et al.*, 1999) because it is toxic and should be completely removed before the extract can be used as a food additive. The use of water, which is certainly suitable for the food industry and considered effective for the extraction of water-soluble phenolic compounds (MILOŠ *et al.*, 2000), was not considered because it requires a high temperature and a long time to be removed, during which time the antioxidant components could easily decompose.

The repeatability of the OSI time of the peanut oil, used as a model lipid system, was checked by analyzing sixteen samples of oil, freshly bleached each time. The mean value of the OSI time was 14.40 h, the standard deviation was ±0.77 h (coefficient of variation was 5.36%). Before analyzing each single extract, some tests were carried out to check if small amounts (up to 3% v/w) of solvents used for the extractions could modify the OSI time of the model lipid system. Ethanol caused a small significant decrease in OSI time (-2.4%), very close to the coefficient of variation of the analysis; no significant effect was recorded for diethyl ether but *n*-pentane or *n*-hexane:2-propanol 4:1 caused a slight increase in the OSI time.

As shown in Fig. 1, the ethanolic extract gave a higher antioxidant activity than the other extracts, obtained with less polar solvents, in increasing amounts, from 0.1 to 0.6% w/w. It is also evident that the hydrolyzed ethanolic extract gave the highest antioxidant activity and this effect increased with increase in the concentration of the extract added to the oil, up to 0.4%.

The effect of the ethanolic extract was tested under a wider range of concentrations, from 0.02 to 5% w/w (Fig. 2). The OSI time of the model lipid system (14.40 h) doubled when the ethanolic extract was added at 0.5% (29.7 h) and it became almost four times as much at 5% (56.9 h). Due to the organoleptic modifications induced by adding high amounts of the extract, percentages higher than 5% were not tested. As reported in Figs. 1 and 2, the addition of 0.1% (25.70 h) hydrolysed ethanolic extract exhibited a protective effect corresponding to 97% of that shown by the addition of 0.04% (26.50 h) BHT, which is a pure standard product. The total polyphenol content of the ethanolic oregano extract was 10.17% ±0.47 (expressed as weight % as gallic acid), calculated on four determinations by UV analysis at 280 nm, near the maximum absorption of most phenols.

The ethanolic oregano extract was then analyzed by TLC (Fig. 3) and nine bands were isolated as black spots, after carbonization with a sulphochromic mixture, and were numbered starting from the top of the plate (column A). The reactions to the solution of 5% aluminum chloride in water, to ammonia fumes and to the Folin-Ciocalteau reagent were positive for bands 3-9 (R_f from 0.86 to 0.05); this response is characteristic of polyphenols, particularly of flavones, flavanones and dihydroflavonols. Bands 1 and 2 with R_f = 0.93 and R_f = 0.89, respectively, were not flavonoids (probably chlorophylls); the spot with R_f = 0.84 (band 4 columns A, B) could contain, as reported in the literature (VEKIARI *et al.*, 1993a), apigenin (flavone, column C), eriodictyol (flavanone, column D), kaempferol (flavonol, column E), quercetin (flavonol, column G and I) or rosmarinic acid (phenolic acid, column M). Bands 6-9 corresponded to polyphenol glycosides because they disappeared when the oregano extract was hydrolyzed (column B) such as: rutin (quercetin-3-

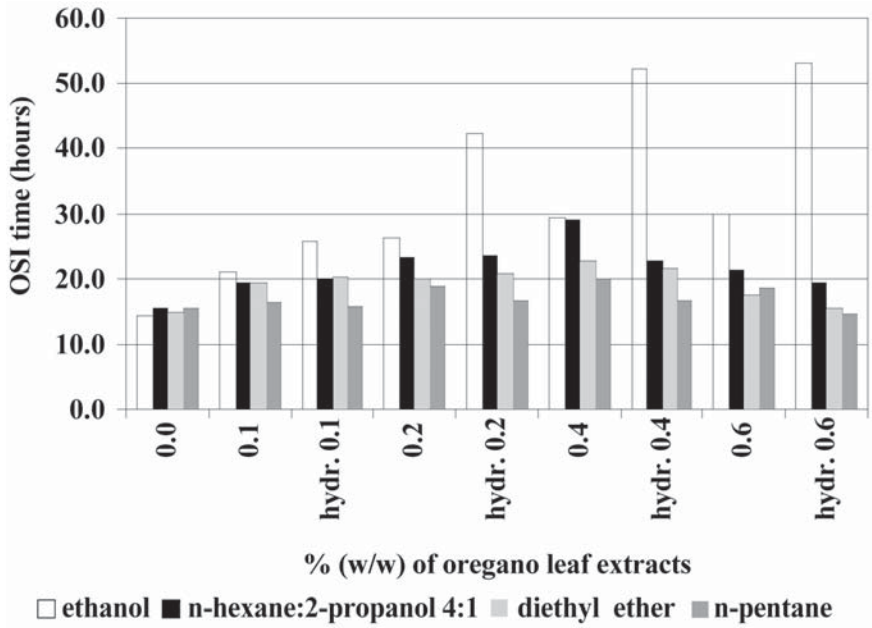


Fig. 1 - OSI time values (hours)[a] of refined bleached peanut oil, with the addition of increasing amounts (from 0.1 to 0.6% w/w) of untreated and hydrolysed oregano leaf extracts, obtained using different solvents (ethanol, diethyl ether, *n*-hexane: 2-propanol 4:1, *n*-pentane). Control group was refined bleached peanut oil, with the addition of 3% (v/w) of each extraction solvent.
[a] Calculated from two determinations.

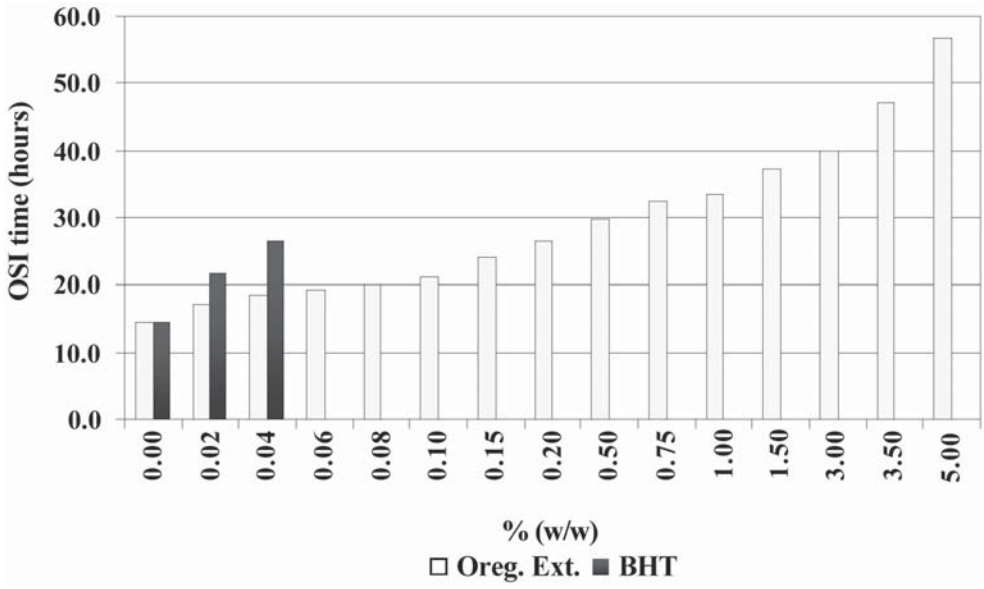


Fig. 2 - OSI time values (hours)[a] of the refined bleached peanut oil, with the addition of increasing amounts (from 0.02 to 5% w/w) of untreated ethanolic oregano leaf extracts and BHT (from 0.02 to 0.04% w/w). Control group was refined bleached peanut oil, with the addition of 3% (v/w) ethanol and diethyl ether (the solvent used to dissolve BHT).
[a]Calculated from two determinations.

rutinoside, R_f 0.19, spot number 12, column H) which, once hydrolyzed (column I), released its corresponding aglycone (quercetin, R_f = 0.84, column G).

CONCLUSIONS

To evaluate the use of vegetable extracts to protect food lipids from oxidation, the antioxidant power of the dried oregano leaf extracts, obtained using different solvents, was measured by OSI time. The dried ethanolic extract had the highest antioxidant activity and, when added to a model lipid system at a percentage of 0.20% w/w (26.40 h of OSI time), showed the same antioxidant power as BHT added at 0.04% w/w (26.50 h of OSI time). Hydrolyzed ethanolic oregano extracts had stronger antioxidant properties, with respect to

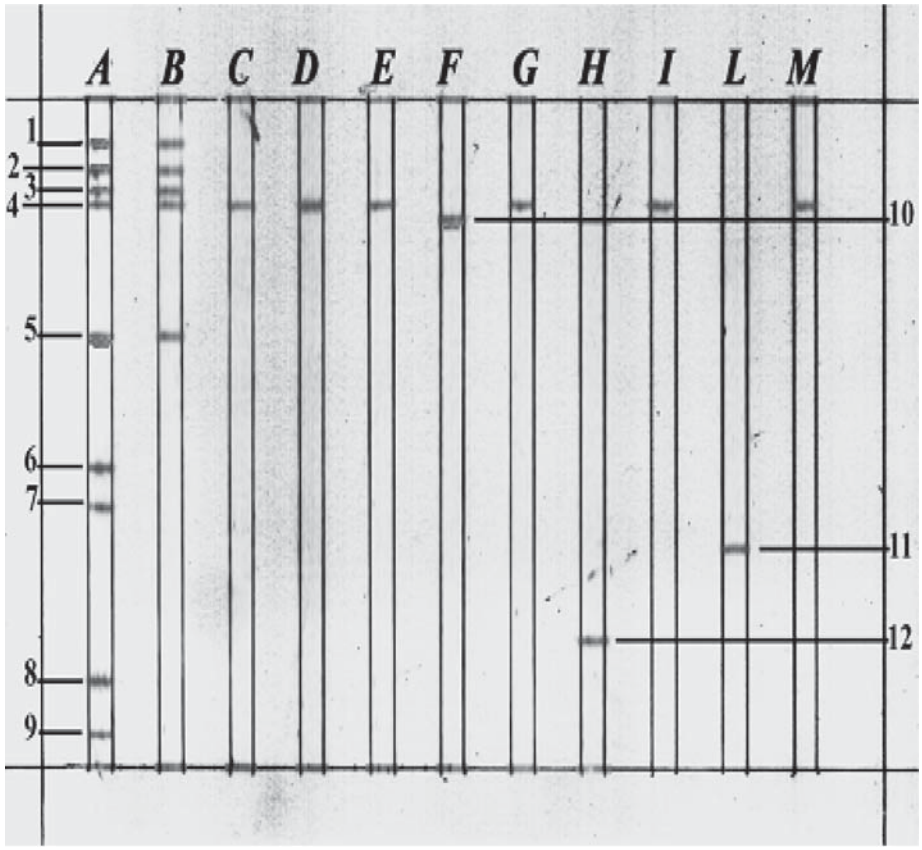


Fig. 3 - Thin layer chromatography of: A = Untreated ethanolic extract of oregano leaves; B = Hydrolysed ethanolic extract of oregano leaves; C = Apigenin; D = Eriodictyol; E = Kaempferol; F = Taxifolin (dihydroquercetin); G = Quercetin; H = Rutin (quercetin-3-rutinoside); I = Hydrolysed rutin; L = Hesperidin (hesperetin-7-rhamnoglucoside); M = Rosmarinic acid.
The bands visualized as black spots after carbonisation with sulphochromic mixture, are numbered from 1 to 9, starting from the top of the plate. The R_f (retention factor) of the bands are:
A1,B1 R_f = 0.93; A2,B2 R_f = 0.89; A3,B3 R_f = 0.86; A,B,C,D,E,G,I,M-4 R_f = 0.84;
A5,B5 R_f = 0.64; A6 R_f = 0.45; A7 R_f = 0.39; A8 R_f = 0.13; A9 R_f = 0.05;
F10 R_f = 0.82; L11 R_f = 0.33; H12 R_f = 0.19.
Mobile phase: toluene/acetone/formic acid 30:60:10 v/v/v.

the non-hydrolyzed ones. In fact 0.20% (42.30 h of OSI time) of the hydrolyzed extract showed an antioxidant power 1.6 times higher than the untreated extract. TLC analysis of the untreated and hydrolyzed extracts showed the presence of polyphenolic compounds in both types of extracts and the lack of phenolic glycosides (spots with low R_f) in the hydrolyzed ones. This evidence suggests that free phenolic aglycones have a higher antioxidant power, as confirmed by the structure activity relationship studies of HERRMANN (1976) and DAS and PEREIRA (1990) and as showed for oregano volatile aglicone, tested by measuring peroxide values of lard, stored at 60°C (MILOŠ *et al.*, 2000). This evidence merits further investigation on the use of phenolic hydrolyzed oregano extracts as a source of natural antioxidants for the food and cosmetic industries.

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PAPER

INITIAL INVESTIGATION ON SOME CHEMICAL CONSTITUENTS OF CAPERS (*CAPPARIS SPINOSA* L.) FROM THE ISLAND OF SALINA

STUDIO INIZIALE SU ALCUNI COSTITUENTI CHIMICI DEI CAPPERI (*CAPPARIS SPINOSA* L.) DELL'ISOLA DI SALINA

D. GIUFFRIDA*, F. SALVO, M. ZIINO, G. TOSCANO and G. DUGO
Università di Messina, Dipartimento di Chimica Organica e Biologica,
Salita Sperone 31, 98166 S. Agata di Messina, Messina, Italy
* Corresponding author: Tel. +39 090 676-5241; Fax +39 090 393895;
e-mail: giuffrid@isengard.unime.it

ABSTRACT

The chemical composition of three different sized buds of raw and pickled capers from the island of Salina (Eolian Archipelago) was determined. Water and carbohydrate contents increased as bud size increased, whereas flavonoid, mineral, ash, lipid and protein contents decreased as bud size increased. Pickled capers had a much lower content of flavonoids and lipids. High amounts of Na, Mg, Ca, and K were determined. Among the polyunsaturated fatty acids, linoleic acid (18:2 ω 6) and γ-linolenic acid (18:3 ω 6) were identified as the major components.

RIASSUNTO

È stata determinata la composizione chimica dei capperi freschi e salati di tre diverse dimensioni dell'isola di Salina (Arcipelago Eoliano). Il contenuto di umidità e carboidrati cresce all'aumentare delle dimensioni dei capperi, mentre il contenuto di flavonoidi, metalli, ceneri, lipidi e proteine diminuisce all'aumentare delle dimensioni degli stessi. La salatura porta ad una notevole diminuzione del contenuto di flavonoidi e lipidi. Sono inoltre stati determinati alcuni elementi minerali e fra questi, Na, Mg, Ca e K risultano i più abbondanti. Fra gli acidi grassi polin-

- Key words: capers, composition, elements, fatty acids, flavonoids, lipids -

Rutin and kaempferol-3-rutinoside were the most abundant flavonol glucosides. Small raw buds were more suitable for processing due to their chemical properties.

saturi, l'acido linoleico (18:2 ω 6), e l'acido γ-linolenico (18:3 ω 6), sono stati identificati come i maggiori componenti. La rutina e il canferolo-3-rutinoside sono i flavonoli glicosidi più rappresentativi. I boccioli freschi di piccole dimensioni risultano i più adatti a successive trasformazioni in riferimento alla loro composizione chimica.

INTRODUCTION

There are about 150-200 species of the genus *Capparis* (Capparaceae) in tropical and subtropical countries throughout the world. *Capparis spinosa* L. is a common shrub in the Mediterranean area; its young shoots, flower buds, fruits and seeds are used for food. Capers are the flower buds of the caper plant and have been used as a seasoning since ancient times. Spain, Marocco, Italy and Turkey are the leading world producers; it is estimated that the world production of capers is approximately 10,000 tons per annum.

There have been a limited number of studies on the chemical constituents of capers (BREVARD *et al.*, 1992; NOSTI VEGA and de CASTRO RAMOS, 1987; ÖZCAN and AKGÜL, 1995, 1998; RODRIGO *et al.*, 1992), but no detailed study on the composition of raw and pickled Italian capers is available. In Italy, during the last three decades, capers have been cultivated mainly on the islands of Salina (Eolian Archipelago) and Pantelleria, where it has become an important economic crop.

The purpose of the present study was to determine some chemical constituents of raw capers from the island of Salina having different bud sizes in order to provide new basic data on the composition of the flower buds of this species. Furthermore the influence of pickling on these constituents was investigated in the final salted product.

MATERIALS AND METHODS

Plant material

Raw and pickled flower buds from *Capparis spinosa* L. were purchased from a local farmer on the island of Salina (Eolian Archipelago, Sicily) in June 2000. Capers were collected from five different fields from three different caper plants in each field and were analysed separately. The buds were divided into three different sizes: large (diameter >14 mm), medium (diameter 9-14 mm) and small (diameter <9 mm). The water content was immediately measured on arrival at the laboratory. A portion of the raw capers was immediately lyophilized and a portion of the pickled capers was washed and then lyophilized.

The usual commercial processing procedure for preparing pickled capers in Salina consists in a pretreatment in which raw capers are mixed with marine salt (25% by weight) for about ten days; during this time a brine is formed and fermentation takes place; at the end of this stage the brine is discarded and the capers are treated with marine salt (15% by weight) for another ten days and then stored to obtain the finished product.

The lyophilised material and the remaining raw material were kept in cold storage (-20°C) until used. Three replicates were performed for each determination and mean values are given in the

tables; the standard deviation was less than 5% for minerals, and less than 1% for the others.

Water, ash, protein and carbohydrate

Water, ash and protein were determined according to official methods (AOAC, 1984). Carbohydrate content was determined by difference.

Lipids

Lyophilized plant material (10 g) was extracted with a mixture of chloroform/methanol (2:1, v/v) according to the method of FOLCH *et al.* (1957). Small aliquots of the extracted lipids were dissolved in hexane and separated into lipid classes by thin layer chromatography (HENDERSON and TOCHER, 1992) on 20x20 cm TLC glass plates coated with a layer (0.5 mm) of silica gel G (E. Merck, Milano, Italy) using hexane/diethyl ether/formic acid (80:20:2, v/v) as the solvent system. Detection of the lipid classes was achieved by spraying with 2,7-dichlorofluorescein (0.2% in ethanol), and long wavelength UV light. The classes were identified by comparison with authentic lipid standards (Supelco, Milano, Italy). Four classes (hydrocarbons, triacylglycerols, free fatty acids, and polar lipids) were isolated in order of decreasing retention factors. Another small aliquot (10 mg) of the extracted total lipids was converted into fatty acid methyl esters (FAMEs) by direct transesterification with 1% sulfuric acid:methanol under reflux and in an argon atmosphere. FAMEs were analysed (EVERSHED, 1992) by a Shimadzu GC 17A gas chromatograph (Shimadzu Italy) equipped with a flame ionization detector (FID). Separations were carried out with a 30 m x 0.32 mm i.d. fused silica capillary column Omegawax 320 (Supelco) having a 0.25 mm film thickness. The oven temperature was programmed from 160°C for 2 min and to 200° at 3°C/min and then from 200° to 220° at 4°C/min. The injector had a split ratio of 84:1; injector and detector temperatures were 250°C. Hydrogen was used as the carrier gas with a flow rate of 1.9 mL/min. The individual FAMEs were identified by comparison with the retention times of standard mixtures (Supelco PUFAs 1-2 and Menhaden Oil Text Mix). Peak areas were automatically integrated and data were recorded with a Hewlett Packard 3394 integrator.

Flavonoids

Lyophilized plant material (2 g) was extracted overnight with 100 mL of a mixture of methanol/water (70:30, v/v), filtered and analysed by liquid chromatography for the determination of the flavonol glycosides. Quercetin and kaempferol were determined after extraction and acid hydrolysis of the flavonoid glycosides with 1.2 M HCl in 50% aqueous methanol as described by HERTOG *et al.* (1992).

Liquid chromatography was performed using a Shimadzu HPLC apparatus, (Shimadzu Italy) equipped with two LC-10AD-Vp pumps, an SCL-10A-Vp system controller, a GT-154 on line degassing device, a Rheodyne injector with 20 µL loop, a BER 1000 P oven, an SPD-M10A Vp diode array detector, and a Digital venturis FX-2 computer. An Alltech Alltime ODS (5 µm, particle size) column (250x4.6 mm) fitted with an Alltech Alltime guard column (5 µm particle size; 7.5 x 4.6 mm) was used. The mobile phase consisted of two eluents: (W) water and (A) acetonitrile, adjusted to pH 3.1 with acetic acid. To achieve separation, flow rate was set at 1.0 mL/min and the following gradient was used: initial 95% W and 5% A; 10 min linear change to 75% W and 25% A; 10 min linear to 60% W and 40% A; 10 min linear to 50% W and 50% A; five min linear to 0% W and 100% A maintained for five min; 10 min to 95% W and 5% A main-

tained for 10 min for re-equilibration at initial eluent composition before a new injection. The column temperature was set at 30°C. Detection was at 365 nm and 254 nm (4 nm band width) and spectrograms were recorded between 220 and 400 nm at the apex of each peak.

Pure flavonoid standards were purchased from Extrasynthese (Genay, France). Flavonoids in the samples were identified by comparison of retention times and spectrograms for each peak with the corresponding flavonoid standard.

Total phenolics

The phenol content was measured by the Folin-Ciocalteu reagent (SINGLETON and ROSSI, 1965) using the method described by VELIOGLU *et al.* (1998). Results are expressed as gallic acid equivalents. All reagents were purchased from Sigma-Aldrich (Milano, Italy).

Elements

Minerals were determined according to a modification of method number 200.3 published by EPA (1992). Merck AAS standard solutions of the minerals analysed were used for preparing calibration standards. Merck Suprapur concentrated nitric acid and peroxide were used during the different stages of dissolutions. Concentrations of Na, Ca, K, Mg, Fe, and Zn were determined with a Varian Model SpectrAA20-Plus atomic absorption flame spectrometer (Milano, Italy) equipped with a single element hollow cathode lamp, having a deuterium lamp background correction system. The concentration of all other elements, except for As and Se, were determined using a Varian Model 55 atomic absorption spectrometer equipped with a single element hollow cathode lamp with a deuterium lamp background correction system and a Varian 110 Model GTA-graphite tube atomizer; pyrolytic graphite coated tubes were used throughout the experiments. As and Se were determined using a Varian Model SpectrAA20-Plus atomic absorption spectrometer equipped with a Varian Model VGA 77 Metal Hydride generating system.

RESULTS AND DISCUSSION

Proximate composition

Table 1 gives the proximate composition of the raw and pickled caper samples. There was a notable increase in water content and decrease in ash, protein and lipid contents as bud size increased in the raw capers. Small raw capers had higher protein and lipid contents than those reported by RODRIGO *et al.* (1992). These differences could be

Table 1 - Proximate composition (%, wet weight) of three different sizes of raw and pickled capers.

	Small raw	Small pickled	Medium raw	Medium pickled	Large raw	Large pickled
Water	80.41± 0.78	64.50±0.62	83.14±0.81	60.80±0.58	84.08±0.81	60.65±0.58
Ash	1.50±0.01	n.d.	1.31±0.03	n.d.	1.21±0.09	n.d.
Protein	8.98±0.06	8.66±0.03	5.41±0.03	6.04±0.04	4.60±0.02	5.32±0.04
Total Lipids	2.01±0.02	1.13±0.01	1.35±0.01	0.80±0.01	1.25±0.06	0.86±0.01
Carbohydrates	7.1±0.05	25.71±0.26	8.79±0.06	32.36±0.28	8.86±0.06	33.17±0.26

n.d. not determined.

due to variety/cultivar, environmental and growth conditions. The highest protein and ash were found in small buds, in agreement with ÖZCAN and AKGÜL (1998). The reduction in the content of these constituents in raw capers as bud size increased, can be ascribed to a dilution effect taking place during bud growth. A significant lipid synthesis may also occur during the first phase of caper growth. There was very little increase in carbohydrates with bud maturation. Pickling had a negligible influence on the protein level but a considerable affect on the lipid values. The high salt levels used during the pickling process which also assure stability of the finished product may cause spoilage of the raw buds, with loss of texture due to osmotic stress and degradative processes, resulting in considerable lowering of the quality of the product.

Lipid composition

Table 2 provides the percentage composition of the four lipid classes making up the total lipids. Polar lipids were the main constituents. The highest level of triacylglycerols was present in large raw capers; triacylglycerol values in raw capers were higher than in the salted ones, whereas free fatty acids were lower in the raw buds. The levels of free fatty acids may have resulted from the hydrolysis of triacylglycerols and polar lipids occurring during the pickling process. Since the stability and quality of capers also depend on the degree of unsaturation of the lipid component, it is interesting to consider the fatty acid composition in the total lipids (Table 3). Among the saturated fatty acids, palmitic (16:0) and stearic (18:0) were the most representative. Among the monounsaturated acids, oleic acid (18:1 ω9) was dominant. Among the polyunsaturated acids, linoleic acid (18:2 ω 6) and γ-linolenic acid (18:3 ω 6) were identified as major components. These findings are in agreement with the reports by NOSTI VEGA and de CASTRO RAMOS (1987). Pickling greatly reduced the polyunsaturated acid content whereas amounts of monoenes and saturates increased; in particular, the content of palmitic acid greatly increased after pickling. The high salt level has a primary effect on accelerating the oxidative processes in the polyunsaturated acids. In raw buds over 50% of the acids were unsaturated.

Total phenolics and flavonoids

The presence of phenolics in food may have an important effect on the oxidative stability and microbiological safety of products. Table 4 gives the percentage composition of the total phenolic content and of the flavonoids identified. The samples contained glycosides of quercetin and kaempferol. The major components were rutin with average wet weight values 0.23% for raw and 0.13% for pickled capers, respectively, and kaempferol-3-rutinoside with average wet weight values of 0.19% for raw and

Table 2 - Lipid classes (%, in total lipids) of three different sizes of raw and pickled capers.

	Small raw	Small pickled	Medium raw	Medium pickled	Large raw	Large pickled
Hydrocarbons	4.20±0.04	3.40±0.02	4.80±0.04	3.40±0.03	4.40±0.03	3.80±0.02
Triacylglycerols	5.40±0.04	4.80±0.02	8.80±0.02	6.80±0.02	10.60±0.04	6.0±0.03
Free Fatty Acids	3.80±0.01	6.80±0.05	4.20±0.02	8.60±0.04	5.20±0.04	8.60±0.06
Polar Lipids	25.0±0.22	21.35±0.16	37.60±0.32	27.80±0.12	36.20±0.28	25.20±0.20

Table 3 - Fatty acids (%, in total lipids) of three different sizes of raw and pickled capers.

	Small raw	Small pickled	Medium raw	Medium pickled	Large raw	Large pickled
FATTY ACIDS						
Saturates						
C14:0 iso	0.085±0.01	0.225±0.03	0.211±0.01	0.186±0.02	0.037±0.02	0.103±0.04
C14:0	0.495±0.02	0.813±0.02	0.914±0.01	0.901±0.03	0.476±0.02	1.217±0.01
C15:0 ai	0.063±0.01	0.034±0.01	0.053±0.01	0.112±0.04	0.036±0.02	0.103±0.01
C15:0	0.139±0.02	0.062±0.01	0.219±0.05	0.236±0.02	0.179±0.01	0.410±0.01
C16:0 iso	0.541±0.03	0.816±0.01	0.618±0.03	0.722±0.01	0.445±0.03	0.902±0.01
C16:0	29.853±0.22	43.622±0.36	31.989±0.28	42.570±0.36	26.997±0.18	52.038±0.46
C17:0 ai	0.148±0.02	0.332±0.04	0.211±0.01	0.336±0.01	0.203±0.02	0.315±0.01
C17:0	0.046±0.01	0.474±0.02	0.438±0.02	0.474±0.02	0.430±0.01	0.507±0.01
C18:0	4.880±0.04	4.479±0.02	6.670±0.04	6.594±0.05	5.647±0.04	5.646±0.05
C19:0	0.037±0.01	0.164±0.01	0.057±0.01	0.051±0.01	0.028±0.01	0.097±0.01
C20:0	0.788±0.03	0.345±0.01	1.179±0.01	0.913±0.01	1.063±0.01	0.422±0.02
C22:0	1.171±0.01	0.332±0.02	0.671±0.01	0.398±0.01	0.824±0.02	0.390±0.01
C23:0	0.042±0.01	0.394±0.01	0.117±0.01	0.056±0.01	0.149±0.01	0.205±0.01
C24:0	0.230±0.03	0.666±0.01	0.459±0.03	0.143±0.01	0.382±0.03	0.000
Monoenes						
C16:1t	0.230±0.01	0.111±0.02	0.073±0.01	0.184±0.02	0.081±0.01	0.164±0.03
C16:1	0.183±0.02	0.404±0.01	0.185±0.01	0.336±0.02	0.152±0.01	0.677±0.01
C17:1	0.040±0.01	0.036±0.04	0.028±0.02	0.077±0.01	0.117±0.04	0.051±0.01
C18:1 ω9	5.164±0.03	7.354±0.06	5.621±0.04	8.090±0.06	4.550±0.03	9.401±0.06
C18:1 ω7	1.065±0.02	2.056±0.01	0.795±0.01	1.745±0.01	1.003±0.02	1.020±0.01
C20:1	0.312±0.01	2.751±0.03	0.711±0.01	0.148±0.02	0.932±0.01	0.502±0.01
Polyenes						
C18:2 ω6	21.359±0.18	17.158±0.12	19.795±0.12	16.077±0.22	25.242±0.24	14.571±0.09
C18:3 ω6	33.128±0.27	17.371±0.11	28.985±0.21	19.650±0.14	31.028±0.28	11.259±0.09
Sum Saturates	38.519	52.759	43.808	53.693	36.896	62.355
Sum Monoenes	6.995	12.713	7.413	10.580	6.834	11.815
Sum Polyenes	54.487	34.528	48.779	35.727	56.270	25.829

Table 4 - Total phenolics and flavonoids (%, dry weight) of three different sizes of raw and pickled capers.

	Small raw	Small pickled	Medium raw	Medium pickled	Large raw	Large pickled
Total Phenolics	4.28±0.03	1.67±0.01	3.40±0.02	1.62±0.02	3.28±0.01	1.64±0.01
Rutin	1.7±0.01	0.47±0.01	1.42±0.02	0.31±0.01	0.88±0.01	0.27±0.02
Kaempferol-3-rutinoside	1.23±0.01	0.21±0.01	1.1±0.01	0.13±0.01	0.95±0.01	0.08±0.01
Kaempferol-3-glucoside	0.11±0.01	n.d.	0.13±0.01	n.d.	0.12±0.01	n.d.
Quercetin	0.8±0.02	0.18±0.01	0.65±0.01	0.16±0.01	0.49±0.02	0.16±0.01
Kaempferol	0.92±0.01	0.21±0.01	0.88±0.02	0.33±0.01	0.8±0.02	0.14±0.02

n.d. not detected.

0.053% for pickled capers, respectively; their contents decreased as bud size increased. The contents of these flavonol glucosides should be of interest from a health and nutrition point of view because of the numerous beneficial effects attributed to flavonoids (HERTOG *et al.*, 1995). A decrease in flavonoid glucoside content with maturation has been reported in other fruits (AMIOT *et al.*, 1986). The higher flavonoid glucoside content in small buds may be due to active phenolic synthesis in young growing flower buds, in which these compounds act as defense and protective agents. The initial rapid growth is followed by maturation during which time some chemical or enzymatic degradation of the glycosides occur and the end products may be reused in other metabolic pathways. It also seems probable that as the maturation of the flower buds progresses, phenolic synthesis is directed more towards the formation of anthocyanins. The relative percent of the different flavonol glucosides identified in raw capers was reported by RODRIGO *et al.* (1992). On the basis of their information and considering the retention time and UV-spectra of peak 1 (Fig. 1), it seems very probable that it could be kaempferol-3-rhamnorutinoside. Pickling greatly reduced but did not completely eliminate the amount of total phenolics, including flavonoids. The general problem of the effect of stress on the secondary metabolism in plant cells has been reviewed by DICOSMO and TOWER (1984); the high quantity of salt used may have caused hydrolysis of flavonoid glucosides and polyphenoloxidase is activated as disruption of cell integrity occurs. The total phenolic content of the plant material investigated by VELIOGLU *et al.* (1998) varied from 169 to 10548 mg/100 g of dry product. The level of total phenolics in capers reported in this study can be regarded as considerable, for example higher than in potatoes and close to that of red onion.

Element composition

The concentration of the 17 elements studied is shown in Table 5. Na, K, Ca, and Mg were the most abundant elements. There were also appreciable amounts of Zn, Mn, Cu and Fe. The contents of Na and Fe were lower, and the content of Cu was higher with respect to results reported for raw buds (NOSTI VEGA and de CASTRO RAMOS, 1987; ÖZCAN and AKGÜL, 1998; RODRIGO *et al.*, 1992). Except for Ca, pickling reduced the mineral concentration.

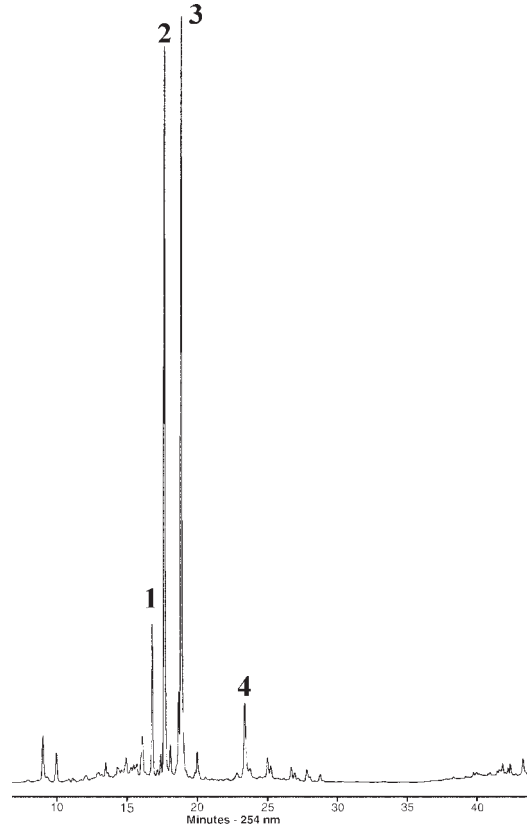


Fig. 1 - Chromatogram of flavonoids present in capers. (1) Keampferol derivate (probably kaempferol-3-rhamnorutinoside); (2) Rutin; (3) Kaempferol-3-rutinoside; (4) Kaempferol-3-glucoside.

Table 5 - Elemental composition (ppm, dry weight) of three different sizes of raw and pickled capers.

	Small raw	Small pickled	Medium raw	Medium pickled	Large raw	Large pickled
Elements						
Na	193.3± 7.72	n.d.	188.6 ± 9.0	n.d.	186.2± 5.4	n.d.
K	22395.4± 666	12702.7 ± 254	21325.2 ± 631	12628.4 ± 375	21121.4± 844	12538.6 ±535
Ca	2420.8± 96	3508.1 ± 105	2360.6 ± 98	3322.4 ± 97	2380.8± 78	3642.4 ±155
Mg	4638.6± 154	2756.8 ± 54	4460.8 ± 98	2628.4 ± 88	4280.2± 196	2614.8 ±57
Fe	13.9± 0.54	9.7 ± 0.08	12.8 ± 0.42	9.6 ± 0.07	11.6± 0.35	9.3 ±0.07
Zn	55.3± 1.78	34.8 ± 1.36	52.6 ± 2.02	32.6 ± 1.97	52.2± 2.06	31.1 ±1.22
Pb	0.42± 0.02	<0.4 ± 0.04	0.4 ± 0.02	<0.4 ± 0.01	0.41± 0.02	<0.4 ±0.03
Cd	<0.4± 0.02	<0.4 ± 0.01	<0.4 ± 0.01	<0.4 ± 0.03	<0.4± 0.02	<0.4 ±0.02
Ni	4.8± 0.156	2.8 ± 0.114	4.4 ± 0.221	2.6 ± 0.114	4.2± 0.222	2.6 ±0.148
Cr	<0.4± 0.04	<0.4 ± 0.02	<0.4 ± 0.02	<0.4 ± 0.02	<0.4± 0.03	<0.4 ±0.01
V	<0.4± 0.02	<0.4 ± 0.01	<0.4 ± 0.04	<0.4 ± 0.02	<0.4± 0.01	<0.4 ±0.01
Mn	18.9± 0.8	10.3 ± 0.3	17.6 ± 0.5	9.6 ± 0.4	16.4± 0.3	9.8 ±0.4
Se	1.5± 0.066	1.1 ± 0.022	1.8 ± 0.014	0.9 ± 0.036	1.2± 0.018	0.8 ±0.018
As	0.4± 0.03	<0.4 ± 0.02	0.6 ± 0.02	<0.4 ± 0.01	0.4± 0.01	<0.4 ±0.02
Cu	14.3± 0.55	11.0 ± 0.42	13.8 ± 0.35	10.2 ± 0.54	13.4± 0.47	10.4 ±0.52
Co	5.0± 0.2	3.8 ± 0.1	4.9 ± 0.3	3.4 ± 0.2	3.98± 0.2	3.4 ±0.1
Ti	2.7± 0.2	<0.4 ± 0.01	2.5 ± 0.2	<0.4 ± 0.02	2.2± 0.1	0.4 ±0.01

n.d. not determined.

CONCLUSIONS

The physical and chemical properties of capers have been reported (ÖZCAN and AKGÜL, 1998; RODRIGO *et al.*, 1992) to be affected by species, growth conditions, environmental factors and bud size. In this study the chemical composition of capers grown on the island of Salina is reported for the first time; some chemical constituents affected by bud size and by the pickling process are also shown. A detailed fatty acid analysis, quantification of total phenolics and of the main flavonoids, and determination of Ni, Cr, V, Se, As, Co, Ti, Cd and Pb in capers are also reported for the first time. Small buds have the advantage of being harder which is desirable to obtain high quality products due to processing without flaking; moreover the higher concentration of important chemical constituents in small buds makes them more beneficial for human consumption. An appreciable content of antioxidant compounds makes capers from Salina a good source of important nutrients.

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PAPER

SENSORY ANALYSIS OF TRADITIONALLY PRODUCED RED WINE FROM THE TACORONTE-ACENTEJO AREA OF THE CANARY ISLANDS

ANALISI SENSORIALE DI VINI ROSSI DELLA ZONA DI ORIGINE TACORONTE-ACENTEJO (ISOLE CANARIE)

G. GONZÁLEZ HERNÁNDEZ*,
A. HARDISSON DE LA TORRE[1] and J.J. ARIAS LEÓN
Department of Analytical Chemistry, Nutrition and Food Science,
University of La Laguna, 38071 Santa Cruz de Tenerife, Spain
[1] Department of Obstetrics and Gynaecology, Paediatrics, Preventive Medicine and Public Health, Toxicology, and Medical Legislation, University of La Laguna, 38071 Santa Cruz de Tenerife, Spain
* Corresponding author: Tel. +34 922 318047, Fax +34 922 318003,
E-mail gglezh@ull.es

ABSTRACT

Traditionally-produced red wines from two consecutive years from the Tacoronte-Acentejo Designation of Origin area of the Canary Islands were assessed by sensory evaluation. The taste testing included four visually perceived attributes: fluidity, clarity, hue and colour intensity; four attributes perceived by smell: cleanliness, intensity, fineness and harmony; two perceived by taste: cleanliness and intensity; and four taste-smell attributes:

RIASSUNTO

Il presente lavoro studia l'analisi sensoriale di vini rossi della zona di origine Tacoronte-Acentejo ottenuti da due raccolti consecutivi. L'analisi prevede quattro fattori sensoriali visivi: fluidità, turbidità, tonalità e intensità colorante, quattro fattori al profumo: odore pulito, intenso, elegante e armonico, due fattori al gusto: pulito e intenso e quattro fattori gusto-profumo: corpo, armonia, persistenza e sensazione finale. I risultati mostra-

- Key words: Factor analysis, principal component analysis, sensory analysis, wine -

body, harmony, persistence and final sensation. The means of the two harvest groups differed significantly at the 5% level with respect to the vintage year, and the attributes of smell and fluidity. Linear discriminating analysis showed a relatively good separation between the two harvests and the varimax rotation contributed some substantial information to the database. The influence of the production process on the sensory characteristics is also discussed.

no che il confronto tra i valori medi dei due gruppi (annate) rivela una differenza del 5% in relazione all'anno di vendemmia e gli attributi di odore e fluidezza. Questa ultima caratteristica è collegata alla tensione superficiale. L'analisi discriminante lineare attesta che esiste una separazione relativamente buona tra le due annate. Dall'altra parte, la rotazione varimax integra sostanzialmente le informazioni del database. In ogni caso, è importante segnalare che sono stati valutati alcuni effetti del processo di produzione sulle caratteristiche sensoriali dei vini.

INTRODUCTION

Three characteristic features distinguish viticulture in the Canary Islands from that of the rest of the world. First, phylloxera is not present in this area, which means that the plant reproduces directly from the vine shoot; this is physiologically advantageous and has allowed many indigenous varieties to be conserved (LÓPEZ ARIAS *et al.*, 1993). Second, the soil is of volcanic origin and is exceptionally rich in minerals. Third, temperatures are usually very mild (20°-25°C) due to the prevailing Atlantic trade winds and the Canary Islands' oceanic current, which make them milder than would be expected at this latitude (MARZOL, 1990, 2000; RODRÍGUEZ RODRÍGUEZ, 1973).

Traditionally-made wines in the Canary Islands are not filtered or stabilised and account for 86% of the total production (LÓPEZ ARIAS *et al.*, 1993). Since little is known about the sensory characteristics of these wines, sensory analyses were conducted on some of the most renowned and popular wines from the Canary Islands, such as those from the Tacoronte-Acentejo area. This area of the archipelago is one of the oldest and most extensive vine-growing regions, with a total of 2,422 hectares. The total annual wine output in the Canaries is 25.3 million litres, of which a mere 3.5 million litres are produced using state-of-the-art technologies (LÓPEZ ARIAS *et al.*, 1993). The remaining 21.8 million litres are produced and sold in bulk in the myriad of small traditional wineries scattered throughout the vine-growing regions (GONZÁLEZ, 1998). The area studied is located on the northern coast of Tenerife (200-600 m asl).

Sensory quality of wine is related to the overall harmony of many substances, some of which come from the grape, while others are a result of fermentation and ageing processes, i.e. environmental factors, winemaking process, and storage techniques (MARTÍN, 1986). Sensory analysis provides different information than chemical analysis and requires the careful training of judges in order to obtain reliable results (SALVADOR *et al.*, 1995). Professionals define a wine on the basis of its sensory characteristics. In addition to appearance and colour, they distinguish aroma, taste and the so-

called common chemical sensations, i.e., astringency, which is a "dust-in-the mouth" sensation. Each of these properties is due to the interaction of the chemical compounds with taste and smell organs (ETIEVANT and ISSANCHOU, 1987; MENNELLA, 1998). While many techniques are used in sensory analysis (CASP and BERNABEU, 1987; GONZÁLEZ VIÑAS and SALVADOR, 1996), descriptive analysis is the most informative (SALVADOR *et al.*, 1995).

The objective of the present study was to classify a large number of sensory parameters in order to characterise traditionally-produced wines from the Tacoronte-Acentejo area, and to evaluate the influence of the production process on their sensory quality. For this purpose, univariate data analyses and multivariate data analyses (principal component analysis, linear discriminating analysis and factor analyses) were carried out.

MATERIALS AND METHODS

Samples

Thirty-two artisanally produced red wines from the Tacoronte-Acentejo Designation of Origin area of the Canary Islands were analysed. Fifteen were from the 1991 harvest and 17 were from the 1992 vintage. Cellars spread throughout the steep terrain of the area were chosen and the samples were obtained directly from the cellars to guarantee that they were representative. The musts used to make the red wines came from a mixture of red and white *Vitis vinifera* grape varieties, mixed in a ratio of 85 to 15%, respectively. The red grape varieties used were "listán negro" (60%), "negramoll" (20%) and other indigenous varieties (5%), and the white grape variety was "listán blanco" (15%). This is the approximate mixture of the musts of this region (LÓPEZ ARIAS *et al.*, 1993).

After the grapes were pressed, they underwent mashing and fermentation in large, shallow vats. The prolonged aeration of the musts accelerated the start of the fermentation process due to rapid yeast growth. After 48-72 hours, the solid material was pressed and the liquid was transferred to 400-600 L chestnut barrels, where fermentation was completed. The wine was then kept in the same barrels to acquire its typical flavour.

Physical-chemical parameters

Data on the physical-chemical parameters of the wines studied were previously reported by GONZÁLEZ HERNÁNDEZ (1994), GONZÁLEZ HERNÁNDEZ *et al.* (1997) and GONZÁLEZ *et al.* (1999, 2002), and are summarised in Table 1. Total acidity, pH, fixed acidity, alcohol content, sugar, density, colour intensity, hue and sulphur dioxide (SO_2) were determined using the reference methods of the European Community (EEC, 1990). Volatile acidity was determined using the Mathieu method (RIBEREAU-GAYON and PEYNAUD, 1962).

Methods

Taste tests were carried out in March and April of 1992 and 1993, i.e., when the wines were expected to be clarified by natural sedimentation. The sensory analysis procedure suggested by RIVELLA (1987) and recommended by the UIŒ (Union Internationale des Oenologues) was used. Tasting was conducted in accordance with the internationally approved criteria for sensory analysis of wines (UIŒ, 1986).

The team of tasters was made up of seven people who usually serve on tasting committees of the regulatory councils from three of the Designation of Origin areas of the Canary Islands: Icoden Daute Isora, Valle de La Orotava and Tacoronte-Acentejo. The seven judges had previously participated in national

Table 1 - Variables for must and wine samples. Data were taken from GONZÁLEZ HERNÁNDEZ (1994), GONZÁLEZ HERNÁNDEZ *et al.* (1997) and GONZÁLEZ *et al.* (1999, 2002).

Variable [a]	Must 1991 [b]	Must 1992 [c]	All samples	Wine 1991	Wine 1992	All samples
Fixed acidity	-	-	-	4.40 (0.73)	4.55 (0.64)	4.47 (0.69)
Total acidity	3.91 (0.62)	4.04 (0.83)	3.97 (0.73)	5.16 (0.62)	5.43 (0.48)	5.30 (0.57)
Volatile acidity	-	-	-	0.76 (0.26)	0.76 (0.23)	0.76 (0.24)
Reducing sugars	278 (52)	238 (43)	250 (48)	2.57 (0.78)	2.83 (0.86)	2.72 (0.83)
Density	1099 (7)	1083 (14)	1088 (14)	992.6 (1.8)	993.5 (1.4)	993.0 (1.7)
Alcohol strength	-	-	-	14.23 (0.67)	13.39 (0.76)	13.67 (0.81)
pH	3.45 (0.18)	3.45 (0.15)	3.45 (0.16)	3.65 (0.17)	3.57 (0.13)	3.61 (0.16)
Free SO_2	-	-	-	17.4 (11.4)	22.8 (44.3)	20.0 (32.0)
Total SO_2	-	-	-	123 (62.4)	107 (86.6)	115 (75.2)
Colour intensity	-	-	-	4.53	3.47	4.01
Hue	-	-	-	0.884	0.924	0.904

[a] Fixed and total acidity, g/L tartaric acid; Volatile acidity, g/L acetic acid; Reducing sugars, g/L inverted sugar; Density d^{20}_{20}; Alcohol strength, % vol. at 20 °C; Free and total SO_2, mg/L; Colour intensity, $D_{420}+D_{520}+D_{620}$; Hue, D_{420}/D_{520}.
[b] Each value is the average of 15 samples with the standard deviation in parentheses.
[c] Each value is the average of 17 samples with the standard deviation in parentheses.

and international tasting events and competitions, and were familiar with the wines to be evaluated.

Procedure

Each taster was given an evaluation form for each sample. The form included 14 sensory attributes: visual (fluidity, clarity, hue and colour intensity), smell (cleanliness, intensity, fineness and harmony), taste (cleanliness and intensity) and taste-smell (body, harmony, persistence and final sensation). The tasters were asked to score all the attributes on a scale of 0-8, except for the visual attributes of fluidity, hue and colour intensity, which were scored on a scale of 0-4. The sum of the individual scores gave the total score for the wine. Once the upper and lower marks were eliminated (since they may be due to error), the average of the total scores given by each taster was calculated (RIVELLA, 1987).

RIVELLA (1987) classified wines according to the sensory evaluations into the following categories (score): excellent (100), very good (86), good (73.5), average (60), below average (46.5), mediocre (24), and negative (0). The total scores obtained were then grouped as follows: excellent, >93.00; very good, 93.00-79.75; good, 79.74-66.75; average, 66.74-53.25; below average, 53.24-35.25; mediocre, 35.24-12.00; and negative, <12.00. In all cases, the intervals were chosen based on the following equation (by way of example):

The wine is "good" if the score lies within the interval

$$x_2 + (x_1-x_2)/2 \text{ to } x_2 - (x_2-x_3)/2$$

Where x_1, x_2 and x_3 are the sums of all the attributes of the scores "very good", "good" and "average", respectively.

Statistical analysis

The data were analysed by using a combination of several multivariate techniques. The data were first screened and pre-treated, and then pattern-recognition tools were applied. The Statgraphics Plus 4.0 software package (INST, CA) was used for the principal component analysis (PCA), linear discriminating

analysis (LDA) and factor analysis (FA) of the data. PCA was carried out to study and illustrate the data structures. The number of principal components was determined through purely mathematical means. Components accounting for less than 5% of the data variance were not investigated. FA was used to ascertain the basic structure of the data. Only the most relevant components were considered in the original data set, which explains the high percentage of variance in the system studied.

RESULTS AND CONCLUSIONS

The statistical parameters of the sensory analysis are illustrated in Table 2. The coefficient of variation was similar for all the visual attributes and was lower than the other parameters; it was very high (above 50%) for the taste, smell and final sensation attributes. The coefficient of variation was even greater for the sensory attributes of body, harmony and persistence, which are part of the mixed taste-smell sensations (58-60%).

The high coefficient of variation values are the result of the great diversity of the wines. While the study area is relatively small in size, it is characterised by a wide variety of geographical features (LÓPEZ ARIAS *et al.*, 1993). Another important factor that contributes to the high coefficient of variation is that the wines are made using traditional methods. In general, there is no temperature monitoring (due to difficulties posed by the wooden vessels used) or analyses aimed at improving the musts and regulating the addition of sulphur dioxide (GONZÁLEZ, 1998).

The sensory results indicated that the taste-smell sensory attributes (body, harmony and persistence) were the best for differentiating the wines. This is in line with the observations of CASP and BERNABEU (1987), although they included colour. In this study, the visual attributes varied little from one wine to another, but they influenced the final appreciation of the wine. Thus, two wines (6.2%) which should have scored a poor or negative quality rating because of their taste and smell were deemed to be mediocre due to the initial rating of their appearance and colour by the tasters. Similarly, ten wines (31.2%), which deserved a mediocre rating based on their taste and smell were eventually rated below average or average due to their visual characteristics; and three samples (9.4%) earned an average rating due to their visual attributes.

Regarding the overall classification of the wines (Table 2), the mean quality was below average, which could be expected since it refers to traditionally-made wines, i.e. without final filtering and stabilisation. However, some of the wines that received a good rating were produced with purely traditional techniques, in wooden barrels and with no parameter control. Experience and intuition were the vintners' only means for determining grape maturity, harvest time and carrying out of the winemaking process.

Only one of the samples was rated as very good. The wine was produced using medium-level technology, namely temperature regulation, stainless-steel vessels and semi-industrial bottling, along with the control of basic physical and chemical properties. Thirteen samples earned a rating of average, representing 41% of the total number. A further 41% were judged below average, while 4 (12%) of the samples received a mediocre rating. None of the wines sampled were rated excellent or negative.

The 1991 and 1992 groups were both rated below average, although 1992 was closer to average. The lower rating for the 1991 group could have been due to the bad weather conditions (strong easterly winds, accompanied by very high temperatures) during the grape harvest and fermentation period. The problem was even greater when wood was used

Table 2 - Basic statistics of the sensory parameters of the red wines.

	Visual				Smell				Taste		Taste-smell					
	flu[a]	*cla*	*hue*	*col*	*cle*	*int*	*fin*	*har*	*cln*	*its*	*bod*	*ham*	*per*	*sen*	*scor*	*qualif*
								1991/92 harvest								
av.[b]	2.54	5.25	2.69	2.76	2.88	2.95	2.39	2.76	2.80	2.76	2.73	2.78	2.54	2.88	40.6	b.av.
SD	0.69	1.24	0.77	0.88	1.57	1.45	1.30	1.55	1.52	1.41	1.60	1.66	1.48	1.60	15.8	-
min.	1.5	1	1	1	0	0	0	0	0	0	0	0	0	0	14.1	medi
max.	3	7	4	3	7	7	6	7	7	6	7	7	6	7	84.4	v.good
c.v.	27.1	23.7	28.6	32.1	54.4	49.2	54.3	56.2	54.2	50.9	58.5	59.6	58.5	55.7	38.8	-
								1991 harvest								
av.	2.19	4.90	2.60	2.71	2.24	2.40	1.77	2.19	2.50	2.50	2.29	2.55	2.24	2.60	35.7	b.av.
SD	0.67	1.27	0.82	0.98	1.31	1.23	0.62	1.07	1.54	1.54	1.40	1.71	1.44	1.58	13.0	-
								1992 harvest								
av.	2.85	5.56	2.76	2.80	3.44	3.44	2.94	3.27	3.08	2.98	3.12	2.98	2.80	3.12	45.0	b.av.
SD	0.55	1.16	0.73	0.83	1.59	1.49	1.50	1.75	1.50	1.27	1.70	1.64	1.52	1.63	17.0	-
α [c]	0.005	0.133	0.581	0.770	0.027	0.039	0.009	0.048	0.290	0.335	0.144	0.468	0.291	0.368	0.096	

[a] *flu*, fluidity; *cla*, clarity; *col*, liveliness of colour; *cle/cln*, cleanliness; *int/its*, intensity; *fin*, fineness; *har/ham*, harmony; *bod*, body; *per*, persistence; *sen*, final sensation; *scor*, total score; *qualif*, qualification; *medi*, mediocre;*v.good*, very good; *b.av.*, below average; *v.c.*, variation coeff.
[b] 15 samples from the 1991 harvest and 17 from 1992.
[c] level of statistical significance of the difference in the means of the two harvests.

in the winemaking, due to its poor thermal conduction, which leads to excessively high fermentation temperatures. Furthermore, when wooden vessels are also used to store the wine, the porosity of the wood allows slight but constant contact with the air, thereby increasing the oxidation of the wine. The negative situation is often compounded by the method used to close the vessels.

However, given the climatic conditions of the Canary Islands, the use of wood in winemaking does not always produce negative results; the 1992 harvest was of better quality and the only wine that received a good rating was from that year.

Table 2 shows that the aromatic quality (sensory attributes of smell) of the 1991 wines was lower than the 1992 wines. This could have been due to the higher temperatures during the fermentation period and the higher alcohol content of the 1991 wines, 14.23% vol. compared to 13.39% vol. for the 1992 wines (Table 1). A characteristic smell of ethanol masked the other smells; high alcohol can considerably reduce the aromatic intensity of a wine (PEYNAUD, 1989).

These results indicate some negative aspects of the production process. On the one hand, the alcohol content could be reduced by harvesting the grapes earlier (reducing sugars, 250 g/L) (Table 1). The tasting panel was positively influenced by the hue; the average hue value was excessively high (0.904) in the wines studied (Table 1). A high hue value is related to the premature oxidation of the wine, strong aeration of the must and the type of container used for maturing and storing, which allows high oxygen input. The SO_2 content, which was also excessively high, 115 mg/L (Table 1), is another factor that is highly relevant to the hue and smell quality. Nevertheless, these wines are greatly appreciated by domestic consumers (20 million litres) (LÓPEZ ARIAS *et al.*, 1993) as a typical regional product.

The correlation coefficient matrix (Table 3) indicates that there is no close correlation between the visual attributes and the other sensory attributes, with

Table 3 - Matrix of correlation between sensory attributes of the red wines from the 1991 and 1992 harvests.

	Visual				Smell				Taste		Taste-smell			
	flu [a]	cla	hue	col	cle	int	fin	har	cln	its	bod	ham	per	sen
cla	0.628													
hue	0.616	0.742												
col	0.508	0.585	0.798											
cle	0.630	0.598	0.628	0.401										
int	0.409	0.523	0.601	0.392	0.844									
fin	0.606	0.604	0.614	0.426	0.856	0.868								
har	0.531	0.550	0.655	0.486	0.853	0.906	0.918							
cln	0.362	0.482	0.619	0.451	0.791	0.837	0.764	0.859						
its	0.403	0.427	0.541	0.411	0.719	0.740	0.668	0.751	0.902					
bod	0.483	0.450	0.594	0.437	0.801	0.799	0.833	0.909	0.893	0.855				
ham	0.475	0.401	0.466	0.394	0.723	0.652	0.692	0.769	0.789	0.815	0.890			
per	0.366	0.419	0.475	0.385	0.757	0.681	0.688	0.776	0.803	0.773	0.870	0.867		
sen	0.526	0.466	0.598	0.433	0.812	0.724	0.723	0.818	0.870	0.877	0.882	0.906	0.830	

[a] *flu*, fluidity; *cla*, clarity; *col*, liveliness of colour; *cle/cln*, cleanliness; *int/its*, intensity; *fin*, fineness; *har/ham*, harmony; *bod*, body; *per*, persistence; *sen*, final sensation.

the exception of hue. Hue is the sensory quality most closely related to the other attributes and to the overall score. Thus, oxidation of the wine is important, i.e. the intensity of orange tones, which is indicative of the storage condition of the wine. In practice, the degree of ageing of a wine is determined essentially by the condition of the colouring material, which is discernible by tasting (MARECA CORTÉS and DÍEZ DE BETHENCOURT, 1963).

A comparison of the means of the two sample groups revealed a moderate statistical significance with respect to the vintage year and the attributes associated with smell, with significance levels of 0.0272, 0.0392, 0.0087 and 0.0483 for cleanliness, intensity, fineness and harmony, respectively. There was no significant difference for the other sensory attributes, except for fluidity. A much more marked statistical significance between year and fluidity, 0.0046, indicated the quality of smooth flow, the opposite of viscosity; this characteristic is related to surface tension (RIVELLA, 1987).

Based on the correlation coefficients, all the flavour sensory components, excluding those referring to visual appearance, were closely related to each other and some showed a high statistical correlation. These were *smell harmony*, i.e. the correct balance of smell sensations triggered by the wine; cleanliness or *purity of taste*; *body* or consistency of chemosensation experienced in an almost tactile manner in the mouth and which is related to the amount and nature of the extract content; and the *final sensation* left in the mouth after swallowing (PEYNAUD, 1989). Harmony of smell and body had the highest correlations, and these two components were also highly correlated with each other (correlation coefficient = 0.909).

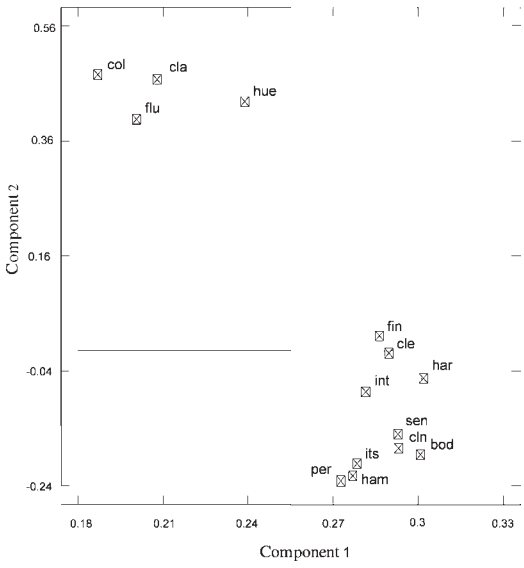


Fig. 1 - Plot of the first two PCs of the sensory attributes of red wine.

Multivariate analysis

Principal component analysis (PCA) was carried out in order to study the data trends and relationships among the variables; correlation matrices were used with autoscaling of the data. The plot of the first two components is given in Fig. 1; the association with visual, smell, taste, and taste-smell characteristics is shown. A similar trend between the taste and taste-smell variables is evident. While this group was positively loaded on the first component, it was negatively loaded on the second.Linear discriminating analysis (LDA) is a method used to visualise data contained in complex databases. Mathematical decision rules obtained from a training set are used to classify unknown samples.

Using an internal cross-validation method, correct predictions of 80.0 and 82.3% were obtained for the 1991 and 1992 harvest, respectively (Table 4). A good separation was found between the two harvests. Generally, classifications based exclusively on sensory data are not very effective (POWERS *et al.*, 1984).

Table 4 - Prediction matrix of the data set by linear discriminating analysis.

Actual group	Group size	Predicted harvest 1991	Predicted harvest 1992
1991 harvest	15	12 (80.0%)	3
1992 harvest	17	3	14 (82.3%)

Using PCA, factors were extracted from the data set. The number of factors was determined on the basis of Kaiser's rule (PEÑA *et al.*, 1996), followed by the application of the eigenvalue rule. The first three factors explained 82.3% of the cumulative variance (Table 5). Factor analysis (FA) was used to search for a latent structure, in order to enhance the information about the sensory data. It was carried out by means of rotation (quartimax, equimax and varimax); Table 5 contains the rotated factors obtained from varimax rotation. Rotation yielded decisive information about the data system, showing how variables contribute to each factor.

The attributes that had the highest loading on the first factor were the taste attributes, cleanliness and intensity, and the taste-smell attributes, body, harmony, persistence and final sensation, while the smell attributes, cleanliness, intensity, fineness, and harmony, had the highest loading on the second factor. Finally, visual attributes had the highest loading on the third factor. These results indicate that all the variables are associated in three groups, i.e. three variables explain the system without important loss of information, persistence for factor 1, smell intensity for factor 2, and liveliness of colour for factor 3.

In conclusion, the ratings of the sensory qualities of the wines studied were low, because of the traditional methods used in winemaking, while the visual attributes scored well. Improved methods for monitoring grape maturity and fermentation could improve the sensory acceptance of these wines. The visual attributes varied little from one wine to another. All the flavour sensory components were closely related with each other, and were very important in the differentiation of the wines. The visual attributes were not closely correlated with the other sensory attributes, with the exception of hue.

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Table 5 - Factor analysis of the sensory attributes: eigenvalues and varimax rotated factor matrix.

Attribute	Rotated factor Eigenvalue (% Variance) 1 8.987 (64.2)	2 1.637 (11.7)	3 0.900 (6.4)
flu[a]	0.114	0.227	0.749
cla	0.126	0.433	0.735
hue	0.291	0.321	0.805
col	0.297	0.034	0.822
cle	0.441	0.676	0.384
int	0.368	0.835	0.205
fin	0.341	0.798	0.367
har	0.532	0.717	0.314
cln	0.759	0.461	0.204
its	0.828	0.280	0.234
bod	0.801	0.465	0.212
ham	0.847	0.262	0.308
per	0.890	0.167	0.194
sen	0.826	0.314	0.150

[a] *flu*, fluidity; *cla*, clarity; *col*, liveliness of colour; *cle/cln*, cleanliness; *int/its*, intensity; *fin*, fineness; *har/ham*, harmony; *bod*, body; *per*, persistence; *sen*, final sensation.

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PAPER

EFFECT OF REFRIGERATION ON THE POTASSIUM BITARTRATE STABILITY AND COMPOSITION OF ITALIAN WINES

EFFETTO DELLA REFRIGERAZIONE SULLA STABILITÀ TARTARICA E LA COMPOSIZIONE DI VINI ITALIANI

A. VERSARI*, D. BARBANTI[1], G.P. PARPINELLO and S. GALASSI
Corso di Laurea in Scienze e Tecnologie Alimentari, Università di Bologna,
Via Ravennate 1020, 47023 Cesena (FC), Italy
[1] Dipartimento di Biotecnologie Agrarie ed Ambientali, Università di Ancona,
Via Brecce Bianche, 60131 Ancona, Italy
*Corresponding author: Tel. +39 0547 636111, Fax +39 0547 382348,
e-mail: versari@foodsci.unibo.it

ABSTRACT

The combined effect of time and refrigeration at three levels (90 days at +4°C, 90 min at 0°C, and 90 min at -4°C) on the stability and composition of white, rosé and red wines was tested in the laboratory. Wines were sampled before and after the industrial cold stabilisation process, then subjected to refrigeration tests in the laboratory and analysed for selected parameters, including pH, total acidity, tartaric acid, potassium and calcium concentration. The time-course of potassium bitartrate precipitation at low temperature was monitored by measuring the conduc-

RIASSUNTO

In questo studio diversi vini, bianchi, rosati e rossi, prelevati in cantina sia prima che dopo il processo industriale di stabilizzazione tartarica a freddo, sono stati sottoposti in laboratorio a refrigerazione (+4°C per tre mesi, e 0° e -4°C per 90 min) per valutare l'effetto del trattamento industriale sulla stabilità e composizione dei vini. Nel corso dell'esperimento i vini sono stati analizzati per i seguenti parametri analitici: pH, acidità totale, acido tartarico, potassio e calcio. La cinetica di precipitazione del bitartrato di potassio durante il raffreddamento è stata misurata se-

- Key words: conductivity, potassium bitartrate, refrigeration, stability, wine composition -

tivity of the wine with time (up to 90 min). An index that can be used to predict the stability of wine with respect to potassium bitartrate precipitation was calculated. White wines were the most unstable followed by rosé and red wines. Analysis of variance disclosed a significant difference ($P \leq 0.05$) in terms of potassium and tartaric acid concentration based on the type of wine and industrial stabilisation process. Factors related to KHT stability in wine are discussed.

guendo la variazione della conducibilità nel tempo (fino a 90 min). In tal modo è stato possibile calcolare un indice di stabilità per ogni vino. I vini bianchi sono risultati i più instabili seguiti da quelli rosati e quindi dai rossi. L'analisi di varianza ha evidenziato una differenza significativa nel contenuto in potassio ed acido tartarico in funzione del tipo di vino e della presenza o meno del processo di stabilizzazione industriale. Il ruolo dei vari fattori coinvolti nel fenomeno della precipitazione tartarica è stato brevemente discusso, con particolare attenzione ai componenti che interferiscono con tale fenomeno.

INTRODUCTION

Grape is rich in tartaric acid and potassium. Therefore grape juice and wine have high concentrations of potassium bitartrate (KHT, potassium hydrogen tartrate, or cream of tartar).

Due to the fermentation process and the consequent production of ethanol, wine becomes a supersaturated solution of potassium bitartrate that spontaneously precipitates (THOUKIS, 1974). Wine quality is not altered by the presence of this crystalline deposit. However, the removal of excess KHT before bottling is always required in order to satisfy consumer preference.

The techniques currently used in wineries to prevent KHT precipitation include: cation exchange (BALDWIN, 1986), electrodialysis (MOUTOUNET *et al.*, 1994) and refrigeration (LESKE *et al.*, 1996), whereby holding the wine at a temperature just above its freezing point, the excess potassium bitartrate is precipitated. This process is called *cold stabilisation*. In this case, the addition of KHT seed crystals to the wine aids KHT precipitation. This technique is known as the *contact process* (MULLER-SPATH, 1979), and the time required for stabilisation depends on several parameters, including wine composition, temperature, rate of cooling, agitation and type of seed crystals (BERG *et al.*, 1968; RODRÍGUEZ-CLEMENTE *et al.*, 1990; GARCIA-RUIZ *et al.*, 1995).

To predict KHT stability in wine, an elaborate calculation has been proposed (USSEGLIO-TOMASSET, 1979). An alternative to this theoretical approach involves the application of refrigeration and mini-contact stability tests (BLOUIN *et al.*, 1979; RHEIN and NERADT, 1979; ANGELE, 1992; DOMEIZEL *et al.*, 1992).

The present work was undertaken to verify the effect of the industrial cold stabilisation process on the KHT stability and composition of three types of Italian wines (white, rosé, and red). Three conditions of refrigeration were tested in the laboratory, and selected wine parameters (pH, titratable acidity, tartaric acid, potassium, and calcium concentration) were monitored before and after each refrigeration test.

MATERIALS AND METHODS

Samples

Commercial table wines, white cv Trebbiano, rosé cv Sangiovese, and red cv Montepulciano were provided by six wineries located in The Marches region (central Italy). The wines were sampled before (BS) and after (AS) similar industrial cold stabilization contact processes using KHT crystals. After sampling, the wines were stored for seven days at 22°C, then used for the refrigeration tests in the laboratory. Wines were also stored for three months at +4°C, as a control.

Refrigeration test

Three conditions currently used in the enological practice were tested: long-time refrigeration at +4°C for 3 months (control), and short-time (90 min) refrigeration at 0° and -4°C (mini-contact stability tests). The time-course of KHT precipitation was followed at 0° and -4°C by monitoring the wine conductivity with time. Wine (100 mL) was poured into an aluminium beaker at low temperature and supersaturated by dissolving 4 g L^{-1} KHT microcrystals (Carlo Erba, Milano, Italy). During cooling, the wine was stirred at 200 rpm using an automatic homogeniser (Crison 2035, Barcellona, Spain). The wine remained in contact with the precipitate and its conductivity was measured at 15 sec intervals until equilibrium was reached. The conductivity meter (Crison microCM 2202, Barcellona, Spain) was equipped with a nickel-platinum probe (cell constant = 0.92) and a temperature compensation probe (PT100). An RS232 serial interface allowed the conductivity data to be transferred to a DEX 386 personal computer.

Analytical determinations

Alcohol, dry extract, ash, alkalinity (AOAC, 1998), total polyphenols (SOMERS and VERETTE, 1988) and turbidity at 570 nm (Hach 2100P turbidimeter, Hach Co, Loveland, CO) were determined before the wines were refrigerated. In addition, pH, titratable acidity, tartaric acid, potassium and calcium concentration were measured before and after each refrigeration test as reported below. The wine was filtered through a filter paper (membrane 589/3, Schleicher and Schell, Dassel, Germany) using a refrigerated filtering device. Calcium and potassium concentrations were determined by atomic absorption spectroscopy using a GBC 905AA instrument (Scientific Equipment Ltd., Victoria, Australia) fitted with an air-acetylene burner and equipped with a hollow cathode lamp (Photron Phy, Victoria, Australia). Measurements were made at 422 nm (5 mA) and 766 nm (10 mA) for calcium and potassium, respectively, with a spectral bandwidth of 0.5 nm (AOAC, 1998). Total acidity (GUCE, 1990) and pH were measured with an automatic microtitrator TT 2050 (Crison, Barcellona, Spain) equipped with a pt4805-s7 electrode. Tartaric acid was determined at 500 nm (VIDAL and BLOUIN, 1978) using a Milton Roy 1201 spectrophotometer (Spectronic Instr., Rochester, NY).

Statistical analysis

The means and standard deviations (mean±SD) were determined. Wines sampled before and after the industrial process of stabilisation were compared by Student's test for paired-samples (*t*-test) using Statistica 5.1 software (StatSoft, Tulsa, OK).

RESULTS AND DISCUSSION

Refrigeration test

After three months of storage at +4°C, several wines had visible deposits, including some wines which had already

Table 1 - Results of the refrigeration tests on wines.

Wine	Sample[a]	Refrigeration Temp. (°C)	Deposit[b]	Conductivity (μS cm⁻¹) at 22° C	start[c]	end	Δ-value	Δ%
White	BS-1	0	yes	1620	675	510	165	24.4
	BS-2	0	yes	2180	933	830	103	11.0
	BS-3	0	yes	1870	929	815	114	12.3
	BS-4	0	yes	1720	697	576	121	17.4
	BS-5	-4	yes		800	687	113	14.1
	BS-6	-4	yes		808	672	136	16.8
	BS-7	-4	yes		781	668	113	14.5
	BS-8	-4	yes		833	705	128	15.4
	AS-1	0	yes	1390	574	417	157	27.3
	AS-2	0	no	1595	771	749	22	2.8
	AS-3	0	yes	1715	885	826	59	6.7
	AS-4	0	no	1415	573	534	39	7.3
	AS-5	-4	no		690	676	14	2.0
	AS-6	-4	no		659	638	21	3.2
	AS-7	-4	yes		731	675	56	7.7
	AS-8	-4	no		694	672	22	3.2
Rosé	BS-1	0	no	1615	661	619	42	6.3
	BS-2	0	yes	1750	704	577	127	18.0
	BS-3	-4	yes		796	720	76	9.5
	BS-4	-4	yes		852	694	158	18.5
	AS-1	0	yes	1570	638	565	73	11.4
	AS-2	0	no	1920	565	554	11	1.9
	AS-3	-4	yes		771	701	70	9.1
	AS-4	-4	no		676	668	8	1.8
Red	BS-1	0	yes	2060	1237	947	290	23.4
	BS-2	0	no	1920	789	767	22	2.8
	BS-3	-4	no		859	804	55	6.4
	BS-4	-4	no		914	900	14	1.5
	AS-1	0	no	1950	940	907	33	3.5
	AS-2	0	no	1910	918	911	7	0.8
	AS-3	-4	no		781	759	22	2.8
	AS-4	-4	no		775	753	22	2.8

[a] Wines sampled before (BS) and after (AS) the industrial cold stabilization process. Laboratory test of refrigeration at 0° and -4° C.
[b] Visible deposit after 3 months at +4 ° C (control).
[c] Mini-contact stability tests: refrigeration at 0 ° and -4° C.

been stabilised in the wineries (Table 1). An attempt was made to relate the stability of the wine to the extent of KHT precipitation obtained with the mini-contact stability test. Fig. 1 shows the changes in conductivity monitored at -4°C for a white wine sampled before and after the industrial stabilisation treatment. Wine conductivity is directly proportional to KHT concentration (Dunsford and Boulton, 1981). Therefore, its measurement provides an effective way to monitor the wine stabilisation treatment.

As expected, the AS-wines were much more stable than the BS-wines. Initially, KHT precipitated rapidly in BS-wines

(without an induction period), and then decreased with time. The analysis endpoint was determined when no substantial decrease in wine conductivity was observed over a 5 min interval (<10 μS cm^{-1}). In the experimental conditions, it took up to 90 min to reach this equilibrium. The absence of an induction step (the time interval, at the beginning of the cooling period, during which there is no decrease in conductivity) was attributed to the nucleation induced by the KHT seeding. The conductivity decay rate of the refrigerated wines decreased with time. This result was mainly due to the precipitation of KHT and the consequent reduction of the supersaturation status of the wine.

The difference between the conductivity of wine at time zero and after 90 min was used as a wine stability index (Δ-value). Table 1 shows the conductivity parameters of the wines. The conductivity Δ-values (<7%) were typical of stabilised wines and the samples lacked visible deposits after three months of storage at +4°C. According to Leske *et al.* (1996) use of the decrease in conductivity of wine at the 5% level as an interpretive criteria may be inappropriate compared to the visual result of the refrigeration test (e.g. 1-3 days at -4°C).

As expected, white wines showed the greatest instability (high Δ-value) followed by the rosé wines. In the red wines no precipitation occurred during the refrigeration test. The only exception was sample BS-1 for which an unusual decrease in conductivity (290 μS cm^{-1}) was recorded. This exception was probably due to improper sampling which resulted in anomalous characteristics being attributed to the wine (i.e. outlier). A large decrease in conductivity occurred in an industrially stabilised white wine (AS-1: 157 μS cm^{-1}; Δ-value 27.3%), implying that the industrial stabilisation process was only partially successful. The stability of the white and rosé wines was greatly affected by the industrial cold stabilisation process, while the red wines were almost stable. This result was probably due to the natural inhibitory effect of several wine components (USSEGLIO-TOMASSET and UBIGLI, 1994), including polyphenols (BOULANGE-PETERMANN *et al.*, 1999), polysaccharides and proteins (VERNHET *et al.*, 1999a, b).

The random adsorption of charged compounds onto the KHT crystal sur-

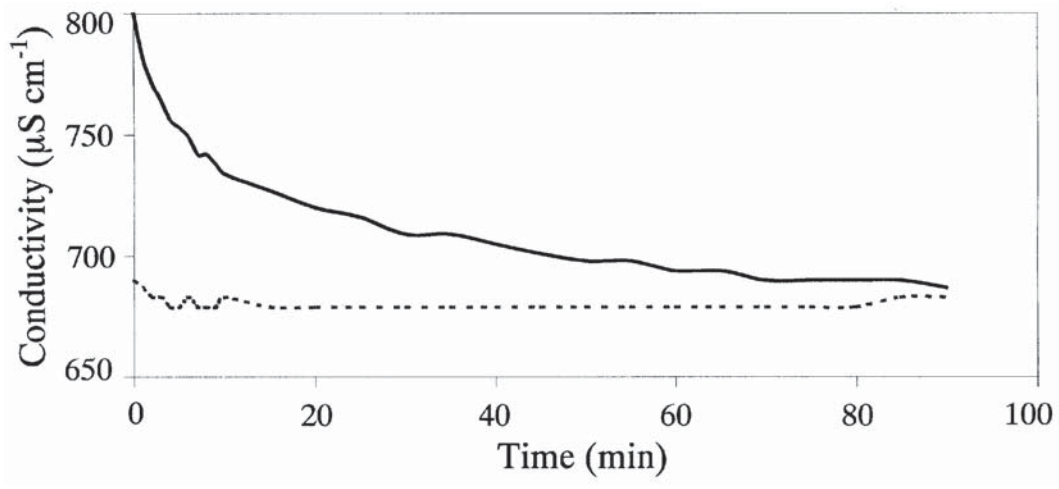


Fig. 1 - Change of conductivity at -4°C for a white wine sampled before (—) and after (- -) the industrial cold stabilisation process.

Table 2 - General composition (mean±SD) of white, rosé and red wines. Due to the lack of differences between composition of samples collected before and after the industrial cold stabilization process, the data were pooled.

	Wine		
	white (n=8)*	rosé (n=4)	red (n=4)
Alcohol (% v/v)	11.4±0.8	11.3±0.2	11.9±0.1
Total polyphenols (g L^{-1} gallic acid)	0.21±0.01	0.24±0.05	1.37±0.15
Turbidity (NTU)	6.9±7.2	4.7±7.1	8.7±6.4
Dry extract (g L^{-1})	19.7±2.6	19.7±0.8	25.3±0.8
Ash (g L^{-1})	1.6±0.3	1.5±0.2	2.0±0.4
Alcalinity (meq L^{-1})	16.1±4.0	16.8±3.4	22.3±1.4
pH	3.25±0.1	3.3±0.10	3.45±0.03
Titratable acidity (g L^{-1})	5.2±0.5	5.1±0.3	5.3±0.1
Calcium (g L^{-1})	0.12±0.03	0.11±0.02	0.11±0.03

*number of samples.

Table 3 - Concentration (g L^{-1}) of tartaric acid and potassium in white and rosé wines before and after the refrigeration tests.

		Tartaric acid (g L^{-1})		Potassium (g L^{-1})	
Wine	sample[a]	initial value	test -4 °C	initial value	test -4 °C
White	BS-1	3.74	2.86	0.63	0.39
	BS-5	3.74	2.85	0.63	0.43
	BS-2	4.48	1.84	0.94	0.65
	BS-6	4.48	1.60	0.94	0.61
	BS-3	1.69	0.80	0.86	0.65
	BS-7	1.69	0.89	0.86	0.69
	BS-4	3.05	2.03	0.79	0.66
	BS-8	3.05	1.90	0.79	0.65
	AS-1	2.64	2.59	0.35	0.32
	AS-5	2.64	2.47	0.35	0.25
	AS-2	2.33	1.58	0.65	0.69
	AS-6	2.33	1.56	0.65	0.68
	AS-3	1.05	0.78	0.72	0.56
	AS-7	1.05	0.66	0.72	0.46
	AS-4	1.84	1.87	0.44	0.42
	AS-8	1.84	1.71	0.44	0.36
Rosé	BS-1	2.16	1.63	0.74	0.59
	BS-3	2.16	1.80	0.74	0.65
	BS-2	2.60	1.48	1.12	0.53
	BS-4	2.60	1.46	1.12	0.52
	AS-1	2.36	1.84	0.74	0.52
	AS-3	2.36	1.68	0.74	0.48
	AS-2	1.50	1.48	0.52	0.51
	AS-4	1.50	1.42	0.52	0.50

[a] Wines sampled before (BS) and after (AS) the industrial cold stabilization process.

faces might modify the K^+/HT^- ratio of the precipitate (RODRÍGUEZ-CLEMENTE and CORREA-GOROSPE, 1988). In our experimental conditions, positively-charged molecules, such as wine proteins, may be adsorbed onto the crystal surfaces. This would bring more HT^- ions onto the precipitate. According to Correa-Gorospe *et al.* (1991a, b), positively charged substances account for 0.011% to 0.064% of the tartrate dry weight and are responsible for anomalies detected in the stoichiometric balance of KHT deposits during wine refrigeration. Moreover, electrostatic attraction between negatively-charged compounds (e.g. polyphenols and/or polysaccharides) and the positively charged KHT crystal faces is expected.

Wine composition

Table 2 shows the general composition of the white, rosé and red wines. There were no differences between samples collected before and after the industrial cold stabilisation process, so the data were pooled.

Among the three refrigeration conditions tested, significant differences ($P \leq 0.05$) were found for the potassium and tartaric acid levels of the white and rosé wines. As expected, wines sampled before and after industrial stabilisation were significantly different from each other, the former having higher potassium and tartaric acid concentration values (Table 3). Moreover, the two mini-contact stability tests at 0° and -4°C showed similar results. The rosé wines, BS-2 and BS-4, had large decreases in potassium (ca. 0.6 g L^{-1}), and high conductivity Δ-values (127 and 158 μS cm^{-1}, respectively). The rosé wines, AS-1 and AS-3, had considerable decreases in tartaric acid (ca. 0.6 g L^{-1}), implying that they were only partially stabilised during the industrial stabilisation process. The instability of this wine was detected earlier by a moderate variation in its conductivity Δ-values (70-73 μS cm^{-1}).

No significant differences were found in the composition of the red wines. The amounts of potassium and tartaric acid that precipitated were consistent with the values found in the literature (MAUJEAN *et al.*, 1985; ESCUDIER *et al.*, 1987). As expected, variations in the calcium levels in the wines were not significant; KHT seeding only induced KHT crystallisation (MINGUEZ and HERNANDEZ, 1998).

CONCLUSION

Due to the complexity of wine it is difficult to predict KHT stability, and therefore a practical evaluation method is desirable. The mini-contact test at constant temperature is a fast, simple method for assessing the stability of wine with respect to KHT precipitation. The results of this preliminary study indicate that a conductivity Δ-value of 7% may be used to verify the stability of wines. However, a long-term survey is needed to compare the reliability of different predictive tests (e.g. refrigeration versus mini-contact) depending on the storage temperature and type of wine. While KHT stability is generally achieved through the industrial cold stabilisation process, some white and rosé wines are only partially stabilised. The consequences of this phenomenon on the sensorial attributes of wines must still be investigated.

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SHORT COMMUNICATION

EFFECTS OF DEHULLING ON SOME PROPERTIES OF COWPEA (*VIGNA UNGUICULATA* WALP. L.) FLOURS

EFFETTO DELLA DECORTICAZIONE SU ALCUNE PROPRIETÀ DELLA FARINA DEL FAGIOLINO DELL'OCCHIO (*VIGNA UNGUICULATA* WALP. L.)

O. AKINJAYEJU* and O. T. ENUDE
Department of Food Technology, Yaba College of Technology, P.M.B. 2011, Yaba-Lagos, Nigeria
*Corresponding author: e-mail:olujayeju@yahoo.com

ABSTRACT

The influence of de-hulling on some properties of cowpea (*Vigna unguiculata* Walp. L.) flours was examined. Brown and white varieties of the legume were manually de-hulled and the flours milled from the de-hulled seeds were compared with those obtained from unde-hulled seeds. The flours were analyzed for proximate composition, physical characteristics and rheological properties. Steamed bean cake ("Moinmoin") samples were prepared from the flours and analyzed for sensory parameters of aroma, texture, taste and colour. De-hulling produced no appreciа-

RIASSUNTO

È stato esaminato l'effetto della decorticazione su alcune proprietà della farina di *Vigna unguiculata* Walp. L. Le varietà brune e bianche del legume sono state decorticate manualmente e le farine ottenute sono state comparate con quelle provenienti da semi non decorticati. Le analisi effettuate sono state la composizione di base e le caratteristiche fisiche e reologiche. Il prodotto alimentare "Moinmoin" è stato preparato da queste farine ed analizzato per le sue caratteristiche organolettiche e reologiche. La decorticazione del seme non produce differenze significative su gran

- Key words: cowpea, de-hulling, flours, physical properties, rheology -

ble influence on the proximate composition and most of the physical characteristics measured. However, de-hulling altered some of the amylograph pasting characteristics of the flours especially maximum viscosity, consistency and setback values. "Moinmoin" prepared from the flour samples had almost equal acceptability mean scores, and they were not significantly different ($p<0.05$) and ($p<0.01$) for all the parameters evaluated for except colour, for which the sample prepared from the unde-hulled brown variety had the lowest acceptability score.

parte dei parametri saggiati. Peraltro la decorticazione modifica alcune caratteristiche amilografiche (viscosità, consistenza e raffermamento). Il "Moinmoin" preparato con le farine decorticate mostra quasi lo stesso punteggio di accettabilità e non presenta differenze significative ($p<0,05$ a $p<0,01$) per tutti i parametri saggiati ad eccezione del colore che risulta migliore di quello del campione ottenuto con farina non decorticata.

INTRODUCTION

Food legumes, belonging to the Leguminosae or bean family, are consumed directly by man as mature dry seeds or as immature, green seeds or as green pods, with immature seeds enclosed (ROBERTS, 1970). Different varieties and types of grain legumes are cultivated in Nigeria, including cowpea, soybean, pigeon pea, African locust bean, bambara nut and winged bean (OGUNTUNDE, 1985). In comparison to cereal grains, these legumes contain more than twice the protein and yield almost as many calories per unit weight. Compared with foods of animal origin, most of these legumes contain similar amounts of protein.

The most common food legume in Nigeria is the cowpea (*Vigna unguiculata* Walp. L.), also called "Black eye" bean. It is used in many local dishes, the most important being steamed bean cake ('Moinmoin') and fried bean cake ("Akara") (ONAYEMI and POTTER, 1976). For most food uses, the seed coats or hulls of the legumes are removed (de-hulled); this reduces the tannin and fiber content, resulting in better appearance, texture, cooking quality, palatability and digestibility of the products. De-hulling can be done manually or mechanically depending on the type of legume and or the quantity involved (EHIWE and REICHERT, 1987).

The objective of this study was to examine the influence of de-hulling on some of the physico-chemical and rheological properties of flours milled from dehulled cowpea compared with flours milled from the whole seeds. The flours from de-hulled and whole beans were used to prepare "Moinmoin", which was sensorially evaluated.

MATERIALS AND METHODS

The materials used were brown and white cowpea (*Vigna unguiculata* Walp. L.), obtained from a local market in Yaba, Lagos, Nigeria. Other ingredients for preparing "Moinmoin" were purchased at the Tejuoso Retail Market in Yaba.

The white (W) and brown (B) varieties were each divided into two parts. One part was manually de-hulled (MD), by soaking the seeds in cold water for five minutes, followed by vigorous hand-rubbing to free the seed coats, which were then removed by flotation. The de-hulled

seeds were then air-dried at 60°C for 36 h. The unde-hulled (UD) samples were cleaned. Each sample was then milled in a Premier Grinding Mill (R. Hunt and Co., England). The flour fraction, which passed through a 425 μ mesh sieve was collected and used for the study.

Moisture content, ash, ether-extractable fat and crude protein (N X 6.25) were determined by using standard A.O.A.C. (1990) methods. The carbohydrate content was determined by difference.

Physical characteristics including bulk density, swelling, solubility, water absorption capacity and reconstitution index were determined by the methods of D'APPOLONIA (1977). Rheological properties were determined using a Brabender Visco-Amylograph (D-4100, OHG Duisburg, Germany). Flour (50 g) was slurried in 450 mL of distilled water; it was then heated uniformly (1.5°C/min), held at this temperature for 15 min and then cooled uniformly to 50°C.

The flour samples were used to prepare the steamed bean cake, "Moinmoin", using the recipe of DOVLO *et al.* (1976). The "Moinmoin" samples were evaluated in the departmental sensory evaluation laboratory for parameters of colour, taste, aroma and texture by 10 untrained panelists made up of staff and students who were familiar with the product. Panelists were asked to taste the samples and to indicate the rating that best described their perception of the samples on a 9-point scale ranging from 1, for liked extremely to 9, for disliked extremely. The responses of the panelists were converted into numerical values and analyzed statistically using analysis of variance (LARMOND, 1982).

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of the flour samples is shown in Table 1. De-hulling had no significant influence on the composition of the flours except for the ash content, which was slightly higher for the unde-hulled samples. This was not surprising since the seed coat is largely responsible for the ash content in cereals and legumes (IHEKORONYE and NGODDY, 1985). These values are in agreement with those previously reported (AKINJAYEJU, 1986 and AKINJAYEJU and GBADAMOSI, 1994). There is no apparent reason for the relatively lower protein content and higher fat of the unde-hulled samples.

Physical characteristics

The physical characteristics of the samples are shown in Table 2. Unde-hulled samples had slightly higher den-

Table 1 - Proximate composition of unde-hulled and manually de-hulled cowpea flours.

Samples	Parameters*				
	Moisture	Protein	Fat	Ash	Carbohydrate
White Variety					
Unde-hulled	10.48±0.27	25.69±1.02	2.79±0.29	4.05±0.88	67.47±1.53
Manually de-hulled	13.92±0.58	24.63±0.96	4.18±0.48	3.02±0.55	68.17±2.01
Brown Variety					
Unde-hulled	9.85±0.16	27.27±0.92	2.00±0.25	3.77±0.75	66.96±1.96
Manually de-hulled	12.5±21.25	24.92±1.75	3.09±0.96	3.66±1.00	68.33±0.85

* Expressed on dry-weight basis except moisture.

Table 2 - Physical characteristics of unde-hulled and manually de-hulled cowpea flours.

Parameters	Samples*			
	UDW	MDW	UDB	MDB
Bulk density (kg/m ^{3})				
Packed flour	750	710	735	710
Loose flour	550	530	525	510
Water absorption capacity (%)	113	143	138	160
Swelling (%)	490	565	524	600
Solubility (%)	3.5	3.5	4.5	4.0
Reconstitution index (cm)	94	93	94	90

* UDW = Unde-hulled white; MDW = Manually de-hulled white; UDB = Unde-hulled brown; MDB = Manually de-hulled brown.

sities than de-hulled samples, due most probably to the weight contribution of the seed coats. However, the de-hulled samples had a higher water absorption capacity and more swelling than the unde-hulled flours. The presence of the seed coat probably restricted water absorption and the consequent swelling of the starch granules. De-hulled had very little effect on solubility and reconstitution of the samples. The drying operation to which the manually-de-hulled samples were subjected to prior to milling could also account for these results. SEFA-DEDEH and STANLEY (1979) observed that the structure and composition of cowpea change considerably during de-hulling and milling, consequently affecting the physico-chemical properties. Their results contrast with our findings in which statistical analysis indicated that there was no significant difference ($p<0.05$ and $p<0.01$) for most parameters measured.

Table 3 - Amylograph pasting characteristics of unde-hulled and manually de-hulled cowpea flours.

Parameters	Samples[a]			
	UDW	MDW	UDB	MDB
Gelatinisation temp. (°C)	80.0	81.5	79.0	79.0
Max. Viscosity (Vm) (BU)[b]	300	75	295	165
Viscosity at 95 °C (BU)	290	65	270	115
Viscosity at 95 °C after 15 min (Vr) (BU)	265	40	255	125
Viscosity at 50 °C (Ve) (BU)	515	135	490	275
Stability (Vm – Vr) (BU)	35	35	45	40
Setback (Ve – Vm) (BU)	215	65	195	110
Consistency (Ve – Vr) (BU)	250	95	235	150

[a] UDW = Unde-hulled white; MDW = Manually de-hulled white; UDB = Unde-hulled brown; MDB = Manually de-hulled brown.
[b] BU = Brabender Units.

Rheological characteristics

Amylograph pasting characteristics of the samples are presented in Table 3. De-hulling produced no appreciable effect on gelatinisation temperature, most probably because the flour samples were reduced to a similar particle size. However, unde-hulled samples had higher viscosity values compared to the de-hulled samples. These results are in agreement with the observations of LINEBACK and KE (1975) and AKINJAYEJU (1986), who noted that bean flours produced amylograph pasting curves characterized by restricted swelling. Their results contrasted with the swelling and water absorption trends in our study for which the values of the unde-hulled samples were lower than those of the de-hulled samples (Table 2). This could be due to differences in swelling and solubility patterns of granules in cold and hot liquids, especially water.

Stability and consistency are important in measuring the mixing tolerance of pastes (PRATT, 1971), during preparation of "Moinmoin". While de-hulling had little or no effect on the stability of the flours, de-hulled samples had lower consistency values compared to unde-hulled samples. The setback value, a measure of the retrogradation tendency of starch products, was lower for de-hulled samples compared to the unde-hulled ones. This value however, could not be related to the compositional or physical characteristics, because these measurements were made when the flours were in a non-gelatinized state.

Sensory evaluation

The mean ratings and calculated variance ratios for sensory parameters of steamed bean cake ('Moinmoin') samples prepared from the flour samples are shown in Table 4. In general, most of the samples received acceptable ratings for all of the parameters. One exception was colour in the unde-hulled brown variety; the mean score range was 6.07-7.73, which indicates low acceptability (9 = disliked extremely). The low acceptability of this sample was due to the brown colour of the cooked product caused by the seed coat.

Analysis of variance indicated significant differences among the samples for colour at both the 5% and 1% levels, and for texture at the 5% level. "Moinmoin" prepared from manually de-hulled brown

Table 4 - Mean scores, standard deviations and calculated variance ratios for sensory parameters of "Moinmoin" from unde-hulled and manually de-hulled cowpea flours.

	Samples*/Variance ratios (F)				
	UDW	MDW	UDB	MDB	F
Aroma	3.80±1.99	4.90±1.87	3.50±2.04	3.90±2.04	1.25
Texture	3.90±2.02	4.50±1.42	3.60±1.86	2.90±1.97	3.21
Taste	3.30±1.27	5.20±1.32	3.50±1.91	4.60±1.80	2.05
Colour	4.20±1.95	2.60±1.20	6.90±0.83	2.90±1.76	8.45

Tabulated Variance Ratios F(0.05) = 2.96 F(0.01) = 4.60

Rating Scale of 1 = like extremely to 9 = disliked extremely

* UDW = Sample prepared from unde-hulled white; MDW = Sample prepared from manually de-hulled white; UDB = Sample prepared from unde-hulled brown; MDB = Sample prepared from manually de-hulled brown.

bean flour was significantly different in colour, while the sample prepared from manually de-hulled white bean flour was significantly different for texture.

CONCLUSION

When the seed coat of cowpea was milled along with the cotyledons, there was little effect on most of the physico-chemical and rheological properties of the flours, or on the "Moinmoin" prepared from the flours. Obviously, the yield of flour obtained from a given quantity of seeds will increase when the seed coats are included in the flour and the flour will contain most of the nutrients present in the seed coat, especially minerals. The seed coat may also provide fibre to the diet, which aids digestion. In addition, the time-consuming seed coat removal operation could be eliminated. The results of this study suggest that unde-hulled white cowpea could be directly milled to make flour to be used for preparing "Moinmoin", while the brown variety should have to be de-hulled prior to milling.

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SHORT COMMUNICATION

OLIVE OIL IDENTITY DETERMINED BY A METHODOLOGICAL AND STATISTICAL PROCEDURE BASED ON EVALUATING THE GLYCERIDIC FRACTION

LA FRAZIONE GLICERIDICA NELL'ANALISI DI IDENTITÀ DI OLI DI OLIVA. PROCEDURA METODOLOGICA E STATISTICA

G. GAMBACORTA*, M. STORELLI[1], V. LIUZZI[2] and E. LA NOTTE
Istituto di Produzioni e Preparazioni Alimentari, Università di Foggia,
Via Napoli 25, 71100 Foggia, Italy
[1]Agenzia delle Dogane, Laboratorio Chimico Regionale,
Corso De Tullio 1/C, 70123 Bari, Italy
[2]Dipartimento di Produzione Animale, Università di Bari,
Via G. Amendola 165/A, 70126 Bari, Italy
*Corresponding author: E-mail: giusgambacorta@unifg.it

ABSTRACT

In this paper a quick method is proposed that enables the identity of imported olive oils and the corresponding exported ones to be determined by applying a methodological and statistical procedure on the glyceride fraction. Fourteen batches were studied, and each batch was made up of the imported oil, used as a reference, and the corresponding exported oils. A statistical "Multitest" program, based on Analysis of Variance, was used on the fatty acid fraction as a screening test, and on the triglyceride fraction as a confirm-

RIASSUNTO

In questo lavoro viene proposto un metodo veloce che consente l'accertamento dell'identità tra campioni di oli di oliva mediante l'applicazione di una procedura metodologica e statistica sulla frazione gliceridica. È stato condotto uno studio su 14 serie di oli, ognuna costituita dal campione di olio importato, usato come riferimento, e dai corrispondenti oli esportati. Per l'accertamento dell'identità degli oli è stato applicato un programma statistico "Multitest", basato sull'Analisi della Varianza, agli acidi grassi, come test di "scre-

- Key words: fatty acids, identity of olive oils, statistical procedure, triglycerides -

atory test, to assess the identity of these oils. Using the Multitest program, 12% of the samples tested were detected as having been partially or totally substituted with other oil. The results obtained confirm the reliability of the method proposed to determine the identity of olive oil samples.

ening" ed ai trigliceridi, come test di conferma. Questa procedura ha consentito di rilevare la violazione del principio di identità per il 12% dei campioni esportati analizzati. I risultati ottenuti confermano l'affidabilità del metodo proposto nel determinare l'identità tra campioni di olio di oliva.

INTRODUCTION

The European Union (E.U.) is the world's leading producer of olive oil. In spite of the great quantity of olive oil produced, many tons are annually imported from Third-World countries such as Tunisia, Turkey and Morocco. In 1998/99 and 1999/00, 225,000 and 146,500 tons, respectively, were imported from these countries (IOOC, 2000). Determining the identity of different olive oil samples is extremely important for commercial exchange, legal purposes and customs, but it is a difficult task due to the lack of specific European regulations. This assessment is necessary within the E.U. in order to obtain "The Temporary Import based on Identity Admission" which is strictly connected to some financial support from the E.U. In order to guarantee that partial or total substitution of the imported oil with other oil has not occurred during storage, customs control authorities, such as the Customs Chemical Laboratories, must be able to assess the chemical identity of the imported oils and that of the exported oils, after operations such as filtration and bottling, in order to verify that they are the same. If this identity is confirmed, the olive oil receives "The Temporary Import based on Identity Admission".

The results of the chemical analyses performed on different olive oil samples are very difficult to evaluate due to the lack of specific national and European regulations. In a previous paper (STORELLI and GAMBACORTA, 1998), a methodological and statistical procedure was proposed to determine the similarity between two different olive oil samples. The Student's *t* test was applied to each identified chemical component of the fatty acid and triglyceride fractions of the exported samples. This approach, however, does not allow determination of "whether all samples belong to one or more populations".

The aim of this study was to develop a quick method based on methodological and statistical procedures to determine the similarity between two or more olive oil samples. This method is based on the analysis of the fatty acid fraction by high resolution gas chromatography (HRGC) as a screening test, and on the analysis of the triglyceride fraction by high performance liquid chromatography (HPLC) as a confirmatory test. In the case of contrasting results between the screening and confirmatory tests, the sample must then be subjected to further analyses such as acidity, sterols, triterpene dialcohols and waxes to determine if it is identical to the corresponding imported oil.

MATERIALS AND METHODS

The chemical and statistical procedures that were used are shown in the

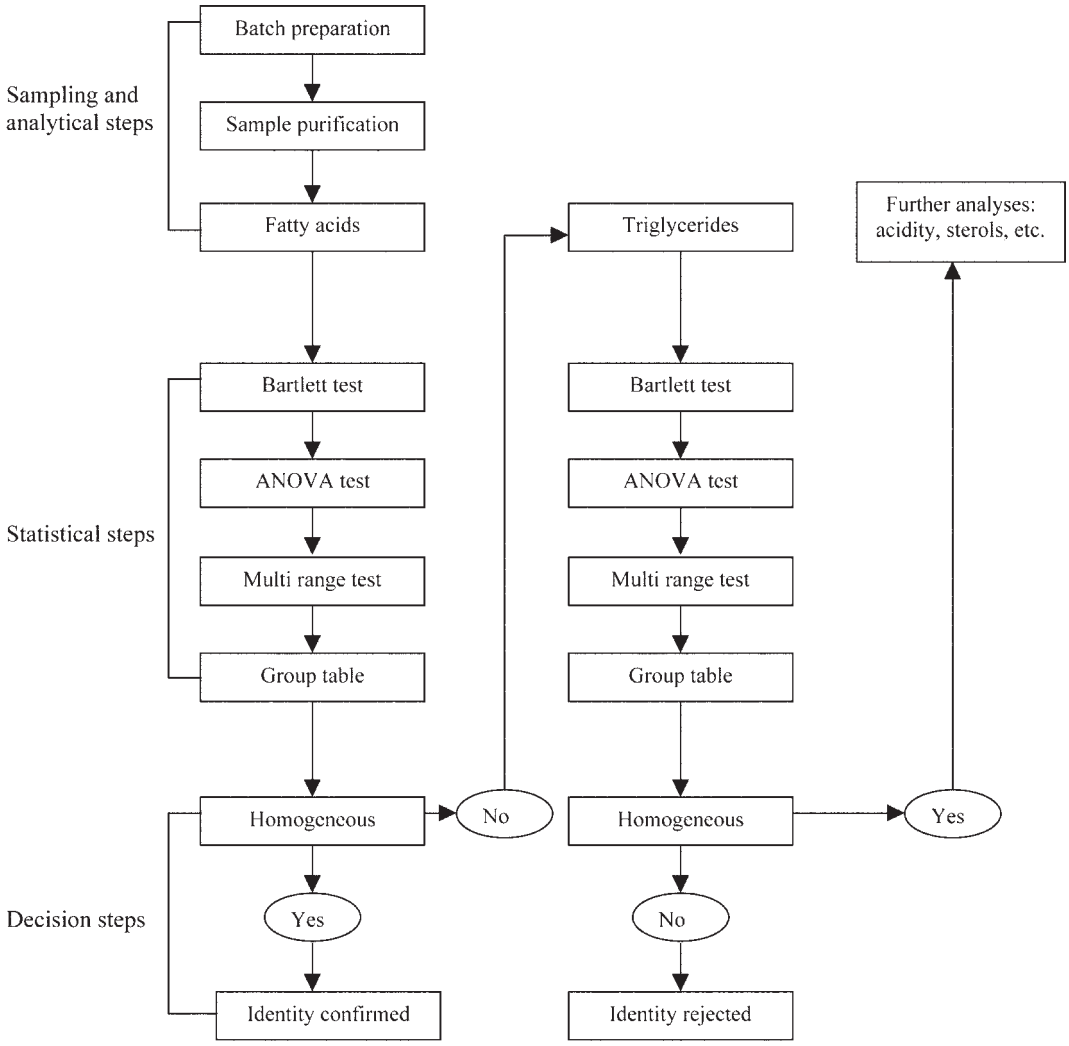


Fig. 1 - Flow chart of the proposed procedure.

flow chart of Fig. 1. Testing was carried out according to Regulation ISO 5555 (ISO, 1991) on 80 samples, which included 14 imported and 66 exported olive and extravirgin olive oils. The chemical analyses were carried out, at the Bari Customs Laboratories (Bari, Italy) in 1998/1999. In the case of the imported oils, samples were taken from vertical cylindrical tanks of 500,000 kg capacity. Small aliquots were withdrawn from the top and the middle of each tank using a cylinder valve sampler, and from the bottom of the tank using a bottom sampler and combined to represent the average properties of the oil. In the case of exported oils, a suitable number of units were randomly chosen within the lot and combined to represent the average properties of the single lot.

Table 1 - Batch batteries of imported oils and the corresponding exported oils tested for their chemical identity. The same letter followed by different numbers indicates small batches of the same oil.

Imported oils	Exported oils
A	A1, A2, A3
B	B1*
C	C1*, C2*, C3*
D	D1*, D2*, D3*
E	E1, E2, E3, E4, E5
F	F1*
G	G1, G2, G3, G4, G5, G6, G7
H	H1, H2, H3, H4, H5
I	I1, I2, I3, I4, I5, I6, I7, I8, I9
J	J1, J2, J3, J4
K	K1, K2, K3, K4, K5, K6, K7
L	L1, L2, L3, L4, L5
M	M1, M2, M3, M4, M5, M6
N	N1, N2, N3, N4, N5, N6, N7

* = Identity rejected.

Fourteen batch batteries of 2 to 10 samples were studied. Each batch was made up of the imported oil, used as a reference, and by 1 to 9 exported olive oils (Table 1). Two of the imported oils were exported in their entirety after some time (B1, F1), while other oils were exported in small batches at different times according to the company exportation plans.

In order to remove any oil oxidation products, each sample was first purified on silica gel according to EC Regulation 2472/97 (EC, 1997).

Fatty acid analysis

The fatty acid composition was determined by HRGC of the methyl esters prepared with BF_3-methanol (MORRISON and SMITH, 1964); an HP 5890 gas chromatograph (Hewlett Packard, Avondale, PA, USA), equipped with an SP-2340 fused silica capillary column, coated with polar stationary phase – poly (biscyanopropylsiloxane) – 60 m x 0.25 mm i.d., 0.25 µm film thickness (Supelco, Inc., Bellefonte, PA, USA) was used. The operating conditions were: Oven 140°C (10 min) to 220° at 1°C/min, hold 5 min; Carrier: helium 20 cm/sec at 140°C; Det.: FID, 240°C; Inj.: 1.0 µL, split (50:1), 220°C.

Triglyceride analysis

The triglyceride fraction was evaluated by HPLC, analysing 20 µL of a 5% oil solution in acetone, previously filtered through a Merck Anotop 10, 0.2 µm disposable filter. The analysis was carried out using an HP system (Hewlett Packard, Avondale, PA, USA) including a Reodyne 7125 injector, a 1090 pump, a 1047A refractometer detector and a 3394A integrator. The HPLC system was equipped with a ChromSpher C18 column (4.6 mm ID x 250 mm, 5 µm) protected by a precolumn Supelguard LC-18 (4.6 mm ID x 20 mm, 5 µm) (Supelco, Inc., Bellefonte, PA, USA). The mobile phase was a mixture of acetone and acetonitrile (63:37, v:v) at a flow-rate of 0.8 mL/min at 20°C. Triglycerides were identified by comparing their retention times with those of pure standards and using the model proposed by TAKAHASHI *et al.*, (1986), MARINI *et al.*, (1989) and GIGLIOTTI *et al.* (1993). Data regarding the accuracy of the fatty acid and trigliceride analyses were reported by STORELLI and GAMBACORTA (1998).

Statistical analysis

Fatty acid and triglyceride analyses were replicated four times. The screening test was performed on the six main fatty acids: palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids. Confirmatory tests were performed by triglyceride analysis of the significant components: trilinoleine (LLL), oleillinoleillinolenine (OLLn), oleildilinoleine (OLL), dioleillinoleine (OOL), palmitiloleillinoleine (POL), trioleine (OOO) and palmitildioleine (POO). The data for

each fatty acid and triglyceride compound, expressed as the % of the total fraction, were analyzed using the Multi Software Program (MEYER and ZUND, 1993).

The sequence of the statistical analysis was the following:

a) the Bartlett test yielded a corrected Chi-square value, which was compared to the critical value;

b) if the standard deviations were not significantly different, an ANOVA test was performed to determine if the data set averages belonged to one or more populations;

c) if at least one standard deviation was significantly different from the others, the program stopped and a new analytical run was required;

d) if more than one population was involved, the Multi-Range Test was used to determine which data set averages could be grouped into homogenous subpopulations. The statistical probability of error *p* was set at 0.05 for each test.

RESULTS AND DISCUSSION

All Multi-Range Test results (for each fatty acid and triglyceride belonging to the same batch) were put together in a "Group" Table. Tables 2 and 3 report the results from two batches in which oil manipulation was proven. In these tables, the averages of the experimental data can be grouped into homogenous subpopulations, Pi. Oil samples having different Pi index values for the same component prove that the analytical data are not derived from the same population; therefore, the olive oil samples are not the same.

As shown in Table 2, the four samples forming the batch of imported oil (D) and the corresponding exported oils (D1, D2, D3) can be grouped into two different subpopulations based on both the fatty acid and triglyceride values. The first subpopulation, corresponds to the reference sample D, and the second one, corresponds to the exported samples D1,

Table 2 - Multi-Range test results related to imported oil D and the corresponding exported oils (D1, D2, D3).

Sample:	D	D1	D2	D3
Fatty acid:				
Palmitic	P1	P2	P2	P2
Palmitoleic	P1	P2	P2	P2
Stearic	P1	P2	P2	P2
Oleic	P1	P2	P2	P2
Linoleic	P1	P2	P2	P2
Linolenic	P1	P2	P2	P1/P2
Triglyceride:				
LLL	P1	P2	P2	P2
OLLn	P1	P2	P2	P2
OLL*	P1	P2	P2	P2
OOL*	P1	P2	P2	P2
POL*	P1	P2	P2	P2
OOO	P1	P2	P2	P2
POO*	P1	P2	P2	P2

* = main peak.

Table 3 - Multi-Range test results related to imported oil C and the corresponding exported oils (C1, C2, C3).

Sample:	C	C1	C2	C3
Fatty acid:				
Palmitic	P1	P2	P3	P4
Palmitoleic	P1	P2	P3	P4
Stearic	P1	P2	P3	P4
Oleic	P1	P2	P3	P4
Linoleic	P1	P2	P3	P4
Linolenic	P1	P2	P2	P2
Triglyceride:				
LLL	P1	P2	P3	P4
OLLn	P1	P2	P3	P4
OLL*	P1	P2	P1	P3
OOL*	P1	P2	P1/P3	P2/P3
POL*	P1	P2	P3	P4
OOO	P1	P2	P3	P4
POO*	P1	P2	P3	P4

* = main peak.

D2 and D3. In this case, substitution of the imported oil was suspected and the Custom Authorities were notified.

With reference to fatty acids, Table 3 shows a more complex situation in which the reference sample, C, and the exported ones, C1, C2 and C3, belong to four different subpopulations. The statistical treatment of the triglyceride data showed that the samples could be grouped in different subpopulations, whereas the OOL triglyceride indicates a partial overlapping between several subpopulations. In this case, the exported oils (C1, C2 and C3) differed among themselves and differed from the imported oil (C). Therefore, the imported oil was substituted partially or totally before each exportation.

Of the 66 exported oils analyzed, the proposed method allowed us to detect and to reject eight oil samples, corresponding to about 12% of those analyzed (Table 1). The confirmatory tests were always in accord with the screening test, so further analyses were not needed.

CONCLUSIONS

The results indicate that fatty acid analysis can be used as a screening test which complies with the typical requirements of good analytical reproducibility and low running expenses. In contrast, the chromatographic separation by HPLC assay of the tryglicerides was not satisfactory because of overlapping of some of the peaks. Therefore, it was performed as a confirmatory test on the screened samples that resulted positive. The proposed methodological and statistical procedure is a quick analytical tool that can be used to determine the identity of olive oil samples.

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SHORT COMMUNICATION

BIOCHEMICAL GENETIC ASSESSMENT OF UNCLASSIFIED MEAT OF COD-LIKE FISH

VALUTAZIONE DI PRODOTTI ITTICI MEDIANTE ANALISI DI GENETICA BIOCHIMICA

C. SUSSI*, F. NONNIS MARZANO[1], F. MARTUZZI, A. SUMMER, G. GANDOLFI[1] and P.G. BRACCHI
Dipartimento di Produzioni Animali, Biotecnologie Veterinarie, Qualità e Sicurezza degli Alimenti, via del Taglio 8, 43100 Parma, Italy
[1] Dipartimento di Biologia Evolutiva e Funzionale, Parco Area Scienze 11 A, 43100 Parma, Italy
* Corresponding author

ABSTRACT

Authenticity testing is often confronted with the problem of high costs and technique reliability for identifying diagnostic markers suitable for species classification. In this paper isozyme/allozyme detection on starch gel electrophoresis was shown to be a fast and useful biochemical technique for assessing species homogeneity in blocks of processed frozen fillets of cod-like fish. Banding patterns of starch gel electrophoresis were informative in 9 out of 22 loci screened, 5 loci were polymorphic in at least one species. Allozyme migration distances were much

RIASSUNTO

Una verifica di autenticità si deve spesso confrontare con il problema dei costi elevati e dell'affidabilità delle tecniche per identificare markers diagnostici idonei alla classificazione di specie. In questo studio l'analisi elettroforetica in gel d'amido di iso-allozimi si è dimostrata una tecnica rapida ed efficiente per la differenziazione e l'identificazione di filetti di diverse specie di pesci, appartenenti ai generi *Merluccius* e *Theragra*, già lavorati e congelati in blocchi. I pattern elettroforetici ottenuti sono risultati informativi in 9 loci dei 22 identificati, 5 dei quali erano poli-

- Key words: allozymes, biochemical genetics, *Merluccius, Theragra* -

more comparable in closely related species of *Merluccius* (Hake) than in the different genus, *Theragra* (Pollack). Genetic relationships were assessed. In addition, preservation quality was investigated, both before and after freezing, by evaluating electromorph resolution and detecting thermolabile enzymes.

morfi in almeno una specie. Le distanze di migrazione enzimatica sono risultate molto più simili nelle specie di *Merluccius*, tassonomicamente vicine, rispetto al diverso genere *Theragra*. Sono state valutate le distanze genetiche ed è stato inoltre controllato lo stato di conservazione delle carni prima e dopo il congelamento in base alla qualità degli zimogrammi ottenuti per proteine termolabili.

INTRODUCTION

Different biochemical and molecular techniques have recently been used to identify the fish species of processed fish meat, particularly when morphological features are no longer distinguishable (BOSSIER, 1999). Among the different protein techniques, Iso-Electric Focusing (IEF) is considered the most reliable method and is the most widely used (REHBEIN *et al.*, 1995). In addition, molecular markers based on PCR-amplified DNA are emerging (BOSSIER, 1999). However, the need for authenticity testing by means of biochemical or molecular classification of species is often confronted with the problem of high costs and the need for specialized personnel.

Iso/allozyme detection on starch gel electrophoresis (SGE) is a reliable, fast and inexpensive technique that can be used to verify taxonomic characterization (UTTER, 1994; CARVALHO and HAUSER, 1998). This paper reports the specific genetic loci encoding for enzyme proteins that can be used to identify different codlike fish and assess species homogeneity and preservation of unclassified fish fillets stored in frozen blocks. Twenty-two genetic loci encoding twelve gene-enzyme systems were investigated in different *Merluccius* and *Theragra* species.

MATERIALS AND METHODS

Twenty-two genetic loci encoding twelve iso/allozymic systems were analyzed in the epiaxial muscle of four different species of Merluccidae: *Merluccius merluccius* (European hake), *M. gayi* (Chilean hake), *M. capensis* (Cape hake), *M. hubbsi* (Argentinian hake) and one species of Gadidae, *Theragra chalcogramma* (Alaskan pollack). All samples were obtained as flesh cores from 50x70x10 cm packages of processed frozen fillets, except for the *M. merluccius* samples that were obtained directly from whole frozen fish. The origin and processing chain of the fish were stated by the firm and proper, constant freezing of the samples was guaranteed. When the frozen fish blocks were received, several (at least 12) still frozen samples (1-5 g each) were taken from each single package, stored at -20°C and analysed within few days. Some samples were stored at -80°C for subsequent analyses.

Homogenates of epiaxial muscle were prepared by mixing 0.5-1 g of frozen fish with equal volumes of cold 0.25 M tris and 0.06 M citrate buffer at pH 8.0, and centrifuging at 12,000 rpm for 7 min; the supernatant was then adsorbed on filter paper for gel application. Horizontal electrophoresis with 11% "Con-

naught" starch gel in continuous tris-citrate buffer was performed (20 g starch in 9 mL buffer diluted to 220 mL with distilled water). Running times were 2-4 h at 40 mA under refrigeration (1-4°C). The staining techniques used were partially modified from the original works of SICILIANO and SHAW (1976) and HARRIS and HOPKINGSON (1976). Locus designation and nomenclature follows the system proposed by SHAKLEE *et al.* (1990). To improve electromorph detection, the stain was applied by using filter paper absorbed on gel slices. A full description of the electrophoretic technique and running conditions is reported in NONNIS *et al.* (2000).

Genetic relationships among the five species were evaluated on both monomorphic and polymorphic loci. In particular, the presence/absence (characters 1-0) of different alleles was assessed and the genetic distance matrix was calculated by means of the "Proportion of differences" program of the Arlequin 2.0 software. Cluster analysis was performed with the "Neighbor" program of the Philip 3.5 package.

RESULTS AND CONCLUSIONS

The diagnostic gene-enzyme systems used to assess the biochemical identifi-

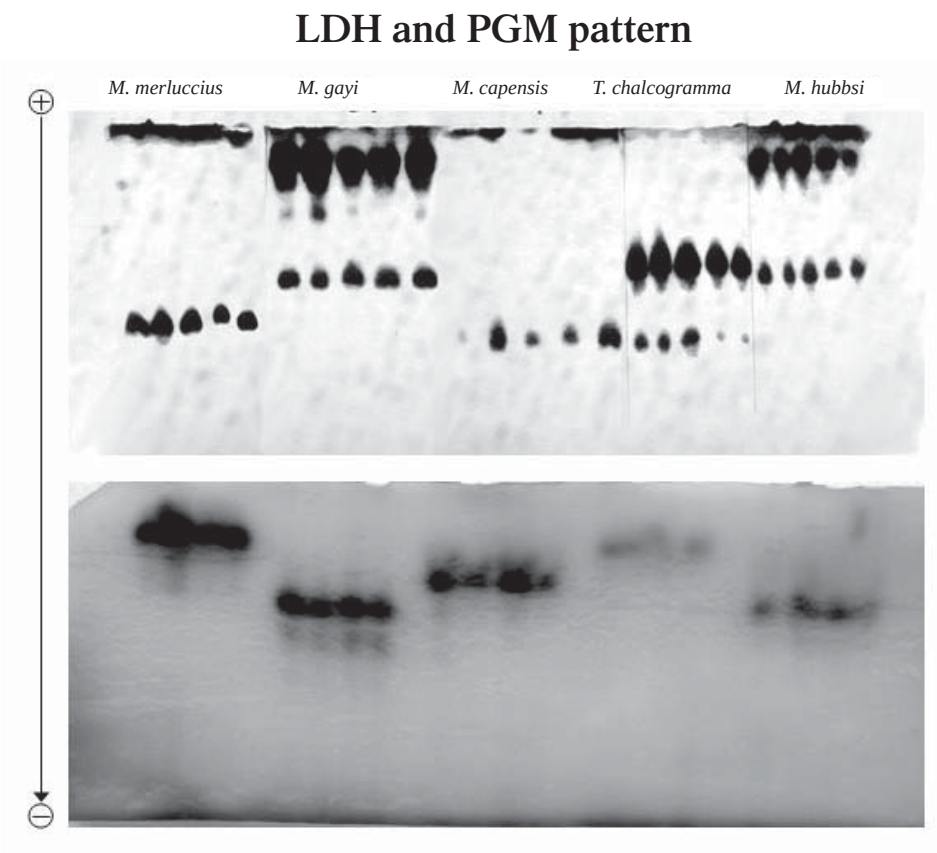


Fig. 1 - Electrophoretic patterns of LDH and PGM in the five fish species.

MATRIX OF GENETIC DISTANCES

	M. merluccius	*M. gayi*	*M. capensis*	*T. chalcogramma*	*M. hubbsi*
M. merluccius	0.0000				
M. gayi	0.3750	0.0000			
M. capensis	0.1250	0.4167	0.0000		
T. chalcogramma	0.6458	0.8542	0.6458	0.0000	
M. hubbsi	0.4167	0.1667	0.4167	0.7292	0.0000

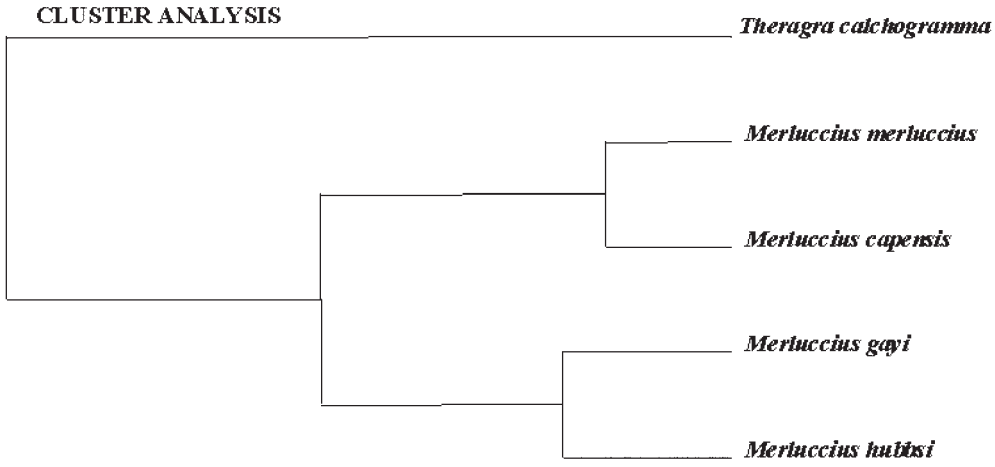


Fig. 2 - Matrix of genetic distances and Cluster analysis of the five fish species.

cation of processed gadoid fish fillets were: alcohol dehydrogenase (ADH, 1.1.1.1), glycerol-3-phosphate dehydrogenase (G3PDH, 1.1.1.8), glucose-6-phosphate isomerase (GPI, 5.3.1.9), lactate dehydrogenase (LDH, 1.1.1.27; Fig. 1), malate dehydrogenase (MDH, 1.1.1.37), mannose-6-phosphate isomerase (MPI, 5.3.1.8) and phosphoglucomutase (PGM, 5.4.2.2; Fig. 1). Banding patterns of SGE were informative in 9 out of the 22 loci screened, 5 loci were polymorphic in at least one species. In particular, the anodal migration distances were much more similar in closely related species of *Merluccius* (Hake) than in the different genus *Theragra* (Pollack). These results are in good agreement with previously published data (ROLDÁN *et al.*, 1999; LO BRUTTO *et al.*, 1998; PIÑEIRO *et al.*, 2000). Starch gel electrophoresis was effective for the most diagnostic loci and is cheaper and easier to use than isoelectric focusing. It can therefore be considered a useful technique for systematic identification. Starch Gel Electrophoresis is less expensive than IEF due to the lower cost of the electrophoretic matrice; starch is less expensive than acrylamide and is less dangerous to the operator. Moreover, ampholites used to create pH gradients in IEF are quite expensive.

Preservation quality during freezing was also determined by evaluating the zymogram quality of thermolabile en-



Fig. 3 - Iso/allozymic genetic loci analysed in the five fish species.

zymes such as G3PDH, GPI and PGM. In particular, good resolution and no protein denaturation were observed.

While reference material for morphological identification was not available, genetic distances calculated by the presence/absence of the different alleles and the related cluster analyses demonstrated the correct biogeographical distribution and similarity of the five species (Fig. 2). Analysis of iso/allozymic genetic loci obtained by SGE is a useful, inexpensive tool for identifying different gadoid fish species. The ADH, G3PDH, GPI, LDH and PGM systems were the most informative for closely related species. No single enzyme was able to characterise the different species, but some enzyme combinations were used to identify the two genera *Theragra* and *Merluccius*, as in the case of loci encoding for MDH, GPI and fumarate hydratase (fumarase, FH, 4.2.1.2). Systematic characterisation of the *Merluccius* species was more complicated and required the simultaneous evaluation of some loci, like LDH or PGM and MPI (Fig. 3).

Diagnostic loci testing can therefore be used to identify mislabelled or fraudulent substitutions of frozen processed fish meat. At the same time, preservation quality can also be verified by evaluating the banding patterns of thermolabile enzymes such as G3PDH, GPI and PGM.

Bioinformatic tools, with special reference to the Arlequin and Philip softwares, can also be used to support the biochemical results and elaborate the genetic distances among species.

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SHORT COMMUNICATION

USE OF POLYMERASE CHAIN REACTION (PCR) AND ELECTROPHORETIC GEL COMPUTER-ASSISTED STATISTICAL ANALYSIS TO SEMI-QUANTITATIVELY DETERMINE PIG/GOOSE DNA RATIO

LA POLYMERASE CHAIN REACTION (PCR), SEGUITA DA ANALISI STATISTICA "COMPUTER ASSISTED" DEL GEL ELETTROFORETICO, MOSTRA UN EFFETTO SEMIQUANTITATIVO PER LA DETERMINAZIONE DEL RAPPORTO TRA IL DNA DI SUINO E QUELLO DI OCA

F. COLOMBO, E. MARCHISIO[1] and C. CANTONI[1]
Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, Università degli Studi di Milano, Via A. Grasselli 7, 20137 Milano, Italy
[1] Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, Università degli Studi di Milano, Via G. Celoria 10, 20133 Milano, Italy

ABSTRACT

A Polymerase Chain Reaction (PCR) method was set up to determine the ratio between pig (*Sus scrofa*) and goose (*Anser anser*) DNA quantities in a mixture. A "universal" primer and two specific primers for goose and pig were used. A highly significant statistical correlation (P ~ 0.01) between goose and pig DNA quantities and the electrophoretic band areas of PCR products was found.

RIASSUNTO

È stato allestito un metodo di Polymerase Chain Reaction (PCR) per misurare il rapporto tra le quantità di DNA delle specie suino (*Sus scrofa*) ed oca (*Anser anser*). Sono stati utilizzati a questo proposito un primer "universale" e due primers specifici per suino ed oca. È stata trovata una correlazione altamente significativa (P ~ 0,01) tra le quantità di DNA dell'oca e del maiale e le aree delle bande del gel elettroforetico corrispondenti ai relativi prodotti PCR.

- Key words: Molecular methods, Mortara goose salami, species identification, statistical interpretation, typical meat products -

INTRODUCTION

"Mortara Goose Salami", a typical product of the Lomellina area (northern Italy), is home-made in accordance with ancient traditions. There are two types of goose salami: the first is a pure goose salami, made of goose meat and fat only and called "ecumenico"; the second is a 2:1 ratio of pig and goose respectively. Pig fat from the "bacon" and/or the "chap" is used to prepare the mixed pig-goose, salami.

Curing is mainly done with salt (280 g/100 kg of mixture), "saltpetre" (potassium nitrate), crushed black pepper and "marsala" wine. After preparing, the salami are boiled at 76°-78°C for 8-9 hours and dried at 18°-20°C for 2-3 days (CATTANEO, 1993; FIOCCA, 1984; FIOCCA and PICCHI, 1989; GIARDINI and SACCHELLI, 1990).

In the last ten years quantitative Polymerase Chain Reaction (PCR) methods have been developed. The best known are "competitive" PCR (RAEYMAEKERS, 1995; SIEBERT and LARRICK, 1992) and "Real Time" PCR (HOLLAND, 1991; HIGUCHI *et al.*, 1992).

In the field of food control, in particular of meat products, MATSUNAGA *et al.* (1999) developed a "multiplex" PCR method to simultaneously identify different meat species. They visually observed variations in the intensity of electrophoretic bands related to the different amounts of each species: intensities of species-specific electrophoretic bands became more intense or fainter as the respective DNA quantities increased or decreased ("semi-quantitative" effect).

The goal of this study was to identify goose and pig species and to determine their quantitative ratio in the typical Italian product "Mortara Goose Salami". To do this a densitometric-statistical method was set up which correlates the varying quantities of the DNA of the two species with the corresponding electrophoretic bands of the PCR products.

MATERIALS AND METHODS

Sampling and DNA extraction

Samples of frozen pig and Italian goose ("paduan" strain) meat were utilised. All template DNA used in the PCR experiments was extracted from these samples.

Genomic DNA was extracted with "Genomic Prep Cells and Tissue DNA Isolation Kit" (Amersham Pharmacia, Uppsala, Sweden) adopting the standard protocol for animal tissues (COLOMBO *et al.*, 2000). Sample amounts were increased for better extraction yields (from 10-20 mg/specimen to nearly 50 mg). DNA concentration and purity were tested measuring spectrophotometric absorbance values at 260 and 280 nm, concentration was calculated using the following formula:

DNA concentration = (absorbance at 260 nm) (DNA dilution factor) (50); where 50 is the standard double-stranded DNA concentration value at an absorbance value equal to 1. The purity factor was obtained by dividing the 260 nm absorbance value by that read at 280 nm.

Mixtures were made with varying percentile ratios of pig and goose DNA, respectively: 88:12, 75:25, 50:50, 25:75, 12:88. The total quantities of DNA used in the experiments differed according to the experimental design reported in Table 1.

Polymerase Chain Reaction

DNA amplification was performed with the thermal cycler "GeneAmp PCR System 2400" (PE biosystems, Foster City, CA), using a "Ready-to-go PCR beads" kit (Amersham Pharmacia, Uppsala, Sweden), according to standard manu-

Table 1 - Parameters used in the trials. Bold indicates statistically significant results.

Trial	Pig DNA percentage (total DNA minus goose DNA) %	Total DNA (ng)	Pig/goose reverse primer ratio	Gel developing time
1	12, 25, 50, 75, 88	250	0.6/0.2	2 min 40 sec
2	50, 75, 88	2.5	0.6/0.4	2 min 40 sec
3	50, 75, 88	2.5	0.6/0.6	2 min 40 sec
4	**12, 25, 50, 75, 88**	**250**	**0.6/0.2**	**5 min 44 sec**
5	**12, 25, 50**	**2.5**	**0.6/0.4**	**5 min 44 sec**
6	12, 25, 50	2.5	0.6/0.6	5 min 44 sec

facturer's protocol and component concentrations, with different amplification conditions (see below).

A "multiplex" PCR method (MATSUNAGA *et al.*, 1999) was used. In this method a "universal" primer is used (SIM, sequence: 5'gacctcccagctccatcaaacatctcatcttgatgaaa 3') with several "reverse" primers, whose targets are different animal species, pig being among them. Pig primer sequence is 5'gctgatagtagatttgtgatgaccgta 3'. The amplification protocol was the following: 30 sec at 94°C, 30 sec at 60°C, 30 sec at 72°C for 35 cycles.

The multiplex PCR was set up utilising a goose reverse primer, which had been tested in a previous work (COLOMBO *et al.*, 2002); its sequence is 5'ctcctgtgtttcaggtttctttgtat 3'.

PCR reactions were performed where the primer ratio was SIM: Reverse pig: Reverse goose = 1: 0.6: (0.2, 0.4, 0.6), where the values inside the brackets are variable values of goose reverse primer.

The electrophoretic gel staining times for all trials were 15 minutes. Entire trials were not replicated but the single phases in previous experiments were repeated at least twice (data not shown).

Electrophoresis

PCR products were revealed with horizontal polyacrylamide electrophoresis using the following system: "Gene Gel Excel 12.5/24 kit" (Amersham Pharmacia, Uppsala, Sweden) on a "Genephor" apparatus (Amersham Pharmacia, Uppsala, Sweden), with the following parameters: V=600, mA=25, W=15, for nearly 80 min, at 15°C. A molecular standard ("100 bp DNA ladder" – Amersham Pharmacia) was also run in the gel and electrophoretic bands were revealed with silver staining ("DNA Plus One Silver Staining Kit" - Amersham Pharmacia, Uppsala, Sweden). The gel standard staining time was reduced from 30 min to 15 min as was the standard gel developing time (6 min to 5 min 44 sec or 2 min 40 sec (Table 1).

Computer-assisted statistical gel analysis

Gel images were obtained by digital scans performed with an Agfa Snapscan 600 scanner (AGFA, Milano, Italy).

Areas of electrophoretic bands were measured by means of the public-domain software "Image Tool" (UTHSCSA, San Antonio, Texas). The area values (arbitrary units) were then imported in the SPSS software (SPSS Science, Chicago). With the "curve estimate" regression procedure the best function describing the mathematical relationship between the DNA percentage of each species and the corresponding electrophoretic band areas was calculated.

RESULTS AND DISCUSSION

Statistically significant (P<0.01) results were only obtained in trials 4 and 5 (Table 1). For goose, this effect was obtained using 250 ng of total DNA template and a 0.6/0.2 ratio of pig and goose reverse primers (Fig. 1, lanes 13-16).

For pig, the (visual) quantitative effect was reached with a total DNA template

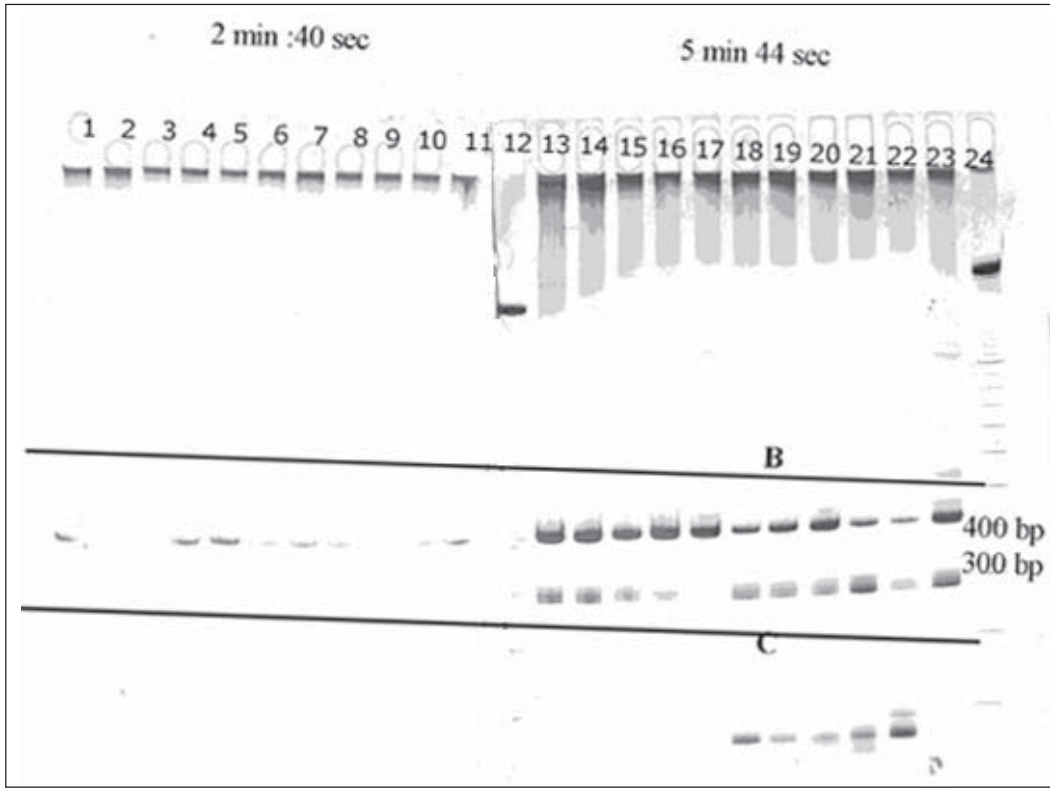


Fig. 1 - Electrophoretic gel: lanes 1-12 were subjected to silver staining with a "developing" step of 2 min 40 sec; lanes 13-24 had a "developing step" of 5 min 44 sec.
Pig bands are around 400 bp and are the first below the B line, goose bands are around 300 bp and are the first above the C line.
The bands below the C line are presumably aspecific products (primer secondary structures).
Lanes 1, 2, 3, 4 and 5: reverse primer ratio (pig/goose) = 0.6/0.2; total DNA quantity = 250 ng; pig/goose DNA ratio (respectively): 12/88, 25/75, 50/50, 75/25, 88/12.
Lanes 6, 7, 8: reverse primer ratio (pig/goose) = 0.6/0.4; total DNA quantity = 2.5 ng; pig/goose DNA ratio (respectively): 50/50, 75/25, 88/12.
Lanes 9, 10 and 11: reverse primer ratio (pig/goose) = 0.6/0.6; total DNA quantity = 2.5 ng; pig/goose DNA ratio (respectively): 50/50, 75/25, 88/12.
Lane 12 and 24: markers.
Lanes 13, 14, 15, 16 and 17: reverse primer ratio (pig/goose) = 0.6/0.2; total DNA quantity = 250 ng; pig/goose DNA ratio (respectively): 12/88, 25/75, 50/50, 75/25, 88/12.
Lanes 18 and 19: reverse primer ratio (pig/goose) = 0.6/0.4; total DNA quantity = 2.5 ng; pig/goose DNA ratio (respectively): 12/88, 25/75.
Lane 20: reverse primer ratio (pig/goose) = 0.6/0.4; total DNA quantity = 2.5 ng; pig/goose DNA ratio = 50/50
Lanes 21 and 22: reverse primer ratio (pig/goose) = 0.6/0.6; total DNA quantity = 2.5 ng; pig/goose DNA ratio (respectively): 12/88, 25/75.
Lane 23: reverse primer ratio (pig/goose) = 0.6/0.6; total DNA quantity = 2.5 ng; pig/goose DNA ratio = 50/50.

of 2.5 ng and a 0.6/0.4 ratio between pig and goose reverse primers (Fig. 1, lanes 18-20).

The computer-assisted statistical analysis revealed a highly significant relationship between DNA quantities and the areas of the related bands.

To calculate the best mathematical function for the relationship between bands areas and DNA quantities, several regression models were tried and the model with the best statistical significance was chosen.

As shown in Figs. 2 and 3 the calculated curve follows the observed data; the exponential curve is likely related to the PCR exponential trend.

The following exponential-type mathematical function describes this relationship: $\%_{species} = b_0 A^{b1}$, where $\%_{species}$ is species-specific percentage, b_0 and b^1 are calculated factors and A is the species-specific electrophoretic band area (Figs. 2 and 3). The resulting calculated quantities were similar to the actual values (Table 2).

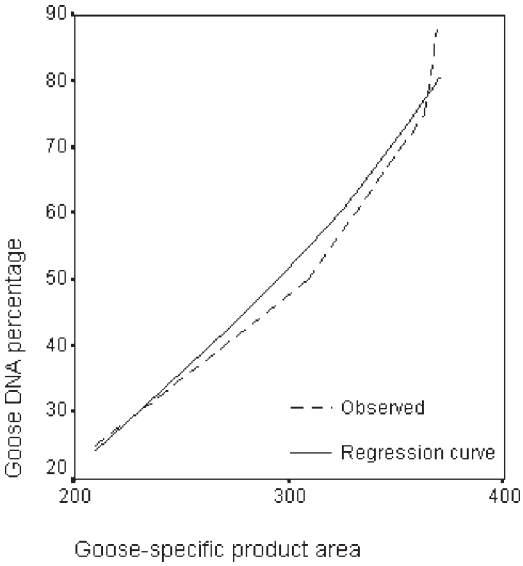


Fig. 2 - Calculated regression curve correlating electrophoretic gel areas of goose PCR product and goose DNA percentage. The values of the parameters of the calculated regression function are: b_0 = 0.0003, b^1 = 2.1193. See text for the regression function formula.

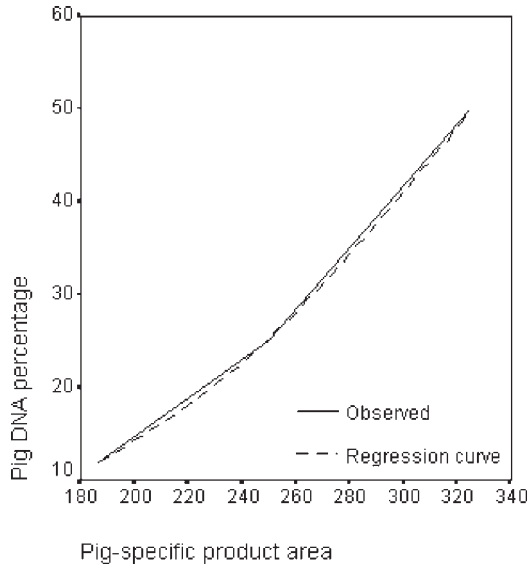


Fig. 3 - Calculated regression curve correlating electrophoretic gel areas of pig PCR product and pig DNA percentage. The values of the parameters of the calculated regression curve are: b_0 = 0,000016, b^1 = 2.5810. See text for the regression function formula.

Table 2 - Electrophoretic band areas. Actual and calculated percentages of pig and goose DNA.

Goose areas (Lanes 13-16)	Pig areas (Lanes 18-20)	Goose % (Actual)	Pig % (Actual)	Goose % (calculated)	Pig % (calculated)
369.00 (13)	187.00 (18)	88.00	12.00	82.68	11.69
363.00 (14)	250.00 (19)	75.00	25.00	79.86	24.73
309.00 (15)	325.00 (20)	50.00	50.00	56.77	48.67
210.00 (16)	Not measured	25.00	75.00	25.04	Not calculated.

The goal of calculating DNA percentages of the species of interest was therefore achieved. Silver staining procedures using a kit are usually reproducible when the phase times are kept constant.

Further experiments are needed to calculate pig/goose ratios in unknown samples. The exponential regression function reported here will be applied to the electrophoretic areas to obtain species' percentages. The results, present an analysis tool applicable to meat mixtures of animal species in general. Further trials will be carried out on specific products, such as Mortara Goose Salami.

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AUTOMATED QUALITY SORTING OF OLIVES AND POTATOES

FFE 414/01/SME12

There is a great need for quality sorting of fruits and vegetables in agriculture and food industry. Traditionally, most quality sorting according to appearance (i.e. size, colour and visual defaults) has been manual or semi-automatic. But these methods prove to be expensive, tedious and slow.

The objective of this cooperative (Craft) project is to build equipment for automatic classification and grading of olives as well as potatoes, with a capacity of 2,500 kg olives per hour and 10,000 kg potatoes per hour. The specific objectives are:

- development of an artificial colour vision system for quality inspection of olives and potatoes. This includes the illumination design, the configuration of the cameras and the development of software;
- implementation of the algorithms over a specific platform for real time processing of the images;
- development of a handling process line for the products at a high speed.

The first report from the project team described the tests for grading olives in four quality classes using a SONY XC003 RGB camera with three CCD colour sensors and a resolution of 768x576 pixel, as well as an algorithm for the classification. The preliminary tests showed promising results and compared machine against manual sorting errors.

Project No: FAIR-CT97-9505 (NIPCO).

Project Co-ordinator: Ricardo Díaz Pujol, Dpto. Instrumentación y Automática, Instituto Tecnológico Agroalimentario (AINIA), Parque Tecnológico de Valencia, Benjamín Franklin, 5-11, 46980-Paterna (Valencia), Spain, Tel. +34 961 366 090, Fax: +34 961 318 008, Videoconference: +34 961 366 009, e-mail: rdiaz@ainia.es URL: http://www.ainia.es

F-FE PROJECT LEADER
Dr. T.R. GORMLEY The National Food Centre Dunsinea, Dublin 15, IRELAND Tel. +353 1 383222 - Fax +353 1 383684

ITALIAN NETWORK LEADER
Dipartimento di Scienze degli Alimenti Università di Udine, Via Marangoni 97, Udine, ITALY Tel. +39 0432 590711 - Fax +39 0432 590719 e-mail: ffe@dsa.uniud.it web site: www.uniud.it/ffe/welcomeflair.htm

FENDING OFF FOOD POISONING FROM SEAFOOD

FFE 415/01/HP10

Shellfish is a good source of high biological value protein and is low in fat. It contains small amounts of long-chain n-3 polyunsaturated fatty acids, and these are known to have significant roles, in reducing risk of coronary thrombosis and atherosclerosis, amongst others. On a weight basis, shellfish contains as much iron as lean meat, although they are rarely eaten in the same quantities.

As a food, shellfish is unique because of the characteristics of the animal (filter feeder) and the way it is eaten (slightly cooked or even raw). The animals are grown in seawater and they can accumulate contaminants, including human enteric viruses, from their watery habitat. The contaminants are able to persist for a long time in the animal and so if not identified, they could enter the food supply and potentially cause disease (including astrovirus, calicivirus, enterovirus, Hepatitis A virus and rotavirus).

At the moment, there is widespread under-identification and ineffective management of shellfish contamination. Epidemiological studies have even linked viral illnesses to the consumption of shellfish that met bacterial safety standards. It has been shown that bacterial indicators are not correlated with viral presence, and so current techniques to identify potentially the harmful viruses need to be improved upon.

The aim of the virus safe seafood project is to provide effective tools for the rapid evaluation of human viral contamination of shellfish, at all stages of the food chain and to develop innovative technology for quality control. These tools will then be used to evaluate the accuracy of EC standards, and to reduce the health risks of seafood consumption. The final objective of the study is to assure the safety of the food supply. This will include the development of guidelines for health risk control, in areas where shellfish are harvested, and during depuration (the process by which shellfish eliminate impurities).

Given the importance of seafood, as a whole in the diet, as a good source of nutrients, this project has the potential to improve the quality and safety of a useful food commodity where increased intake could have potential health benefits.

Project No: QLK1-1999-00634 (V-S SEAFOOD) http://www.ifremer.fr/vsseafood.

Project Co-ordinator: Dr. Monique Pommepuy, Ifremer – DEL, Laboratoire de Microbiologie, B.P. 70, 29280 Plouzané, France, Tel. +33 2 98 22 43 49 - Fax: +33 2 98 22 45 94 or +33 2 98 22 45 48, e-mail: pommepuy@ifremer.fr.

EAT UP YOUR GREENS (AND REDS, YELLOWS AND ORANGES)

FFE 425/01/HP14

The evidence base for eating at least 5 portions of fruits and vegetables every day is increasing. The health benefits of eating these foods, which are rich in antioxidants and a wide range of other micronutrients (vitamins, minerals and

so-called phytochemicals), are now well established both in Europe and worldwide.

Studying the potential health benefits of increased vegetable and fruit consumption within the EU was the topic of a major AAIR project. This project provides new data for the development of hypotheses relating to the health benefits of consumption of specific fruits and vegetables.

One part of the project studied the relationship between increased vegetable and fruit intake (as providers of the carotenoids lycopene, lutein, beta-carotene) and the effects of these compounds on reducing the oxidation of DNA (which contains our genetic information) and one of the fractions of cholesterol (the so-called LDL fraction, or "bad" cholesterol) in human blood. LDL oxidation is linked to arterial disease because oxidised LDL forms part of arterial fatty streaks.

It was shown that higher consumption of carotenoid-rich fruits and vegetables can decrease the susceptibility of LDL to oxidation and that there are significant relationships between high blood carotenoid concentrations and low levels of oxidative DNA damage, and vice versa. But carotenoid supplementation showed no additional protective effect.

The researchers suggest that it is possible that some other element of the diet, itself associated with carotenoids, is the active ingredient. However, the simplest explanation is that the balance and amounts of carotenoids obtained from the food sources do, indeed, have a protective role in the body. This at least supports the antioxidant hypothesis and, in turn, the proposed role of antioxidants in fruits and vegetables in protecting against oxidative damage.

These results refocus attention on the concept of a healthy diet, including increased consumption of carotenoid-rich fruits and vegetables, and encourage nutritional scientists to consider whole food effects in parallel with investigations of isolated food components.

Project No: AIR2-CT93-0888 http://www.ifr.bbsrc.ac.uk/Diet/Micronutrients_EUinit1.html

Project Co-ordinator: Prof. Susan Southon, Institute of Food Research, Norwich Research Park, Colney, Norwich, UK, Tel. +44-1603-255176; Fax: +44-1603-255237; e-mail: sue.southon@bbsrc.ac.uk.

THE TRUTH ABOUT YOGHURT

FFE 429/01/CG15

The term "probiotic" is generally applied to the live microorganisms we ingest with the aim of modifying the characteristics of the gut microflora and exert in this manner a beneficial effect on our health. The intake of foods with live microorganisms, particularly lactic bacteria, is a very old practice: evidence has been found as far back as the Old Testament! However, scientific studies measuring the effects of this intake on health are no more than a few decades old.

These studies enumerate a great deal of potential benefits. However, it is difficult to analyse the actual effects of this complex phenomenom, as ingested bacteria join the microorganisms naturally present in the gut. To throw some light onto this, the European Commission gathered in one project several research teams from different parts of Europe. A big challenge in this project was to define a methodology which could be accepted by all and would allow the analysis of the real influence of probiotic consumption on health.

Researchers studied the action of some bacteria strains on the inmmune system. They underlined that consumption of some strains of lactic bacteria effectively strenghtened the defense mechanisms. This effectiveness seemed to depend not only on the strain, but also on the way the bacteria were ingested: an increased effect was demonstrated when they were consumed in yoghurt rather than in milk. This fact suggests that the compounds resultant from the fermentation of the milk also play a role in this probiotic action.

The most conclusive result shown by the scientists was the benefits of probiotic consumption in babies from 3 to 6 months who suffer from food allergies with eczema outbreaks. Encouraging results have been shown on the effect of probiotics on infant diaorrhea and on gastric ulcer in the elderly, but these results are now to be confirmed with further clinical data. Also, many interesting preliminary results were obtained on inflammatory bowel syndrome and children respiratory diseases. Finally, it was shown as well that probiotics did not cause any harmful immunological side-effects clinically.

In summary, this work allowed to review a very old topic about which many things have been said. It also showed the complexity of the subject and the need for further scientific studies to distinguish between the supposed virtues and the real effects of probiotics and support this with clinical evidence.

Project No: FAIR-CT96-1028 (PROBDEMO) http://www.vtt.fi/bel/new/rovaniemi/backr.htm

Project Co-ordinator: Prof. Tiina Mattila-Sandholm, VTT Biotechnology, P.O. Box 1501, FIN-02044 VTT, Finland, e-mail: tiina.mattila-sandholm@vtt.fi, Tel. +358 9 456 5200, Fax: +358 9 455 2103.

FOLATE IN FOODS

FFE 430/01/SME16

During the last decade, special attention has been given to the influence on human health of vitamin amounts beyond the strictly necessary to avoid the classical deficiency diseases.

There is now good evidence that an increased intake of folate (a B-group vitamin) in early pregnancy significantly reduces the risk of neural tube defects in newborn babies.

Folate (together with vitamins B_6 and B_{12}) also reduces plasma homocysteine concentrations, an emerging risk marker for cardiovascular diseases and stroke,

and possibly age-related cognitive decline. Some evidence also exists of a relation to some cancers, notably colon and colorectal.

Because of these health related effects, there has been much debate on how to raise intakes in the general population - by recommending foods high in folate, by supplements, or by fortification of selected foods.

Many aspects are to be clarified, e.g. the bioavailability of the many forms of natural folate in foods, the possibilities of increasing the folate content in foods, and conclusive intervention studies on the effects on atherosclerosis and other chronic diseases.

The European Commission is currently funding a 3 ½-year research project on these issues. The project started in February 2000 and concentrates on:

- Developing new and refined food processing techniques for foods with enhanced folate content and availability;
- Increasing our knowledge of the bioavailability of different forms of natural folate *versus* synthetic folic acid;
- Developing dietary strategies, especially foods high in folate, for lowering plasma homocyteine levels in the general population;
- Verifying the efficacy of folates in moderating specific risk factors for chronic diseases within human intervention studies.

During the first year of the project many results were attained. For instance, researchers found that one of the main natural forms of folate, (6S)-5-methyltetrahydrofolate, has 26% higher absorption than folic acid. New *in vitro* and *in vivo* models for studying folate absorption are now underway. Scientists will in future use these models to study the influence of the food matrix and processing on bioavailability. For more information, please visit www.ifr.bbsrc.ac.uk/folate

Project No: QLK1-1999-00576 (FolateFuncHealth)

Project Co-ordinator: Paul Finglas, Nutrition & Consumer Science Division, Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, UK, Tel. +44 1603 255318, Fax: +44 1603 507723, e-mail: paul.finglas@bbsrc.ac.uk.

CONJUGATED LINOLEIC ACIDS IN HEALTH AND NUTRITION

FFE 432/01/SME18

Conjugated linoleic acids (CLAs) have been the subject of an increasing number of studies in the past few years. The reason for this is that many of them have a significant role to play in health (see also FFE 321/99).

CLAs are isomers (geometrical or positional) of the fatty acid known as linoleic acid (cis 9, cis 12- C18:2). This acid is converted to CLAs by microorganisms occurring in the gut of ruminant animals. For this reason, CLAs are predominantly found in dairy products and meat from ruminants in levels up to 30 mg/g food.

There are several possibilities for CLA structures, all containing conjugated double

bonds. Often several isomers are found simultaneously in foods, with cis 9, trans 11- C18:2 being one of the most abundant isomers. Thus, this isomer represents 91, 82 and about 60% of the total CLA content of butter, yoghurt and T-bone steaks, respectively. The second most abundant isomers are trans 10, cis 12- and the 7, 9- C18:2.

Because of the indications of effects on carcinogenesis but also on diabetes, platelet aggregation and the immune system, CLA has gained much interest among nutritionists and the food industry. However, most results until now have arisen from animal studies, and good clinical and intervention studies are lacking. Besides, further knowledge is required on the effects of the different isomers, how to influence the concentration in foods, good methods of analysis, nutritional needs, etc.

The objective of this completed Concerted Action was to create a network of scientists with the ability to assess the impact of CLAs on health, in particular human health.

Many results were achieved, e.g.:

a. Inter-laboratory calibration of analytical methods;
b. Feeding strategies for increasing the CLA content of ruminant foods;
c. Progress in safety evaluation and metabolic studies;
d. Reduction of body fat mass (up to an intake of 3,4 g/day);
e. Studies the effect on human cancer cells;
f. The natural isomer is protective against mammary tumour.

The scientists in the Action have now initiated a new shared cost project entitled "Conjugated linoleic acid in functional foods: a potential benefit for overweight middle-aged Europeans" (QLK1-1999-00076). Its development will also be followed up by Flair-Flow during the coming years

Project No: FAIR-CT98-3671

Project Co-ordinator: Prof. J.L. Sebedio, Institut Nationale de la Recherche Agronomique, Unité de Nutrition Lipidique, Rue Sully, 17, FR-21034 Dijon Cedex, France, Tel. +33-38069-3123; Fax: +33-38069-3223, e-mail: sebedio@dijon.inra.fr.

CAPTIVATING CAROTENOIDS

FFE 434/01/HP17

There is substantial and consistent evidence from research that high intakes of carotenoids, as found in foods, decrease the risk of cancers at various sites.

Fruits and vegetables contain various fat-soluble carotenoids such as alpha- and beta- carotene (in Europe, mainly from carrots), lycopene (mainly from tomatoes and tomato products), lutein (mainly from peas and spinach) and beta-cryptoxanthin (mainly from citrus fruit).

However, there is very little information on how much is absorbed from different types of foods, or how food preparation affects absorption of carotenoids.

Using a range of analytical methods it is possible to accurately determine the nutrient content of foods but this does not necessarily tell us about the "nutritional value" of those foods.

This is because only a portion of nutrients present in food can be absorbed into the body, and this portion depends on the type of food, what we eat it with, and how it is prepared before we eat it. In an earlier AAIR project, researchers found that carotenoids seem most beneficial when eaten as carotenoid-rich foods, rather than as a supplement. But how should foods be prepared for maximum "carotenoid" benefit?

This EU FAIR project explored where carotenoids are located in the cells of fruit and vegetable foods, how this affects their release from the food during digestion (so that they can be absorbed) and whether different types of food preparation can increase the amount absorbed into the body.

One of the major findings was that, for good release and absorption of carotenoids, the cells of fruits and vegetables need to be fractured (broken open) before entering the stomach. Cutting, dicing, chopping fruits and vegetables fractures the cells, as does chewing. In addition, cooking and processing of fruits and vegetables does not reduce the carotenoid content and in most cases will result in greater release and absorption of the carotenoids.

This project shows that it is not only whole raw fruits and vegetables that have health benefits: processing can also offer benefits. Some vitamins, such as vitamin C, are preserved by freezing and the freezing will also help to fracture cells to release carotenoids. However, while carotenoids are largely preserved during cooking, vitamin C may be lost. The answer is that a mixture of both raw and processed (shredded, cooked, pasted, juiced, canned, jarred and frozen) fruits and vegetables all form part of a healthy diet.

Project No: FAIR-CT97-3100 (MODEM)

Project Co-ordinator: Prof. Susan Southon, Institute of Food Research, Norwich Research Park, Colney, Norwich, UK, Tel. +44-1603-255176; Fax: +44-1603-255237; e-mail: sue.southon@bbsrc.ac.uk.

BEYOND FRUIT AND VEGETABLES: ANTIOXIDANTS

FFE 436/01/CG16

Many studies have shown the beneficial effects on health of a diet rich in fruit and vegetables, particularly those with a high carotenoid content**.** Fruits and vegetables seem to limit the risk of cancer**,** cardio-vascular diseases and age-related eye disorders. This protective effect is thought to be due to their high concentrations of carotenoids such as lycopene (in tomatoes and water melon), beta-carotene (in carrots, some fruits and green vegetables) and lutein (in yellow/green vegetables). It is thought that these compounds are able to limit the oxidation of DNA and some fractions of blood cholesterol in our body, and form part of the body's antioxidant defence.

The objective of a European Commission-funded research project was to verify on a high number of volunteers the above hypothesis. In this study, no evidence was found of the antioxidant activity of beta-carotene, lutein and lycopene isolates.

However, foods rich in carotenoids were also studied in addition to the carotenoid supplements. These other studies pointed towards a protective effect of a higher consumption of carotenoid-rich fruits and vegetables, by decreasing low-density cholesterol (LDL) sensitivity to oxidation and reducing oxidative damage to DNA. Possibly other elements of fruits and vegetables, alone or associated to carotenoids, take part in these processes.

It seems that it is the natural balance of carotenoids achieved through a diet rich in fruits and vegetables what provides the most effective protection. This refocuses attention on the concept of a healthy diet containing increased amounts of carotenoid-rich fruits and vegetables. It also encourages experts in nutrition to consider, and study, the effects of whole food as well as the isolated food components.

Project No: AIR2-CT93-0888 http://www.ifr.bbsrc.ac.uk/Diet/Micronutrients_EUinit1.html
Project Co-ordinator: Prof. Susan Southon, Institute of Food Research, Norwich Research Park, Colney, Norwich, UK, Tel. +44-1603-255176; Fax: +44-1603-255237; e-mail: sue.southon@bbsrc.ac.uk

VEGETABLE PROTECTION: PHYTOESTROGENS

FFE 438/01/CG18

Phytoestrogens are natural compounds present in plant foods with a chemical structure similar to that of oestrogens, the hormones taking part in many biological processes in humans.

There are several categories of phytoestrogens: isoflavones, mainly present in soy and other legumes; coumestans, present in some fruit; and lignans, widely present in cereals.

Some scientific studies have shown that phytoestrogen consumption may have a beneficial effect on health. Populations that have a large intake, such as the Japanese, have lower incidence of breast cancer, osteoporosis and have less unpleasant menopausal symptoms. Researchers believe that supplementation of women's diet with isoflavones at the initial stages of menopause might help decrease the risk of cardiovascular disease and that of bone fracture, by preventing bone loss. Other studies lead to think that isoflavone intake at a younger age could also reduce the incidence of breast cancer and possibly that of prostate cancer in men.

The objective of this European project is to create a database of phytoestrogen content in foods, to review the scientific evidence on absorption and metabolism, to critically evaluate the experimental models to demonstrate the effects of these compounds in humans and to recommend research protocols to confirm the health benefits of several phytoestrogen categories. The project will

also evaluate different options to increase phytoestrogen intake in Europe, by dietary change, by the development of enriched foods or by the marketing of phytoestrogen formulations, taking into account consumer attitudes and safety considerations.

Project No: FAIR-PL98-4456 (VENUS) http://www.venus-ca.org/

Project Co-ordinator: Dr. Francesco Branca, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, Via Ardeatina 546, 00179 Roma, Italy, Tel. +39-06-50.32.421, Fax: +39-06-50.31.592, e-mail: f.branca@agora.it.

NEWS

First Announcement

DAIRY-NATURE'S CHALLENGE

Old St Jans Hospital Conference Centre
Bruges, Belgium
8-12 September 2003

The IDF World Dairy Summit 2003 will have a broad appeal to dairy, food and related industry professionals in management, processing, policy, farming and academia. It will also appeal to professionals working in marketing, communications and public relations.

The International Dairy Federation is a forum for discussion and exchange and a dairy information centre, created by and for the dairy sector. We amplify communication within the dairy sector through our 41 member countries and serve as a link between the dairy sector and other international organizations. We are a source of scientific and technical expertise and of influence in the dairy sector.

Built on the theme "natural", the IDF World Dairy Summit 2003 will reflect on how to position dairy products in the food market. With this in mind, key areas will be explored: Nutrition, Trade, Technology, and Farming. This IDF World Dairy Summit will open with an international satellite Symposium to honour the Centenary of the IDF.

The IDF World Dairy Summit 2003 will stimulate debate and net-working. International industry leaders and experts will share their expertise.

Belgian Committee of IDF, C/O Technologisch Instituut, Ingenieurshuis - KVIV, Desguinlei 214, B-2018 Antwerpen 1, Belgium, www.fil-idf.org

Preliminary Notice
IDF

2nd WORLD SYMPOSIUM OF DAIRY PRODUCTS IN HUMAN HEALTH AND NUTRITION

24-27 August 2003
Melbourne, Australia

This major symposium, part of the 2003 Foods for Life Conference in Australia, will be devoted to scientific and commercial discussion of nutritional and physiologically functional characteristics of milk and dairy products. The impact of the consumption of dairy products on human health and nutrition at any stage of life around the globe, together with the application of new technologies for preparation of novel dairy products, will be featured.

Invited international speakers will review and update the impact of dairy products on human health and nutrition and the revolution being introduced by modern biotechnology concepts and technologies. The role of dairy products as your partner throughout life will be highlighted.

Topics to be covered include:

- Milk composition and production and the influence of biotechnology;
- Dairy nutrition from birth throughout life;
- Milk as a functional food and source of functional food ingredients;
- Advances in the processing of dairy streams and production of novel dairy ingredients;
- Communication and promotion of dairy products in human health and nutrition.

Please direct expressions of interest to: Pamela Tyers, Food Science Australia, Private Bag 16, Werribee Vic 3030, Australia, E-mail: pamela.tyers@2003foodsforlife.com, Tel. +61 3 9731 3484, Fax +61 3 9731 3366 - www.2003foodsforlife.com

Announcement of competition

"PREMIO DEL MUSEO" EUROPEAN MUSEUM PRIZE XI EDITION 2002

Section for journalism

The Board of Scientific consultants of the National Museum of Pasta, the only one of its kind in the world, will award a Prize for journalism in 2000.

The Prize aims to promote knowledge and appreciation of Italian pasta food, education in learning the nutritional value of this typical national food, which can contribute to alleviate the world-wide problem of hunger. The theme for 2002 is "Pasta-World Money".

For more information: Piazza Scanderbeg 114-120, 00187 Roma, Italy, Tel. +39 06 6991119-6991120 - Fax +39 06 6991109.

EUROPEAN COMMUNITY POST- AND PREDOCTORAL FELLOWSHIPS WITHIN THE FRAMEWORK OF EUROPEAN GRANT "CENTER OF EXCELLENCE"

The 6 and 12 month fellowships are available during 2002-2003 for the citizens of the European Union and the Candidate Countries.

Within the fellowship we offer 1043 EU monthly salary and the two-way ticket.

For the application, the candidate is kindly requested to send curriculum vitae and the recommendation letter.

If you are under 40 years old and interested in:

- food chemistry, particularly in non-enzymatic glycation of proteins, please contact: Prof. Dr. Henryk Kostyra, Department of Food Chemistry, Polish Academy of Sciences, Division of Food Science, ul. Tuwima 10, P.O. Box 55, 10-747 Olsztyn 5, Polonia, Tel. +48 89 5234675, Fax +48 89 5240124, e-mail: kos@pan.olsztyn.pl, http://www.pan.olsztyn.pl/struct/pb/anchem.html.

- analytical chemistry, particularly in the molecular recognition processes at the border of two liquid phases, please contact: Dr. hab Jerzy Radecki, Analytical Chemistry Department, Polish Academy of Sciences, Division of Food Science, ul. Tuwima 10, P.O. Box 55, 10-747 Olsztyn 5, Polonia, Tel. +48 89 5234612/5234613, Fax +48 89 5240124, e-mail: radecki@pan.olsztyn.pl, http://www.pan.olsztyn.pl/struct/pb/anchem.html.

FINAL PROGRAM SET FOR IAFP 2002

June 30-July 3 2002
Manchester Grand Hyatt San Diego, California

The International Association for Food Protection has set the final program for IAFP 2002 - the Association's 89th Annual Meeting. The meeting has earned recognition as the leading food safety conference.

This year's program is well diversified with over 400 presentations including 23 symposia, 6 technical sessions and 224 poster presentations. A sample of symposia topics include: Enhancing Agricultural Security, Antibiotic Resistance in Humans and Feed Animals, Cooperating to Improve Foodborne Outbreak Investigations, Chronic Wasting Disease and Other Transmissible

Spongiform Encephalopathies, Customized Approaches to Microbial Risk Assessment, Applications of DNA Chip Technology in the Food Safety Area, Current Practices in Produce Safety, and many others.

Online registration and program information is available on the Association Web site at www.foodprotection.org.

Contact: Bev Corron, Public Relations, Tel. +1 515.276.3344, Fax: +1 515.276.8655, e-mail: bcorron@foodprotection.org.
Mailing address: 6200 Aurora Avenue, Suite 200W, Des Moines, Iowa 50322-2861, USA.

MEETING OF MEDITERRANEAN GROUP PESTICIDES RESEARCH 2002

Pesticide research in food and environment; cooperation between Mediterranean Countries
24-26 April 2002
Sousse, Tunisa

The Mediterranean Group of Pesticide Research (MGPR) is an association of scientists from Mediterranean countries working on pesticides.

It aims to promote the collaboration between groups, contribute to research in this field and disseminate scientific information. It was formed during the International Symposium "Pesticides in Food in Mediterranean Countries" held in Cagliari, Italy in April 1999.

This year, the main objective of the meeting is to activate the Cooperation between pesticide researchers from European and African Mediterranean Countries, and to promote the elaboration of project proposals into the different National and International Research Cooperation Programmes related with the impact of Pesticides in the Environment and Foods.

Main Topics

- EU-Cooperation Research Programmes;
- Pesticides in Soils, Water, Air;
- Pesticide Regulation and Registration;
- Food Processing Studies;
- Natural Pesticides;
- Integrated Pest Management Systems;
- Pesticide Controls for Import/Export;
- Quality Assurance in Pesticide Analysis.

Call for Poster Presentations

Poster presentations on recent research works of the participants will also be included in the programme as an effective tool to meet partners and to define suitable objectives for the cooperation project proposals. Authors are invited to submit by e-mail and fax to the Meeting Secretariat one abstract page not later than 15th March 2002.

Meeting secretariat: Pr. Ag. Rafik Hadidane, Faculté de Médecine de Monastir - 5019 Monastir - Tunisia, Tel. +216 73 462200 - Fax +216 73 460737, Tel./Fax: +216 73 237719, e-mail: rafik.hadidane@fmdm.rnu.tn, E-mail: r_hadidane@yahoo.fr.

BOOKS

DURUM WHEAT, SEMOLINA AND PASTA QUALITY
Recent Achievements and Trends

By J. Abecassis, J.-C. Autran, P. Feillet, ed.
2001, 202 p., 16x24 cm, réf. C0099
Les Colloques series
ISBN: 2-7380-0997-2
Text entirely in English
Price: 38 € (249,26 FF)
Publisher: INRA Editions - RD 10, 78026 Versailles Cedex, France, Tél. 33.(0)1.30.83.34.06, Fax 33.(0)1.30.83.34.49 - Minitel 3614 INRA - INRA-Editions@versailles.inra.fr., Catalogue & order on line: http://www.inra.fr/Editions/

To improve the composition and texture of durum wheat for use in pasta products (i.e. genetic and agronomic adjustment of the grain properties to meet market requirements) and to optimize milling and pasta-making processes to insure an optimum quality of pasta to fit the consumer demands are essential goals of the durum wheat chain operators (breeders, farmers, millers and pasta processors).

Conventional breeding together with progress in genomics, better control of agronomic practices, understanding grain fragmentation mechanisms (with the dual aim of increasing semolina quality and yield), unravelling interactions between the main components of semolina (proteins, enzymes, starch, lipids, pentosans) after hydration and energy input (shearing and heating) during pasta processing and their relations with pasta appearance, cooking and nutritional quality are all important and vivid fields of investigations.

The aim of this book, based on the six plenary conferences and on posters presented at the International Workshop "Durum Wheat, Semolina and Pasta Quality", (November 27, 2000, Montpellier), is to update knowledge and to identify the future priorities in the following areas of research: Genetic engineering; Breeding; Crop management; Processing; Physicochemical bases of quality; Analytical methods.

It is published for scientists in biochemistry, physico-chemistry, technology, process engineering, agronomy, plant molecular biology, genetics and breeding; for university teachers and students; for managerial staff of technical institutes, control laboratories, or various companies involved in durum wheat breeding, production, milling and pasta-making.

IDF Publications

CHEESES IN ALL THEIR ASPECTS
An eclectic assembly of topics formed the programme of the IDF Analytical Week symposium in San Pellegrino (Italy) in 2001

- Traditional local cheese production (Giangiacomo and Pagani) illustrates the link between the cheese and geography

and climate, race of animal, etc. in local production.

- Cheese ripening - General mechanisms and specific cheese varieties (Ardo). Analysis. of casein components, peptides and amino-acids characterizes the ripening. Unique criteria can be established for specific cheese varieties.

- Casein in relation to cheese (Boutrou and Gagnaire). Different cheesemaking processes, coagulants and starter strains produce different casein matrices. Different cheeses result from different forms of proteolysis and from differences in interaction between the components of the casein matrix.

- Factors associated with hygienic control and quality of cheese prepared from raw milk (Donnelly) summarizes earlier reviews of epidemiological literature on human illness outbreaks involving such cheeses and considers what is known about the technological background to the cheeses in question. Stress adaptation of raw milk pathogens in the cheesemaking environment is considered. Environmental contamination appears to be a greater threat.

- Sampling of cheese (Lamprecht). Cheese is an nonhomogeneous product. Furthermore there is a requirement to provide a sample of cheese "as it is usually consumed" but this requirement cannot be achieved in practice. It is proposed to report on how the sampling has been accomplished in a transparent fashion.

- Tasting the cheese and sensory analysis (Giorno) introduces the concepts of brain function in relation to sensory perception and an approach to the process of purchase choice. Communicating the characteristics determined for a cheese is considered, as well as the transfer from one language (and culture) to another.

32 pp - English only.

DETECTION OF ANTIBIOTIC RESIDUES IN LIQUID WHEY AND DEMINERALIZED WHEY POWDERS

J.-M. Diserens, A. Beck, M.-C. Savoy-Perroud, W. Heeschen & G. Suhren

While tests exist for the detection of antimicrobial agents in milk and milk derivatives, in practice the interpretation of the results frequently presents a difficulty because the history of the process from raw material to product cannot be traced and the performance criteria of the analytical procedure are insufficiently well-known. The study of several methods described here indicates that before methods are applied their performance characteristics must be established.

10 pp - English only.

SAFETY PERFORMANCE CRITERIA FOR A MICROBIOCIDAL STEP (TREATMENT)
IDF Task Force on Alternatives to traditional heat treatments

The Task Force's report establishes safety performance criteria for treatments for producing safe drinking milk and dairy products, in comparison with pasteurization. A 9-step process is described to achieve the establishment of adequate process criteria for a microbiocidal step.

3 pp - English only.

47 pages total.
Bulletin N. 369/2001- 40 Euro (1,614 BEF)

Fédération Internationale de Laiterie - Diamant Building - Boulevard Auguste Reyers 80 - B-1030 Brussels, Belgium - Tel. +32 2 7339888 - Fax +32 2 7330413 - e-mail: info@fil-idf.org - Web site: http://www.fil-idf.org

GUIDE FOR AUTHORS

ITALIAN JOURNAL OF FOOD SCIENCE - IJFS

1. Manuscript Preparation

(1) Manuscripts must be typed, double-spaced and **four** copies submitted along **with** the computer disk. There should be liberal margins on top, bottom and sides (2.5 cm). English is the official language. Authors who are not fluent in written English should seek help from a fluent person before the final version is typed. The Assistant Editor reserves the right to make literary corrections and to make suggestions to improve brevity.

The paper must also be submitted on a Macintosh or Windows floppy disk. Indicate which word processor was used to generate the file and save the file also in format "Text only", DCA-RTF or ASCII, if you do not have programs for Macintosh; graphics, pictures and diagrams must be saved in TIF, JPEG, EPS, CGM or PICT formats (not included in MsWord documents).

(2) Every paper should be divided under the following headings in this order:

Title. Informative of the content of the article (<50 characters + spaces). Author(s). Initials and Surname, omit professional and official titles. The Institute and address where the research was carried out and the current address of each author should be given as a footnote on the title page.

Abstract. Clearly state the objective of the study, give a concise description of experiment(s), observations, results and conclusions. No references should be cited. DO NOT EXCEED 100 WORDS. An abstract and title in Italian must also be included. DO NOT EXCEED 200 WORDS.

Keywords. Up to six words, in alphabetical order, which describe the document must be given to aid data retrieval and indexing.

Introduction. Review pertinent previous work and cite appropriate references. State the purpose of the investigation.

Materials and Methods. Indicate apparatus, instruments, reagents, etc., giving sufficient detail to allow the work to be repeated.

Results and Conclusions. Results and Conclusions may be presented together or separately. Concisely present results using tables and figures to help justify conclusions (do not present the same information in both forms). Use statistical analysis when appropriate. Unsupported hypotheses should be avoided. Conclusions should point out the significance of the findings and, if possible, relate the new findings to some problem in Food Science and Technology.

Acknowledgments. Acknowledgments of assistance are appropriate provided they are not related to analyses, or other services performed for a fee. Financial support, thanks for assistance, article number or thesis fulfillment may be included.

Units. A list of units particular to the paper may be included.

References. References should be arranged alphabetically, and for the same author should be arranged consecutively by year, typed double-spaced. Each individual citation should begin flush left (no indentation). Refer to attached examples taken from "Style Guide for Research Papers" by the Institute of Food Technologists (Chicago - Illinois - USA). Literature citations in the text should be referred to by name and year in parentheses (only the initials in capital letters). If there are more than two authors, mention the first author and add et al.

(3) Lines on all pages, including those pages for "References" and figure legends, must be numbered (by pen) in the left margin, beginning with number one at the top of the page.

(4) Tables should be as few and as simple as possible and include only essential data. Each table must be on a separate sheet and saved on floppy disk, and have an Arabic number, e.g. Table 4 NOT Tab. 4. Legends must be self-explanatory and on a separate sheet. Use lower-case letters for footnotes in tables and explain below the table in the order in which they appear in the table.

(5) Figures must be drawn on separate sheets of paper and saved on floppy disk in TIF, JPEG, EPS, CGM or PICT formats. They should be drawn so that on 50% reduction, lines, figures and symbols will be clearly legible and not overcrowded. A photocopy of how the figure should appear must be included. Photographs must be unmounted, glossy prints or slides. All figures must be given Arabic numbers, e.g. Fig. 3, in the text and in the final copy only on the back where the title of the paper, the senior author's surname and the top of the illustration must also be marked; for reviewing procedures, do not include this information in the first submitted copies. Legends for figures must be self-explanatory and should be typed on a separate sheet under "Legends to Figures".

(6) Standard Usage, Abbreviations and Units. The Concise Oxford and Webster's English Dictionaries are the references for spelling and hyphenation. Statistics and measurements should always be given in figures, e.g. 10 min, except when the number begins a sentence. When the number does not refer to a unit of measurement it is spelled out unless it is 100 or greater. Abbreviations should be used sparingly, only when long or unwieldy names occur frequently, and never in the title; they should be given at the first mention of the name. International Standard abbreviations should generally be used except where they conflict with current practice or are confusing. For example, 3 mm rather than 3x10-3m. Abbreviations should be defined the first time that they are used in the text and they should be used consistently thereafter. Temperatures should be expressed in the Celsius (centigrade) scale. Chemical formulae and solutions must specify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by the Latin binomial (italics) at the first mention. For subsequent use, the generic name should be contracted to a single letter if it is unambiguous.

2. Review Policy

Scientific contributions in one of the following forms may be submitted:

Opinions and Reviews - Papers may be sent directly to the Editor-in-Chief who will decide upon publication or articles will be requested directly from the authors by the Editor-in-Chief.

Short Communications and Surveys - They do not need to have the formal organization of a research paper; they will receive priority in publication;

Papers - The paper must follow manuscript preparation.

Short Communications, Surveys and Papers will be subjected to critical review by the referees. Upon receiving papers from authors, the Advisory Board with the Editor-in-Chief will select papers in relationship to innovation and originality and send copies to the referees. A letter stating that the paper has been accepted for refereeing will be sent to the authors. Papers needing revision will be returned to the author, and the author must return the revised manuscript to the Editor-in-Chief within 1 month, otherwise the paper will be considered as withdrawn. Papers not suitable for publication will be returned to the author with a statement of reasons for rejection.

3. Editorial Policy

Referees may not be from the same institution as the author. Referees should make their comments and questions in detail and return the paper to the Editor-in-Chief as soon as possible, usually within 4 weeks. The identity and the report of the referees are made known to the Editor-in-Chief, but only the anonymous report is routinely sent to the author. If all referees recommend acceptance or rejection, the decision stands. If the opinions of the referees tie, the Editor-in-Chief has the freedom to decide upon acceptance or rejection of the paper. Manuscripts will be edited in the order received and accepted papers will be published as closely as possible in this order. A letter announcing the issue of publication will be sent to the author after the manuscript has been accepted by the Editor-in-Chief. Each paper is accepted with the understanding that it is the sole document under active consideration for publication covering the work reported (it has NOT been previously published, accepted or submitted for publication elsewhere). Upon acceptance of the paper for publication, the author agrees to pay the page charges ad published on the first page of each issue. Authors take full responsibility for all opinions stated in their papers and published in this journal.

4. Mailing Instructions

Papers for publication and communications regarding editorial matters should be sent to:
Prof. Paolo Fantozzi or Dr. Mary F. Traynor, F.S.E.
Istituto di Industrie Agrarie, Università di Perugia, S. Costanzo, I
06126 Perugia, Italy
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REFERENCE EXAMPLES

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ASSOCIATIONS

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Tecnoalimenti - Milano	Fax +39-02-67077405

RESEARCH INSTITUTES

Istituto di Tecnologie Alimentari, Facoltà di Agraria
Dipartimento di Agrobiologia e Agrochimica (D.A.B.A.C.),
Università della Tuscia, Viterbo — Fax +39-0761-357498

Dipartimento di Ingegneria e Tecnologie Agro-Forestali
Università di Palermo, Palermo — Fax +39-091-484035

Dipartimento di Scienze Ambientali Agrarie
e di Biotecnologie Agro-Alimentari (Di.S.A.A.B.A.),
Università di Sassari, Sassari — Fax +39-079-229276

Dipartimento di Scienze degli Alimenti, Università di Udine, Udine — Fax +39-0432-501637

Dipartimento di Scienze e Tecnologie Agroalimentari e
Microbiologiche (DI.S.T.A.A.M.), Università del Molise, Campobasso — Fax +39-0874-404652

Dipartimento di Scienze e Tecnologie Alimentari
e Microbiologiche (DI.S.T.A.M.), Università di Milano, Milano — Fax +39-02-70638625

Dipartimento di Valorizzazione e Protezione delle Risorse
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