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PAPER

DETECTION OF GENETICALLY MODIFIED BT-MAIZE IN COOKED FOOD PRODUCTS BY PCR

IDENTIFICAZIONE DI MAIS BT GENETICAMENTE MODIFICATO IN ALIMENTI COTTI MEDIANTE PCR

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ABSTRACT

A comparison was made of eight known and published PCR primer sets to detect zein, cryIA(b), bla and bar genes and the 35S cauliflower mosaic virus promoter of transgenic Bt "Maximizer" maize "event 176" (Novartis) in cooked food products. Transgenic pop corn, polenta and cookies were used as models. The polenta and cookies were made with different percentages (100, 1, 0.1% w/w) of transgenic maize flour. The DNA extracted from samples taken at different times of cooking revealed a progressive decrease in the average

RIASSUNTO

Sono stati comparati per il rilevamento di mais transgenico Bt "Maximer" "evento 176" (Novartis) otto differenti metodi PCR, precedentemente pubblicati, aventi come bersaglio i geni zeina, cryIA(b), bla, bar ed il promotore 35S del virus del mosaico del cavolfiore. Come modelli di alimenti processati sono stati preparati polenta e biscotti a diverse percentuali (100, 1, 0,1% w/w) di farina di mais transgenico e pop corn transgenici. Il DNA estratto da campioni prelevati a diversi tempi di cottura, pur mostrando una progressiva frammen-

- Key words: GMO detection, heat processed food products, "Maximizer" maize "event 176", Polymerase chain reaction (PCR) -

DNA fragment length, which was, however, still amplifiable by PCR. Of the primer sets tested, Cry 03/04 showed the best performance and led to the detection of transgenic DNA in final cooked products containing amounts of Bt-maize DNA as low as 0.1%.

tazione all'aumentare del tempo di cottura, si dimostrava idoneo per l'analisi PCR. Il più efficiente tra i primer set confrontati si è dimostrato il primer set Cry 03/04 in grado di rilevare DNA transgenico nei prodotti alimentari contenenti 0,1% di mais Bt.

INTRODUCTION

The cultivation of genetically modified plants (GMP) is in continuous expansion, and thirteen genetically modified crops and their products have already entered the food market in Europe (EU, 2001). According to European Community regulations (EC, 1997, 1998, 2000a, b), products derived from genetically modified organisms (GMO) that are "no longer substantially equivalent" to the conventional counterpart require labelling if adventitious genetically modified material is higher than 1% w/w of the food ingredients considered individually.

As many GMP will reach the market in a processed stage, reliable detection methods applicable to both raw materials and processed products are needed. The polymerase chain reaction (PCR) is a highly specific and sensitive method for the detection of small amounts of target DNA and provides a reliable tool for the identification of GMO derived products. For this purpose, several PCR systems differing in target, length of the amplicon and mode of reaction (single or nested) have been described (MEYER, 1999).

A prerequisite for using PCR for processed food analysis is the quality (degradation, inhibitors) of the DNA present in the final product (MEYER and JACCAUD, 1997; HUPFER et al., 1998; STRAUB et al., 1999a; b). A few studies using PCR (HUPFER et al., 1998; 2000; VAïTILINGOM et al., 1999; TENGEL et al., 2000) have been published on the detection of DNA from "Maximizer" maize "event 176", Novartis (Bt-maize), in cooked food products. An evaluation of the feasibility of using already existing PCR methods and their limits of detection may help in choosing the most suitable primer sets for developing efficient quantitative PCR systems (HUPFER et al., 2000). To our knowledge a direct comparison of the various published primer sets and PCR protocols with regard to the detection of Bt-maize has never been published. Such a comparison would give information on the suitability of different primer sets for quantitative PCR (HUPFER et al., 2000). This kind of comparison was done to detect the 35S cauliflower mosaic virus (CaMV) promoter in different GMP and GMP-derived products resulting in the definition of the most suitable PCR primers (WOLF et al., 2000).

The aim of our study was to evaluate the applicability of several different existing PCR-based methods (EHLERS et al., 1997; HUPFER et al., 1997; 1998; PIETSCH et al., 1997; STUDER et al., 1997; MEYER, 1999) for the detection of transgenic DNA in heat-processed maize products.

MATERIALS AND METHODS

Certified reference materials

Certified reference materials (CRMs), produced by the Institute for Reference Materials and Measurements (IRRM, Geel, Belgium) were purchased from Fluka (Sigma, Milan, Italy). The powders contained different percentages of Bt-

maize (2.0, 0.5, 0.1, 0.0% w/w).

Preparation of polenta, cookies and pop corn

Food products were prepared following procedures which resemble as closely as possible those used during household preparation. Polenta was prepared by adding 60 g of maize flour (containing 0.1, 1 and 100% of Bt-maize) to 250 mL of boiling water to which salt (NaCl) had been added (0.4% w/v). The mixture was boiled continuously with stirring for 45 min. For the cookies, 160 g maize flour (containing 0.1, 1 and 100% of Bt-maize) was mixed with 150 g wheat flour, 50 g sucrose, 150 g butter and 1 egg. After mixing, the cookies were oven baked at 160°C for 30 min. Samples of the polenta and cookies were taken before and at different times of processing. The pop corn was "popped" in a microwave oven, exposing the maize kernels (event 176, hybrid "Brama", Novartis) to 600 W power for 4 min, followed by 1 min of resting. Individual samples of polenta, cookies and pop corn were homogenized at maximum power in a blender and stored at –20°C.

Isolation and characterization of genomic DNA

Three hundred milligram samples were taken of the polenta, cookies and pop corn, and a 100 mg sample of CRM; to each sample, 860 μL of 1 × TNE buffer (10 mM Tris-HCl pH 8, 150 mM NaCl, 2 mM EDTA, 1% SDS), 100 μL (5 M) guanidine hydrocloride (Sigma) and 40 μL (20 mg/μL) proteinase K (Amersham Pharmacia Biotech, Milan, Italy) were added; the samples were then incubated in a water bath at 60°C for 3 h. After centrifugation (13,000 × g for 10 min) 5 μL RNase (10 mg/mL) was added to the supernatant (500 μL) and incubated for 30 min at 37°C. The released DNA was purified according to the Wizard Protocol (Promega, Milan, Italy). The DNA was eluted in 50 μL Tris buffer (10 mM, pH 9.0). The quality and efficiency of the DNA extraction was evaluated by agarose gel electrophoresis (1.5% agarose) using lambda DNA digested with HindIII/EcoRI (Boehringer Mannheim, Milan, Italy) as the molecular weight marker. The concentration of DNA in solution was determined by UV absorption at 260 nm; its purity was checked on the basis of 260/280 nm absorption ratio.

Tenfold serial dilutions of Bt-maize DNA were prepared by diluting the DNA extracted from 100% Bt-maize in sterile distilled water.

DNA target sequences and primer oligonucleotides

The primers specific for a maize storage protein zein gene and primers for the specific detection of Bt-maize are listed in Table 1. All the primers were synthesized by Amersham Pharmacia Biotech (Milan, Italy).

Polymerase chain reaction

DNA amplifications were carried out in a final volume of 50 μL in 0.2 mL tubes containing 1× PCR buffer (Amersham Pharmacia Biotech), 200 mM of each dNTP (Amersham Pharmacia Biotech), 0.5 mM of each primer (Amersham Pharmacia Biotech), 1 unit of Taq DNA polymerase (Amersham Pharmacia Biotech) and variable amounts (0.01-100 ng) of DNA. Extra $MgCl_2$ (4 mM final concentration) was added for the CryIA 1/2-3/4 nested system and for the Zein 1/2-3/4 nested system. PCR with primer sets 35S 1/2, Zein 1/2-3/4, CryIA 1/2-3/4 were supplemented with 2 μg/mL BSA (PIETSCH et al., 1997; STUDER et al., 1997), while PCR with primer sets Amp, Bar, CryIA(b), Cry 01/02, Cry 03/04 were supplemented with 5% DMSO (EHLERS et al., 1997; HUPFER et al., 1997; 1998).

All amplifications were carried out in

Table 1 - Primer sets used.

Primer set	Target	Amplicon length (bp)	Reference
Zein 1/2-3/4	zein (methionine-rich storage protein) gene	485-277	STUDER *et al.* 1997
35S 1/2	CaMV 35S-promoter	195	PIETSCH *et al.* 1997
Amp	*bla* (ampicillin resistance)	828	EHLERS *et al.* 1997
Bar	*bar* (phosphinothricin resistance)	264	EHLERS *et al.* 1997
CryIA(b)	Synthetic, truncated c*ryIA(b)*	184	EHLERS *et al.* 1997
CryIA 1/2-3/4	Synthetic, truncated c*ryIA(b)*	420-189	STUDER *et al.* 1997
Cry 01/02	Synthetic, truncated c*ryIA(b)*	1914	HUPFER *et al.* 1997
Cry 03/04	CDPK promoter/*cryIA(b)*	211	HUPFER *et al.* 1998

a GeneAmp 2400 Thermal cycler (Perkin Elmer Applied Biosystems, Milan, Italy). Temperature profiles varied, depending on the primer set. After an initial denaturation at 95°C for 5 min, 40 cycles of amplification were performed with denaturation at 95°C for 30 s, primer annealing for 30 s and primer extension at 72°C for 30 s. A final extension step was performed at 72°C for 3 min. The annealing temperatures were: 57°C (primer set Cry 01/02); 59°C (primer set Amp); 63°C (primer set Cry 03/04); 64°C (primer sets Bar and CryIA(b)). For the nested PCR reactions with primer sets Zein 1/2, Zein 3/4, and CryIA 1/2, CryIA 3/4, 25 cycles of amplification were performed for the first PCR (Zein 1/2 and CryIA 1/2), followed by 25 cycles with the nested primer sets (Zein 3/4 and CryIA 3/4). Two microliters from the first reaction were used as the template for the second PCR. The thermal conditions for the reactions were: initial denaturation at 95°C for 5 min followed by denaturation at 95°C for 40 s at each cycle. Primer annealing was at 60°C for 40 s and primer extension at 72°C for 3 min. For the primer set 35S 1/2, a 40 cycle program according to LIPP et al. (1999) was used.

PCR products were subjected to agarose gel electrophoresis and stained with ethidium bromide (1 μg/mL). The gel images were recorded under UV light by the Gel Doc 2000 image system (Biorad, Milan, Italy). As size reference a 50 bp or 100 bp ladder (Amersham Pharmacia Biotech) was used.

RESULTS AND DISCUSSION

To investigate the limitations of the existing PCR methods to detect transgenic Bt-maize DNA in cooked food products, 100% Bt pop corn, polenta and cookies (the latter two prepared with flour containing different percentages of transgenic Bt-maize) were used. The sensitivity of different existing PCR systems was determined, and the primers showing the best sensitivity were used to detect DNA in maize-derived products both during the cooking process and in the finished product.

The sensitivity of the eight different primer sets was evaluated with tenfold serial dilutions of Bt-maize DNA extracted from 100% Bt-maize flour (Table 2). The sensitivity of the eight primer sets was also evaluated in CRMs containing different percentages of transgenic flour (2.0, 0.5, 0.1%, w/w) in conventional maize flour (Table 3). Table 3 shows that for all the Bt-maize specific primers (except Cry 01/02 amplifying the longest sequence) the detection limit was at least 2 ng; primer set Cry 03/04 and nested primer set CryIA 1/2-3/4 showed the best sensitivity, permitting the detection of at least 0.1 ng of Bt-maize per

Table 2 - Detection limits of Bt-maize flour DNA with different sets of primers evaluated with tenfold dilution series of transgenic DNA.

Primer set PCR	ng/reaction of Bt-maize DNA used in			
	10	1	0.1	0.01
Zein 1/2-3/4	+	+	+	-
35S 1/2	+	+	±	-
Amp	+	-	-	-
Bar	+	+	-	-
CryIA(b)	+	+	-	-
CryIA 1/2-3/4	+	+	+	-
Cry 01/02	+	-	-	-
Cry 03/04	+	+	+	-

PCR result: + positive, - negative, ± unreliable result.

single PCR reaction when supplemented as DNA extracted from 100% Bt-maize (Table 2), or from a mixture of Bt and non Bt-maize (CRMs standards; Table 3). Hence, it was possible to detect Bt-maize DNA in mixtures containing as little as 0.1% Bt-maize flour. As previously reported (JANKIEWICZ et al., 1999), these results confirm that the presence of conventional maize scarcely influenced the sensitivity. The data obtained for Zein 1/2-3/4, CryIA 1/2-3/4 are in accordance with those reported by STUDER et al. (1997); the sensitivity determined for Cry 03/04 with dilution series of pure Bt-maize DNA was lower than reported previously (HUPFER et al., 1998; JANKIEWICZ et al., 1999), probably because a hot-start PCR protocol was not used.

Table 3 - Detection limits of Bt-maize flour DNA with different sets of primers evaluated with mixtures of DNA from transgenic and conventional maize (CRMs 2, 0.5, 0.1% w/w).

Primer set	%w/w of Bt-maize over non Bt-maize (ng/reaction of Bt-maize DNA used in PCR)		
	2 (2)	0.5 (0.5)	0.1 (0.1)
35S 1/2	+	+	±
Amp	+	-	-
Bar	+	±	-
CryIA(b)	+	+	-
CryIA 1/2-3/4	+	+	+
Cry 01/02	-	-	-
Cry 03/04	+	+	+

PCR result: + positive, - negative, ± unreliable result.

Primer set 35S 1/2 showed unreliable results when used with 0.1 ng/reaction (Tables 2, 3) of Bt-maize DNA: in nine separate reactions this primer set gave three positive and six negative amplifications.

Gel electrophoresis of total DNA extracted from pop corn and from samples of polenta and cookies taken at various cooking times revealed a decrease in the average DNA fragment length with respect to the cooking time (Fig. 1). HUPFER et al. (1998) and STRAUB et al. (1999a) also reported that the thermal treatment caused increasing fragmentation of the DNA with cooking time. The average size of the DNA isolated from the finished polenta and cookies was mainly in the 100-1,500 bp range (Fig. 1A, B), while the DNA extracted from pop corn had fragment sizes below 800 bp (Fig. 1C). Control experiments for the presence of amplifiable DNA were performed with the primer set Zein 1/2-3/4, yielding a 277 bp PCR product. The control PCR carried out with the three different Bt-maize products showed the good PCR-quality of the isolated DNA; the absence of DNA polymerase inhibitors and the sufficient amount of the DNA indicated that the DNA was suitable for further PCR analysis.

Primer pairs CryIA 1/2-3/4, Cry 03/04, 35S 1/2 and Bar were used for the specific identification of Bt-maize DNA in heat processed products at different times of cooking, as well as in the final products. These primer sets were chosen as they gave the best sensitivities (Tables 2, 3). In samples prepared from 100% transgenic flour, bands of the expected sizes were observed for all the tested primers for all the cooking

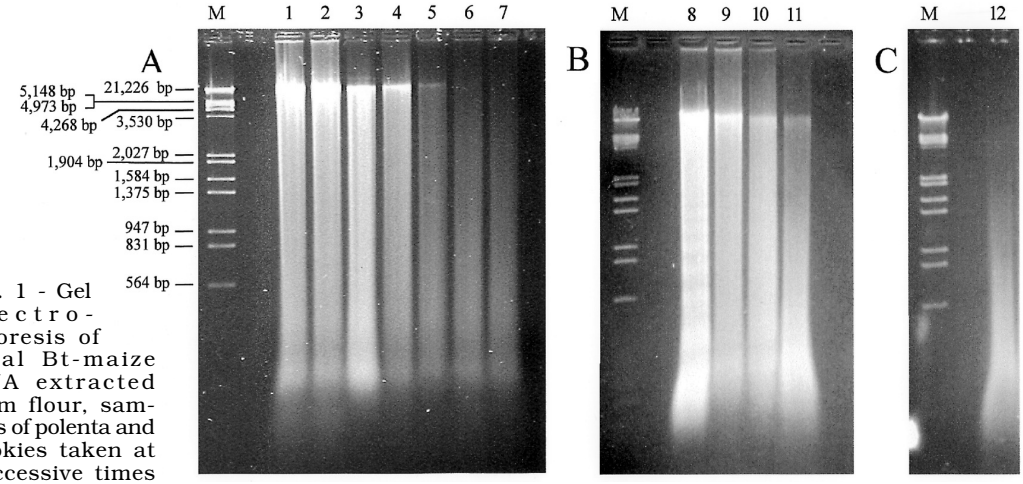


Fig. 1 - Gel electrophoresis of total Bt-maize DNA extracted from flour, samples of polenta and cookies taken at successive times of cooking and pop corn. An equal amount of DNA was loaded in each lane. (A) polenta (B) cookies (C) pop corn. Lanes M, molecular size marker (EcoRI/HindIII); lane 1, conventional maize flour; lane 2, 1% Bt-maize flour; lane 3-7, 1% Bt-maize polenta at 0, 5, 15, 30, 45 min of cooking; lane 8-11, 1% Bt-maize cookies at 0, 5, 15, 30 min of cooking; lane 12, "popped" pop corn from 100% Bt-maize kernels.

times.

The data obtained from samples prepared using 0.1 and 1% Bt-maize flour are summarized in Table 4. The difference in the detection efficiency of the tested PCR systems is evident in the products with a low content of Bt-maize

Table 4 - Detection of Bt-maize DNA in samples of 1% and 0.1% Bt polenta and cookies withdrawn at various times of cooking and in 100% Bt-maize pop corn using primer sets 35S 1/2, Bar, CryIA 1/2-3/4 and Cry 03/04. Data were obtained from at least two sample extractions and relative PCR in duplicate.

	Cooking time (min)	Primer set			
		35S 1/2	Bar	CryIA 1/2-3/4	Cry 03/04
Polenta 1% Bt	0	+	+	+	+
	5	+	+	+	+
	15	+	±	+	+
	30	+	±	+	+
	45	±	±	±	+
Polenta 0.1% Bt	0	+	-	+	+
	5	±	-	+	+
	15	-	-	-	+
	30	-	-	-	+
	45	-	-	-	+
Cookies 1% Bt	0	+	+	+	+
	5	+	+	+	+
	15	+	±	+	+
	30	±	-	+	+
Cookies 0.1% Bt	0	+	-	+	+
	5	±	-	+	+
	15	-	-	+	+
	30	-	-	±	+
Pop corn 100% Bt	0	+	+	+	+
	5	+	-	+	+

PCR result: + positive, - negative, ± unreliable result.

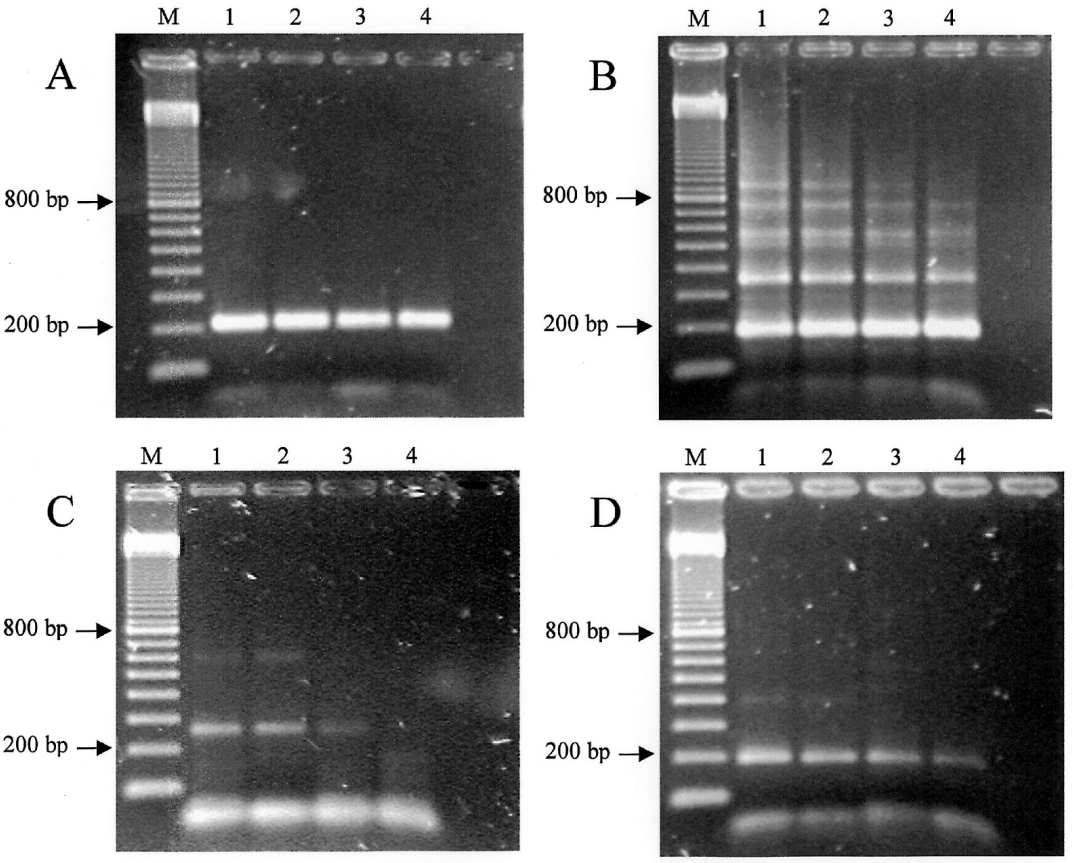


Fig. 2 - Detection of transgenic Bt-maize DNA using different primer sets in 1% Bt-maize cookies at various times of cooking. Primer sets: Cry 03/04 (A); CryIA 1/2-3/4 (B); Bar (C); 35S 1/2 (D). Lanes M, 100 bp ladder; lanes 1-4, 1% Bt-maize cookies at 0, 5, 15, 30 min of cooking. Lanes without notation indicate negative controls (reactions added with non transgenic maize DNA).

DNA. Fig. 2 shows the results obtained with cookies. In detecting 1% Bt-maize in the polenta and cookies, the primer set Bar and 35S 1/2 gave weaker signals than the other primer sets used and failed to detect the target DNA in the final products made with 0.1% Bt-maize; only primers CryIA 1/2-3/4 and Cry 03/04 yielded comparable amounts of amplification products from all samples collected at different times of cooking.

Detection of the target DNA in polenta and cookies containing 0.1% Bt-maize DNA failed with primer sets Bar and 35S 1/2; with primer set CryIA 1/2-3/4, it was unreliable after 5 and 30 min of cooking the polenta and cookies, respectively, whereas with primer pair Cry 03/04, it was still successful in the finished products (Fig. 3).

With respect to the nested reaction with primer set Cry IA 1/2-3/4, the PCR test with primer set Cry 03/04 is less time-consuming and since it requires fewer manipulations it decreases the risk of false positive results due to contamination with exogenous DNA.

It is interesting to note that the 35S 1/2 primer set, recommended in the official European Community protocol for

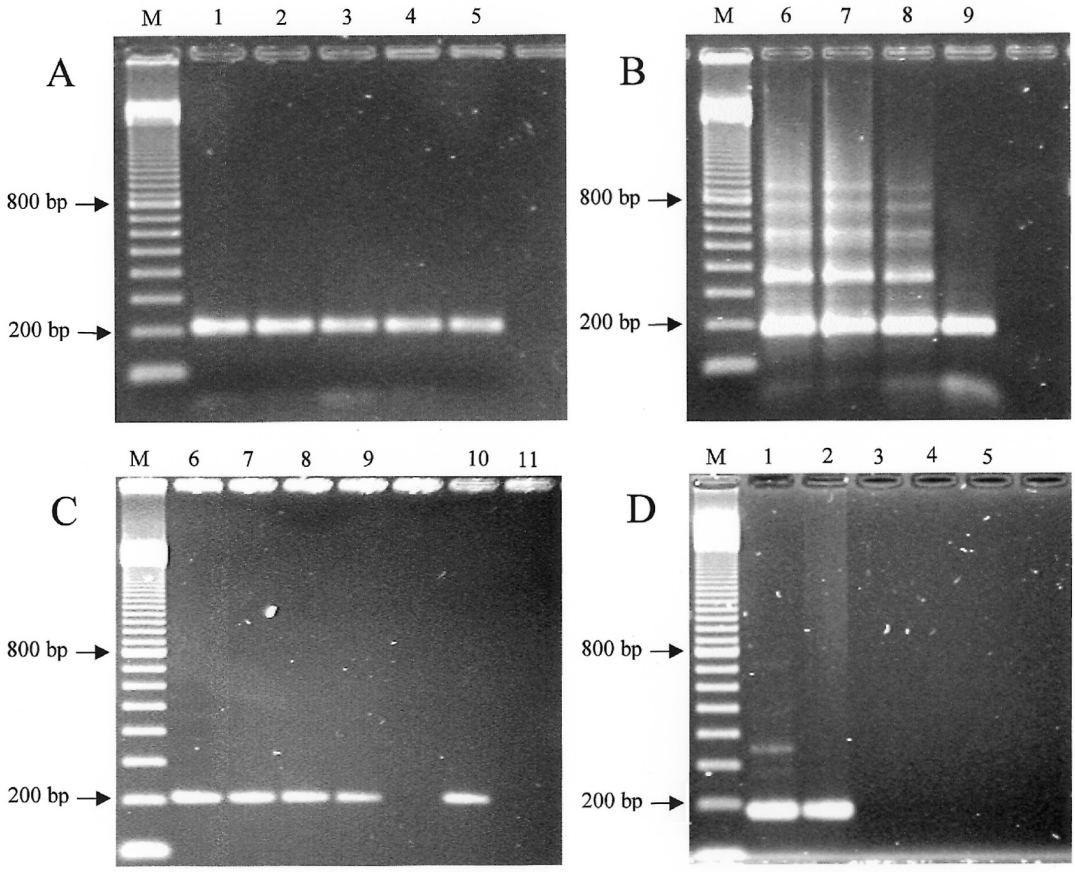


Fig. 3 - Agarose gel electrophoresis after PCR of 0.1% Bt-maize polenta and cookies at various times of cooking. PCR products obtained with primer sets: Cry 03/04 in polenta (A); CryIA 1/2-3/4 in cookies (B); Cry 03/04 in cookies (C); CryIA 1/2-3/4 in polenta (D). Lanes M, 100 bp ladder; lanes 1-5, 0.1% Bt-maize polenta at 0, 5, 15, 30, 45 min of cooking; lanes 6-9, 0.1% Bt-maize cookies at 0, 5, 15, 30 min of cooking; lane 10, positive control; lane 11, negative control (reaction without DNA). Lanes without notation indicate negative controls (reactions added with non transgenic maize DNA).

the detection of genetically modified agricultural products (LIPP et al., 1999), gave less reliable performance than primer set Cry 03/04. Although this could be due to slight differences (e.g. reagent brand) in the PCR protocol with respect to those of original publications, primer set 35S 1/2 has also been shown by WOLF et al. (2000) to be less specific than other primer sets, yielding many unspecific fragments. Possibly, the unspecific amplicons compete with the target, decreasing the PCR yield when the reaction is performed on low-amount/low-quality templates such as those obtained from cooked products.

CONCLUSIONS

All the PCR methods tested were able to detect transgenic DNA in cooked products containing 100% Bt-maize DNA. The results obtained with products containing 1 and 0.1% Bt-maize DNA show that the detectability of transgenic DNA in processed food is affected by the extent of DNA degradation as well as

by the choice of the primers. Primer set Cry 03/04 gave the best results, permitting the detection of transgenic DNA in processed food containing amounts of Bt-maize DNA as low as 0.1%.

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PAPER

DSC CHARACTERISATION OF COCOA BUTTER POLYMORPHS

CARATTERIZZAZIONE DELLE FORME POLIMORFICHE DEL BURRO DI CACAO MEDIANTE DSC

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ABSTRACT

Cocoa butter polymorphs were identified by Differential Scanning Calorimetry (DSC) which is a simple and economic technique compared to X-ray diffraction or similar techniques. The principal aim was to provide the cocoa butter processing industry with a simple method to identify unambiguously the polymorphic form obtained during cooling of chocolate. DSC experiments were carried out on a set of cocoa butter samples to study crystallization as a function of cooling rate. Results were compared to literature X-ray diffraction data

RIASSUNTO

È stata studiata l'identificazione dei vari polimorfi del burro di cacao mediante Calorimetria Differenziale a Scansione (DSC), una tecnica semplice ed economica rispetto alla diffrazione ai raggi-X o tecniche simili. L'obiettivo era quello di fornire all'industria del cioccolato un metodo facile per effettuare correttamente il raffreddamento e la conservazione del cioccolato. Le prove DSC sono state condotte su alcuni campioni di burro di cacao al fine di investigare la cristallizzazione in funzione della velocità di raffreddamento. I risultati ottenuti sono stati confrontati

- Key words: cocoa butter, crystallization, differential scanning calorimetry, DSC, polymorphism, X-ray diffraction -

elaborating two polymorph identification diagrams, found to be as reliable as the X-ray diffraction ones.

con dati di letteratura di diffrazione ai raggi-X elaborando due diagrammi di identificazione del polimorfo risultando confrontabili con questi ultimi.

INTRODUCTION

It is well known that the chemical and physical properties of vegetable fat principally depend on the presence of triacylglycerols (TAG). The TAG composition and the different lengths of the hydrocarbon chain and unsaturation of their fatty acids (FA), as well as their different melting points, are responsible for their complex thermal behavior.

Phase transitions (solidification and melting) of cocoa butter (CB) are accompanied by phenomena which can be explained in terms of fat polymorphism, that is, the capacity of the lipid to crystallize into several forms.

In CB 80% of the TAG fraction is monounsaturated, of which 95% is the sum of POP, SOS and POS. Polymorphism results from the different possibilities of lateral packing of the fatty acid chains and of longitudinal stacking of molecules in lamellae, resulting in each polymorphic form having a typical X-ray diffraction pattern (GIBON et al., 1985). These patterns can be divided into two zones. The first is related to the short-spacings (SS), which are the small reticular distances between the FA hydrocarbon chains of the TAG; they arise from the different possibilities of lateral packing of the FA chains and define the polymorph sub cell. The second is related to the long-spacings (LS), which result from the longitudinal packing of molecules in lamellae; they are the repeat distances in the direction perpendicular to the lamellae. Double or triple chain lengths are common and both SS and LS allow the polymorph to be identified. According to VAECK, (1960), the three main organizations frequently observed for the lateral packing are α, β' and β sub-cells, in order of their increasing thermal stability (LOISEL et al., 1998). There is also a fourth crystalline form (often called γ or sub-α) with a β' sub-cell but it is less stable than the α form. Many authors describe polymorphism in terms of six different polymorphic forms noted from I to VI in Table 1 (WILLE and LUTTON, 1966).

It has been postulated for many years, however, that forms I and III correspond to phase mixtures, while form VI may result from phase separation during solidification of form V (LOISEL et al., 1998). Contrary to the β form which is thermally resistant, polymorphs sub-α, α and β' are metastable. It has been recently demonstrated that γ, α and β' phases can crystallize directly from the

Table 1 - Nomenclature of the cocoa butter polymorphs (VAECK, 1960; WILLE and LUTTON, 1966) and their melting range (VAN MALSSEN et al., 1999).

Lutton Nomenclature	Vaeck Nomenclature	Melting range (°C)
I	γ	-8-+5
II	α	17-22
III	β'	20-27
IV		
V	β	29-34
VI		

melt and that β' formation, via transformation from α, is much quicker than directly from the melt. The two β phases V and VI are obtained via phase transformation from the β' phase only or from the melt if there is a so-called β-memory effect (even a few β crystallites persisting in molten CB are capable of causing complete β solidification of the sample) (VAN MALSSEN et al., 1999; LOISEL et al., 1998; GIBON et al., 1985).

In CB, unlike pure TAG, transition from γ to α is irreversible such as from α to β' and from β' to β. The latter phase, in particular, is very interesting for processing because, being the most stable polymorph, it influences the characteristics of the final chocolate products, both rheological properties, which determine the workability in the production processes, and physical properties such as gloss, snap, texture, heat resistance, fat bloom stability, etc. (CHAISERI and DIMICK, 1995 a).

The least stable solid phase is the γ phase which can stay unchanged for at least 10 days only at temperatures < -10°C (otherwise within a short time it transforms into α) and can only be obtained after fast cooling. (VAN MALSSEN et al., 1999). Under practical conditions the γ phase never forms and α will usually be the first to crystallize.

Many different techniques have been used to investigate crystallization, such as thermal analysis with Differential Scanning Calorimetry (DSC) (GIBON et al, 1985; SCHLICHTER et al., 1988; DAVIS and DIMICK, 1989; CHAISERI and DIMICK, 1995 a, b; KELLER et al., 1996), X-ray diffraction (VAECK, 1960; WILLE and LUTTON, 1966; CHAPMAN et al., 1971; ADENIER et al., 1975; VAN GELDER et al., 1996), viscosimetry and the more complex system of X-ray diffraction in combination with programmed temperature regulation (XRTD) (VAN MALSSEN et al., 1996 a, b, c, 1999; LOISEL et al., 1998).

Three main approaches for studying the solidification process are reported in the literature: the seeding of molten CB with fat crystals HACHIYA et al., 1989 a, b); static or dynamic crystallization (CHAISERI and DIMICK, 1995 a, b; LOISEL et al., 1998), and cooling by means of DSC (SCHLICHTER ARONHIME et al., 1988).

VAN MALSSEN et al. (1996 a, b, c) obtained some very interesting results with X-ray powder diffraction, studying solidification as a function of both final temperature and cooling rate. Since XRTD allows very precise and unambiguous information about the crystalline form to be obtained, the results of VAN MALSSEN et al. (1996 a), were used as reference data to interpret DSC results.

MATERIALS AND METHODS

Experiments were conducted on three CB samples from Ghana: two were extracted by pressure (named plus and medium quality butters) and one by solvent (named inferior quality butter). The fatty acid compositions were determined by gas chromatography (Table 2) according to the official method AOAC (1995).

Thermal analysis was carried out by means of a Setaram DSC-92 (Setaram, Caluire-France): the working temperature and scanning rate ranges were -140° to 600°C and 0.01°C/min - 30°C/min, respectively. The instrument

Table 2 - Fatty acid composition (%) of the three cocoa butter samples.

	Butter plus	Butter medium	Butter inferior
C14	0.1	0.1	0.1
C16	26.1	25.6	25.3
C16:1	0.2	0.2	0.2
C17	0.2	0.2	0.2
C18	36.2	33.9	34.8
C18:1	33.2	35.6	34.4
C18:2	2.6	3	3.6
C18:3	0.1	0.1	0.1
C20	1	0.9	0.9

was calibrated with tin (melting point 231.8°C and melting enthalpy ΔH= 58.6 J/g) according to the Setaram calibration procedure. The sample was put in an aluminum crucible and the reference was an empty aluminum crucible which is thermally inert at the working temperature range of the instrument. Cooling temperatures were achieved by connecting a Setaram liquid nitrogen refrigerating system to the DSC equipment. Melting and solidification enthalpies were calculated using integration software (SPIGNO et al., 1999).

CB samples were directly weighed (40-100 mg) in the open aluminum crucible and then put in the sample cell. The sample was heated to 50°C, kept at this temperature for 10 minutes in order to destroy any memory effect and cooled to 45°C by the refrigerating system; the cooling program was then started at a constant rate ranging from 0.2 to 10°C/min. Solidification patterns were recorded and related enthalpies calculated.

RESULTS AND DISCUSSION

The tests provided information concerning the crystalline component, temperature (onset value) and enthalpy (ΔH) of solidification and polymorphism and polymorphic transitions.

The X-ray results of VAN MALSSEN et al. (1996 a) revealed that controlled cooling allowed the following to be obtained:

- Pure β' form at a cooling rate < 0.25°C/min;
- Pure α form at a cooling rate between 0.25°C/min and 2°C/min;
- α-crystallization followed by some γ-crystallization at a cooling rate > 2°C/min.

Based on data from VAN MALSSEN et al. (1996 a, 1999) DSC results (Table 3) could be correlated to the different crystalline forms. Crystallization is an exothermic phenomenon and the area of the peak is related to the solidification enthalpy which depends on the crystalline form.

Interpretation of the DSC patterns of the three CB samples (Figs. 1, 2 and 3) revealed three aspects of great importance:

a) for the scanning rate from 0.4°C/min to 5°C/min, two exothermic phenomena occurred with the main peak always preceded by a "shoulder" (named δ form);

b) the β' form was obtained only at a very low scanning rate (0.2°C/min);

c) at high cooling rates α and γ forms could not be separated because of temperature thermal delay in following the established scanning rate (the peak was larger and tailing).

All the recorded DSC patterns agree with LOISEL et al. (1988). In fact as a general trend an increase in the cooling rate induced a decrease in the exothermal temperature. At the lowest scanning rate, <0.2°C/min, a peak formed at about 29°C (Figs. 1, 2). Since the β form can be obtained via phase transformation from the β' phase only if no memory effect is present (the present experimental procedure was aimed to avoid this effect), it must be related to the β' phase.

The so called "shoulder" or δ phase, was already visible at 0.2°C/min and became larger at higher scanning rates, tending to overlap the main peak and then disappear at 5°C/min (Fig. 3). DAVIS and DIMICK (1989) reported that CB seed crystals, formed during early crystallization contain high concentrations of glycolipids, phospholipids and TAG. Furthermore LOISEL et al. (1998) pointed out that lipid segregation occurs in CB on cooling and the fraction that crystallizes in addition to the usual V form exhibits a higher melting point. So the δ phase was related to the presence of a fraction of TAG and other impurities which crystallized apart from the mass.

Table 3 - DSC tests results on cocoa butter samples.

Scanning rate °C/min	T global range (C°)	Cocoa butter	Observed phases	1st Phase T Range (°C)	2nd Phase T Range (°C)
0.2	44-0	plus	β'		21.5-12.5
0.4	44-0	plus	δ[a], α	25-20.5	20.5-11
0.8	40-0	plus	δ, α	23.7-18.8	18.8-8.5
1	50-(-10)	plus	δ, α	23.2-18.0	18-7.4
1.5	44-(-5)	plus	δ, α	22.2-17.2	17.2-4.7
2	45-0	plus	δ, (α+γ)	21.4-16.6	16.6-2.5
2.5	50-0	plus	δ, (α+γ)	20.6-16.2	16.2-1.0
3	50-(-10)	plus	δ, (α+γ)	20.2-16.0	16.0-0
4.5	44-(-5)	plus	δ, (α+γ)	19.0-15.5	15.5-0
5	47-(-10)	plus	δ, (α+γ)	19.4-15.4	15.4-0
6.5	44-(-10)	plus	α+γ	17.0-0	
8	47-0	plus	α+γ	17.0-0	
10	45-0	plus	α+γ	15.5-0	
0.2	45-0	medium	δ, β'	24.7-19	20-12.5
0.4	45-0	medium	δ, α	24-22	21.0-10
0.8	45-0	medium	δ, α	23-17.5	17.5-5
1	50-(-10)	medium	δ, α	22.5-16.5	16.5-0
2	50-0	medium	δ, α	19.5-15.3	15.3-0
5	50-0	medium	(α+γ)		17.0-0
8	60-(-10)	medium	(α+γ)		12.5-0
0.2	40-0	inferior	δ, β	23-21	18-14
0.45	40-0	inferior	δ, α	22.7-20.5	17.5-15
0.8	40-0	inferior	δ, α	22.2-19.2	17.2-8
2	45-0	inferior	δ, α	21-17	17.0-5
3	55-0	inferior	(α+γ)		18.0-0
5	45-0	inferior	(α+γ)		17.5-0
8	50-(-10)	inferior	(α+γ)		13.0-0

a: δ phase indicates a shoulder preceding the main peak.

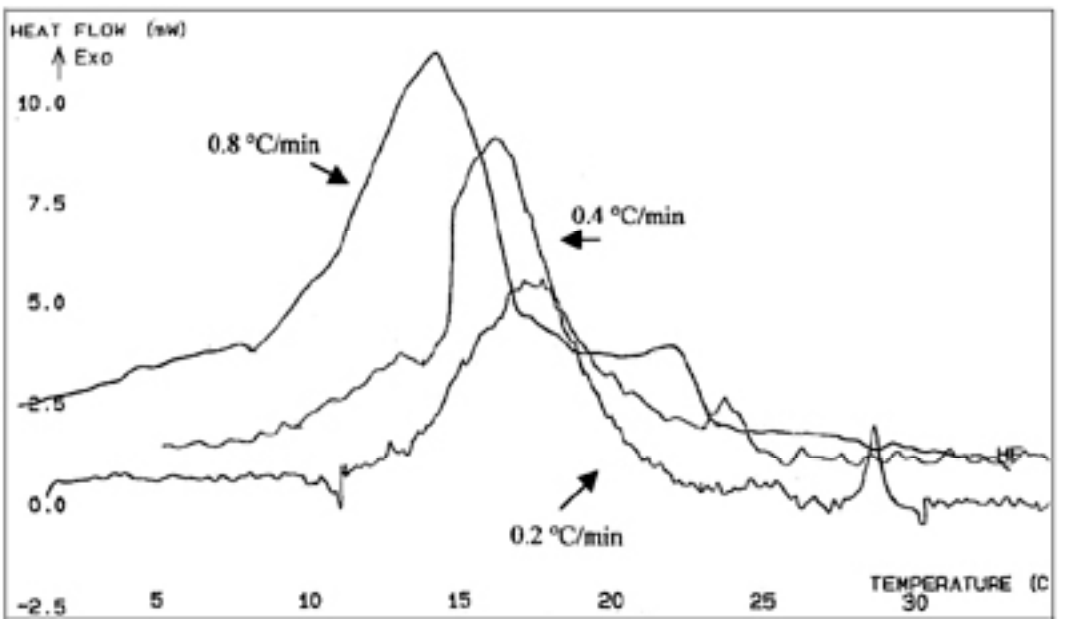


Fig. 1 - Solidification curves of CB plus obtained at different cooling rates.

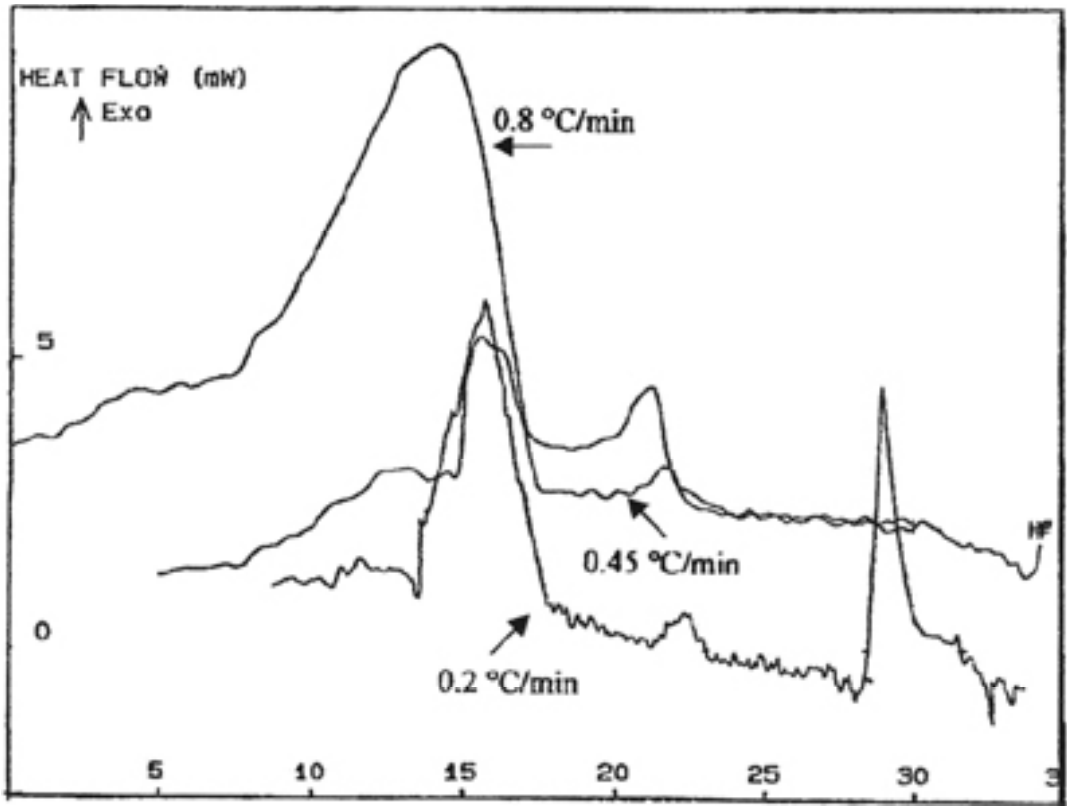


Fig. 2 - Solidification curve of CB inferior obtained at different cooling rates.

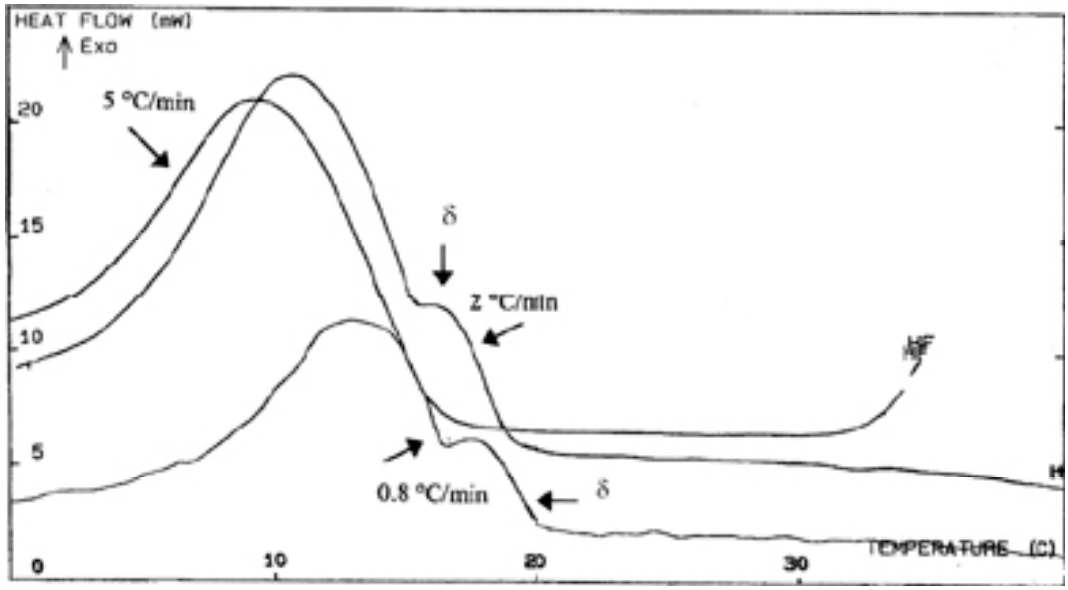


Fig. 3 - Solidification curve of CB medium obtained at different cooling rates (δ indicates shoulder).

It seems that the impossibility of distinguishing α and γ forms is due to the fact that the γ phase has a wide and not exactly determined melting range (-8 to +5°C) together with a small exothermic effect when crystallization occurs. Moreover, some α is formed before γ formation starts even when liquid CB is cooled at a rate of 360°C/min (VAN MALSSEN et al., 1999).

During cooling the first components to crystallize are the higher melting ones so that CB will consist of both a solid and liquid fraction till solidification is completed. Fast cooling of fats leads first to the formation of a liquid crystalline phase which is probably partly organized as a crystal with a compact β' type sub-cell and partly as a liquid (LOISEL et al., 1998).

Very slow cooling (scanning rate <0.2°C/min) makes the more stable polymorph β' form and if the process is completely carried out at a constant rate, the entire sample will solidify into the β' form without further "secondary phase" crystallization. It has been recently demonstrated that both α and β' polymorphs transform into β (the form typical of chocolate products) after some minutes, hours or days depending on the solidification temperature (VAN MALSSEN et al., 1999).

DSC results were elaborated into a phase transition or polymorph identification diagram (Fig. 4) from which, for a particular type of CB, the final polymorphic structure can be predicted, depending on the cooling rate applied during tempering. The graph was obtained plotting the cooling rate on a logarithm scale versus the onset temperatures of solidification peaks. X-ray diffraction results from VAN MALSSEN et al. (1996 a) concerning solidification were treated in the same way, producing a second phase transition diagram (Fig. 5).

Scanning rate data (v_s) were elaborated, by linear regression, as a function

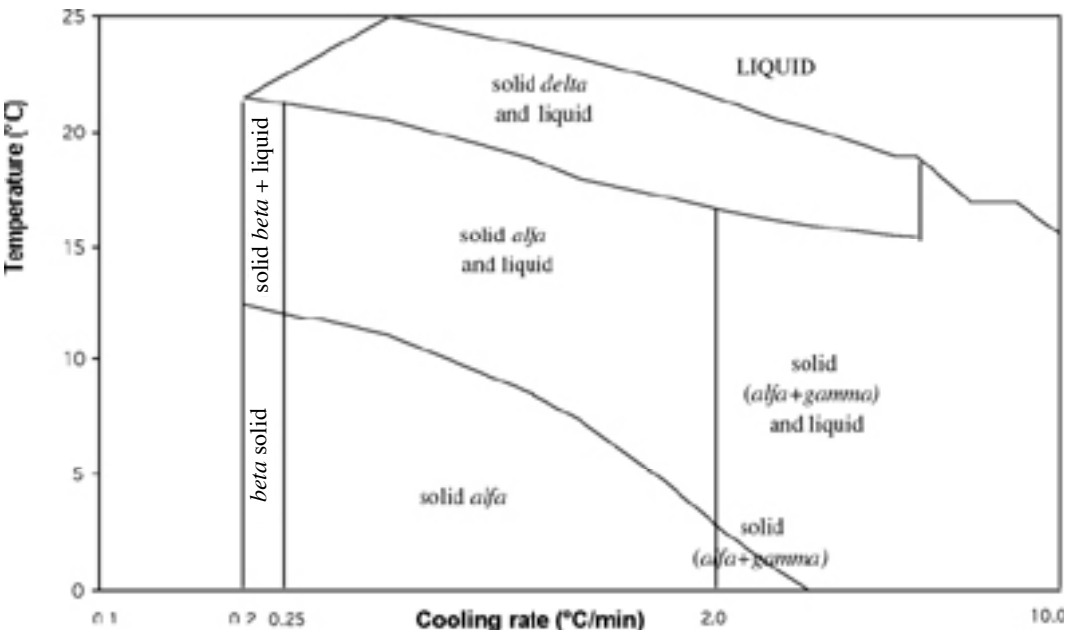


Fig. 4 - Polymorph identification diagram elaborated from DSC results on butter plus.

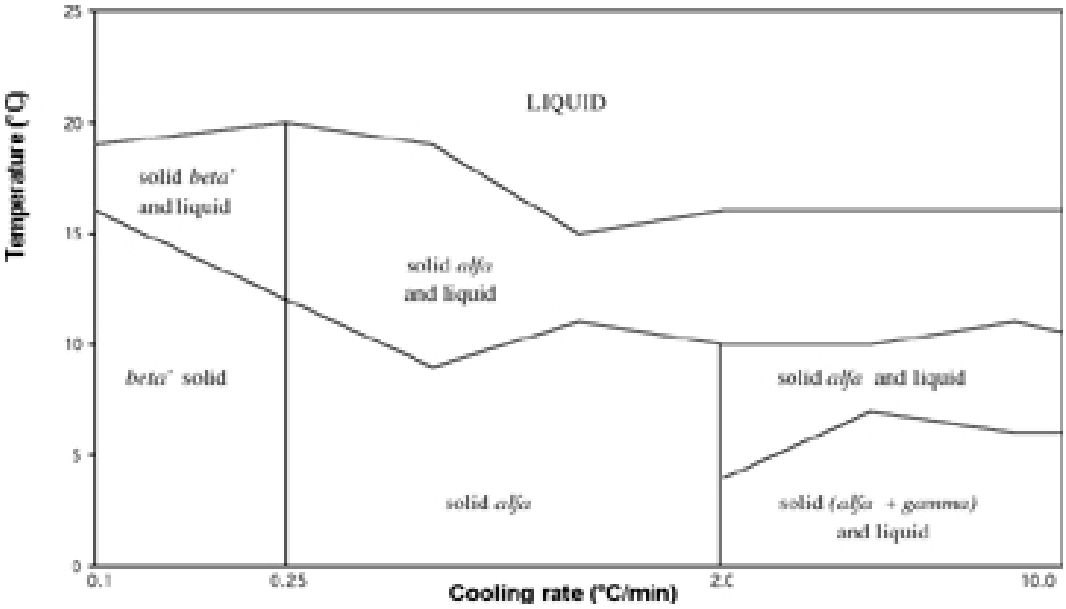


Fig. 5 - Polymorph identification diagram from Van Malssen's X-ray results.

of onset temperatures, according to the relationship: $T = a \cdot v_s^b$.

The parameters correlated well with a linear trend (Fig. 6). This agrees with the decrease in solidification temperature observed with the scanning rate increase.

CONCLUSIONS

Single coexisting phases or crystal-

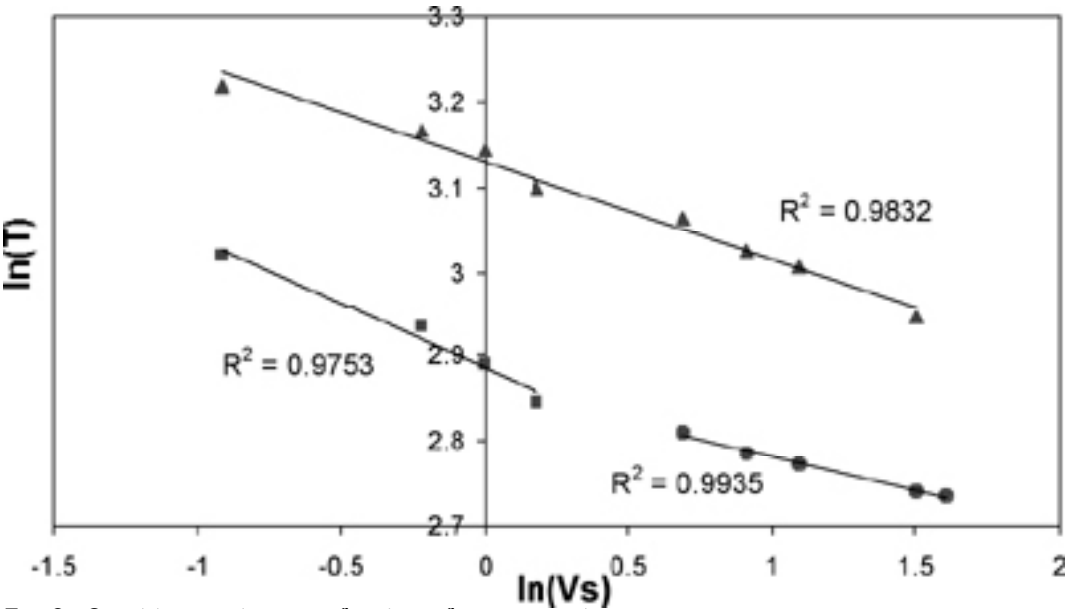


Fig. 6 - Onset temperature as a function of scanning rate.

line transitions can be identified in the DSC plots. By integrating the peak area the percentages of solid and liquid fractions can be calculated if the enthalpy of solidification typical of each form is known (SCHLICHTER et al., 1988). However, a qualitative interpretation of the different thermal events is difficult since each peak can be surely attributed to the presence of a particular crystallite only if supported by different literature information, i.e. X-ray diffraction values.

By comparing DSC and XRTD techniques, the latter is more precise because each polymorph is collocated into a well delimited area, with quite narrow temperature intervals (starting and ending formation T), whereas in the DSC phase diagram, single forms cannot be separated because of thermal analyses limits and because the temperature ranges for the peak formation are wider than in XRTD. However, if temperature and scanning rate, together with the general trend are considered, the two transition phase diagrams nearly overlap each other. From a technological point of view, it is much simpler and overall much cheaper to operate with DSC instruments than with an X-ray diffractometer.

The purpose of this work was to provide the chocolate industry with a simple-to-use tool for the best butter and cocoa processing to obtain good quality end products. DSC patterns recorded at different cooling rates were similar for all three CB samples. Fig. 4 shows polymorph formation for CB plus but can be used as a reference diagram for any CB. Of course, if TAG composition varies slightly, temperature limits for each phase formation vary slightly, too. Anyway, if the central areas are considered, it is possible to predict the crystalline state of CB depending on the cooling rate and final temperature applied after the tempering phase. For example, if tempered chocolate is cooled at 1°C/min till 18°C, CB will crystallize in the a form and a liquid fraction will still be present. Even if it has been demonstrated that γ always transforms into α and α into β' and that the β phase is formed via phase transformation from β' at temperatures below 25°C (VAN MALSSEN et al., 1999), β could take a long time to completely crystallize depending on the solidification temperature with subsequent possible fat bloom problems. That is why it would be preferable to obtain the β' form as soon as possible (by cooling at low rates). Furthermore if a chocolate industry is equipped with a DSC instrument, thermal analysis carried out according to the methodology described in this paper allows each stock of CB being processed to be characterized quite rapidly. As a consequence, the correct temperature and cooling rate can be selected to obtain a time stable chocolate.

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PAPER

SENSORY PROFILE OF EIGHT TOMATO CULTIVARS (LYCOPERSICON ESCULENTUM) AND ITS RELATIONSHIP TO CONSUMER PREFERENCE

PROFILO SENSORIALE DI OTTO VARIETÀ DI POMODORO FRESCO E STUDIO DELLE RELAZIONI CON LE PREFERENZE DEI CONSUMATORI

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ABSTRACT

A sensory profile was created for 8 fresh tomato cultivars (Cherry-Pachino, Lobato, Rita, Furora, Bravo, ES200, Cherry-Licatese, and Sardegna) and their relationship to consumer preference was evaluated. A panel of 11 trained judges was used to evaluate the sensory profile. Results were processed by Analysis of Variance. Mean scores of sensory attributes (red, green, colour uniformity, fruity aroma, acid, salty, sweet, astringent, fruity flavour, crispy, firm, thick-skinned, juicy and mealy) were then subjected to Principal Component Analysis. In order to identify the

RIASSUNTO

Scopo di questo lavoro è stato la messa a punto del profilo sensoriale di 8 cultivars di pomodoro fresco (Cherry-Pachino, Lobato, Rita, Furora, Bravo, ES200, Cherry-Licatese e Sardegna) e lo studio delle relazioni con le preferenze dei consumatori. Per la valutazione del profilo sensoriale è stato utilizzato un panel costituito da 11 giudici addestrati. I risultati ottenuti sono stati inizialmente elaborati con l'Analisi della Varianza, quindi i punteggi medi degli attributi sensoriali (rosso, verde, uniformità di colore, aroma fruttato, acido, dolce, salato, astringente, flavour frut-

- Key words: acidity, consumer preference, reducing sugar, sensory profile, tomato -

relationship between the sensory profile and consumer preference, a preference test was conducted and results were processed by Internal Preference Mapping. This procedure showed that there were two distinct consumer groups having preference for different tomato cultivars. The first group preferred the Cherry-Pachino cultivar for its redness and sweetness and the second group preferred the Sardegna cultivar for its acidity and texture characteristics.

tato, croccante, duro, buccia coriacea, succoso e farinoso) sono stati sottoposti all'Analisi delle Componenti Principali. Al fine di identificare le relazioni tra profilo sensoriale e preferenze dei consumatori, è stato condotto un test di preferenza. I dati ottenuti sono stati elaborati tramite la procedura dell'Internal Preference Mapping. Con questo metodo è stato possibile mettere in evidenza l'esistenza di due gruppi di consumatori le preferenze dei quali erano legate a differenti varietà di pomodoro: per un gruppo, la cultivar Cherry-Pachino è risultata maggiormente preferita per le sue caratteristiche di colore rosso e di dolcezza, mentre per un altro gruppo la cultivar Sardegna per le sue caratteristiche di acidità e consistenza.

INTRODUCTION

Tomato is the most cultivated crop in Italy. In 1998 357,000 tons of fresh tomato, corresponding to 15.2% of the overall fresh fruit and vegetable consumption, were consumed (ISMEA, 1998). Italians are among the greatest tomato consumers, and have highly contributed to its fame and diffusion throughout the world (FAO, 1996).

Tomato is being increasingly investigated in genetic, chemical and technological studies after having been shown that lycopene, a carotenoid precursor of vitamin A, characteristic of this fruit, plays an important role in reducing cardiovascular disease and digestive tract tumours (SOUTHON, 1996; STAHL and SIES, 1996; GERSTER, 1997).

Based on the market demand for fresh and processed tomato, genetic research has focused on developing products having specific quality and agronomic characteristics. To be used fresh, tomatoes should have palatable colour, flavour, aroma and texture characteristics; those to be processed should have intrinsic rheological properties such as viscosity and high soluble solids content (STEVENS et al., 1977; DAVIES and HOBSON, 1981; SCHUCH, 1994).

Various studies have been carried out on the relationship between chemical-physical and sensory parameters of industrial tomato and some methods have been set up to evaluate the quality of finished products. Despite this, the scientific literature concerning sensory studies on fresh tomato is poor (KADER et al., 1977; HOBSON and BEDFORD, 1989; GOUGH and HOBSON, 1990; HETHERINGTON et al., 1990; WOLTERS and VAN GEMERT, 1990; PAGLIARINI and RATTI, 1999).

The aim of this work was to create a sensory profile for eight different randomly selected fresh tomato cultivars. Furthermore, the relationship of the profiles to consumer preference was investigated as a basis for developing new cultivars with specific sensory characteristics to meet consumer demand.

MATERIALS AND METHODS

Eight fresh tomato cultivars (Lycopersicon esculentum) from winter production were randomly selected from a market in Milan (Italy). Seven cultivars were supplied from Sicily and one from Sardinia as follows: Cherry-Pachino (A), Lobato (B), Rita (C), Furora (D), Bravo (E), ES200 (F), Cherry-Licatese (G) and Sardegna (H).

All samples were stored in a thermostated cell at 4°C and then brought to room temperature for at least 3 h prior to each sensory session.

Chemical-physical analyses

Tomatoes were chopped, placed in a 17106 Omnimixer (Sorvall Dupont Instrument, Milan, Italy), homogenized at speed 7 for 2 min and then subjected to chemical-physical analyses.

The soluble solids were determined using a DBX55 digital refractometer (Atago, Cinisello Balsamo, Italy), previously calibrated with distilled water. Values are expressed in °Brix (1°Brix corresponds to 1% soluble solids in water). Analyses were carried out in duplicate.

Sugars, expressed as glucose and fructose, were determined with a Uvidec-610 spectrophotometer (Jasco, Milan, Italy) at 340 nm wavelength using a D-glucose/D-fructose enzymatic kit (Boheringer GmbH, Milan, Italy). Analyses were carried out in duplicate.

A PHM62 Standard pHmeter (Radiometer, Copenhagen, Denmark) was used to determine pH as well as acidity by adding 0.1 N NaOH to the homogenate up to pH=8.1. Values are expressed in g citric acid/100 g product. Analyses were carried out in duplicate.

The dry matter content was determined in triplicate by drying in an oven at 105°C for 1 h. Table 1 shows that the composition of the eight cultivars was typical, though very different.

Sensory panel

Two different panels were used for sensory evaluations. A panel consisting of 11 trained judges was used for the sensory profile evaluation and a panel consisting of 100 habitual tomato consumers was used for the preference test.

Sensory profiling

The training period of the 11 judges lasted for 2 months. The aim of this training session was to develop a com-

Table 1 - Means for composition of the eight tomato cultivars.

Cultivars	Reducing sugar (glucose+fructose) (g/100g)	Soluble Solids (°Brix)	Acidity (g citric acid/100g)	pH	Dry matter (g/100g)
A (Cherry-Pachino)	4.90^{d}(*)	5.65^{e}	0.65^{f}	4.07^{b}	7.82^{e}
B (Lobato)	1.70^{a}	3.10^{a}	0.62^{e}	4.04^{b}	6.47^{c}
C (Rita)	3.15^{b}	3.50^{b}	0.40^{b}	4.17^{c}	5.59^{b}
D (Furora)	3.85^{c}	4.05^{c}	0.42^{b}	4.30^{e}	5.36^{ab}
E (Bravo)	3.15^{b}	4.00^{c}	0.36^{a}	4.24^{d}	5.41^{ab}
F (ES200)	2.90^{b}	3.85^{c}	0.49^{c}	4.27^{de}	5.31^{a}
G (Cherry-Licatese)	4.15^{c}	5.30^{d}	0.54^{d}	4.16^{c}	6.83^{d}
H (Sardegna)	6.60^{e}	6.65^{f}	0.74^{g}	3.99^{a}	9.04^{f}

(*) Means within a column with different superscripts are significantly different at $p<0.05$.

mon sensory vocabulary to describe the tomatoes. Also, reference standards for each descriptor were defined in agreement with the panel leader. Analysis of variance was performed for each attribute in order to identify those that were not significant in discriminating amongst the samples. The final list of descriptors with the relevant definitions and standards is reported in Table 2.

The sensory profile was evaluated according to the UNI Regulation U590A1950 (1997) and the panel evaluated the eight tomato cultivars in triplicate. Four tomatoes were evaluated in each session.

Judges were presented with unpeeled whole tomatoes. They were instructed to cut the tomatoes into segments, to score external appearance and aroma successively and then to take a bite of the tomato segment and score the texture. They were asked to score texture, flavour and taste during chewing. If necessary, they could taste more than one tomato segment, which was then swallowed. Reference standards were available during each session.

Within each session the design was balanced for order and carry over effects (MACFIE et al., 1989). Judges were requested to evaluate the intensity of each attribute by assigning a score between 1 (absence of the sensation) and 9 (extremely intense).

Consumer test

The same eight cultivars were then used for the preference test. The 100 consumers (54 females and 46 males between 21 and 58 years of age) were presented with four samples per session. They were requested to express their preference based on the overall sensory characteristics perceived and to rate each sample using a 9-pt hedonic scale. The design was balanced for order and carry over effects.

Data analysis

The sensory data for each attribute were submitted to Analysis of Variance (ANOVA) with cultivars, judges, replicates and their interactions as effects. The significance of these effects was tested with F tests. The means of the cultivars averaged across judges and replicates were submitted to Principal Component Analysis (PCA) in order to interpret sensory differences among tomato cultivars using Unscrumbler® 7.5 software (Camo As, Trondheim, Norway).

Internal Preference Mapping (MDPREF) (CARROLL, 1972) was performed on the consumer preference scores for tomato cultivars in order to examine discrimination between the samples and identify different subgroups among consumers. Internal analysis, unlike external preference mapping, provides a multidimensional product space based on the preference scores alone and may be viewed as a variation of PCA, where the products are the objects and the consumers are the variables (GREENHOFF and MACFIE, 1994). Recent developments in internal preference methodology have resulted in techniques for testing the significance of individual consumers fitted by the preference model and whether differences between product preferences are significant (JAEGER et al., 1998; MONTELEONE et al., 1998). Although internal preference mapping is a mature methodology, research to improve its performance continues. To assist interpretation of the preference dimensions it has become customary to project external information about the stimuli onto the internal preference map (JAEGER et al., 2000).

In our study, statistical analysis of preference data was performed using a SAS procedure developed at the Institute of Food Research, UK (WAKELING, 1996). This procedure performs a permutation test (EDGINGTON, 1987) to evaluate whether each subject's data

Table 2 - Flavour vocabulary, definitions and references for the tomato cultivars.

DESCRIPTORS	DEFINITIONS	STANDARD
Appearance		
Round	Product is round in shape	Round tomato of Dutch origin
Extended	Product is more or less cylindrical in shape	S. Marzano-type tomato
Inside and outside red	Red colour characterized by Hunter's coordinates: L=50.73, a=60.54, b=34.12	Coloured card characterized by Hunter's coordinates: L=50.73, a=60.54, b=34.12
Inside and outside green	Green colour characterized by Hunter's coordinates: L=81.28, a=28.53, b=15.97	Coloured card characterized by Hunter's coordinates: L=81.28, a=28.58, b=15.97
Uniform colour	Colour uniformity at the external fruit surface	Above-mentioned red a nd green cards
Watery	Product contains liquid inside the fruit (locules)	Lychee
Jelly-like	Product has jelly appearance	Passion fruit
Aroma		
Grassy	Cutty grass aroma perceived by the sense of smell	500 ppm Cis-3-esen-1-olo (Sigma, Milan) in water solution
Fruity	Typical tomato fruit aroma perceived by the sense of smell	30 ppm 2-isobutyl-thiazole (Aldrich Chem., Milan) in water solution
Hay	Dry grass aroma perceived by the sense of smell	5 ppb 2E-4E-decadienale (Sigma, Milan) in water solution
Taste		
Sweet	One of the four basic tastescaused by water solutions of various substances perceived on the tip of the tongue	Ripe kiwi fruit
Salty	One of the four basic tastescaused by water solutions of vari us substances perceived on the tip and sides of the tongue	3 g/L NaCl (BDH, Milan) in tomato homogenate

DESCRIPTORS	DEFINITIONS	STANDARD
Acid	One of the four basic tastes caused by water solutions of vari us substances perceived on the central part of the tongue	1.5 g/L citric acid (Sigma, Milan) in tomato homogenate
Bitter	One of the four basic tastes caused by water solutions of various substances perceived at the rear part of the tongue	0.05 g/L caffeine (BDH, Milan) in water solution
Flavour		
Astringent	Characteristic perceived by some sensations caused by narrowing and wrinkling (dryness) of the mucous membrane in the oral cavity	Unripe kiwi fruit
Grassy	Cutty grass aroma, resulting from placing tomato in the mouth, involving taste and smell at the moment ofswallowing	500 ppm Cis-3-esen-1-olo (Sigma, Milan) in water solution
Fruity	Typical tomato fruit aroma, resulting from placing tomato in the mouth, involving taste and smell at the moment of swallowing	30 ppm 2-isobutyl-thiazole (Aldrich Chem., Milan) in water solution
Texture		
Crispy	After biting into the product, it crunches between the teeth	Yellow Golden Delicious apple
Firm	Strength required to compress a substance between the molars (for solids) or between the tongue and the palate (for semisolids)	Unripe Sardegna cultivar tomato
Thick-skinned	Sensation in the mouth caused by tough tomato-peel during chewing	Unripe Sardegna cultivar tomato
Juicy	Wet sensation in the mouth caused by a product after compression between the teeth	Lychee
Mealy	Mouth sensation during chewing caused by product crumbling similar to starch and/or flour texture	Renetta apple

were significantly fitted by the preference mapping model. Also, to assess how differently products are discriminated from each other, the statistical method involves a bootstrapping procedure (EFRON AND TIBSHIRANI, 1986) in order to obtain 90% confidence regions for the product positions.

RESULTS AND DISCUSSION

Sensory profile of tomato varieties

Analysis of Variance on sensory data for each attribute indicated a significant effect of tomato cultivars at a 0.1% level for all the attributes. F values for replicates and interactions between Judges x Replicates and Cultivars x Replicates were not significant for nearly all the attributes.

The results indicate that the mean scores for each tomato cultivar given by the panelist for each descriptor could be assumed satisfactory estimates of the sensory profile of the samples. As a result, they were averaged across assessors and submitted to Principal Component Analysis (PCA).

A multidimensional space based on significant sensory data is reported in Fig. 1a-b. The variance explained by the first two principal components was 87%.

In Fig. 1a (score plot) the tomato cultivars appear to be well separated into three groups. From right to left along the first component (explained variance 62%), samples **A** and **G** (cherry variety), **C**, **D** and **E** (round variety) were separated from samples **H** (camone variety), **B** and **F** (beef heart variety). The second component (explained variance 25%) distinguished the cultivars **C**, **D** and **E** from the others. Fig. 1b shows the loading plot of the attributes, used to identify the importance of each attribute on each

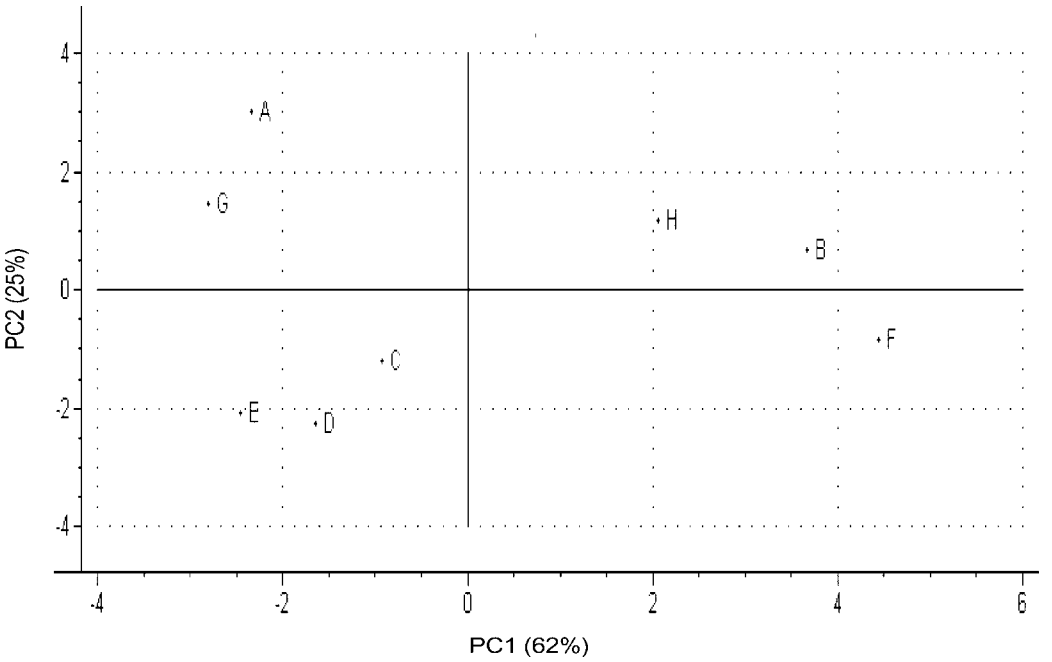


Fig. 1a - Principal Component scores from sensory analysis of tomato cultivars: A=Cherry-Pachino; B=Lobato; C=Rita; D=Furora; E=Bravo; F=ES200; G=Cherry-Licatese; H=Sardegna.

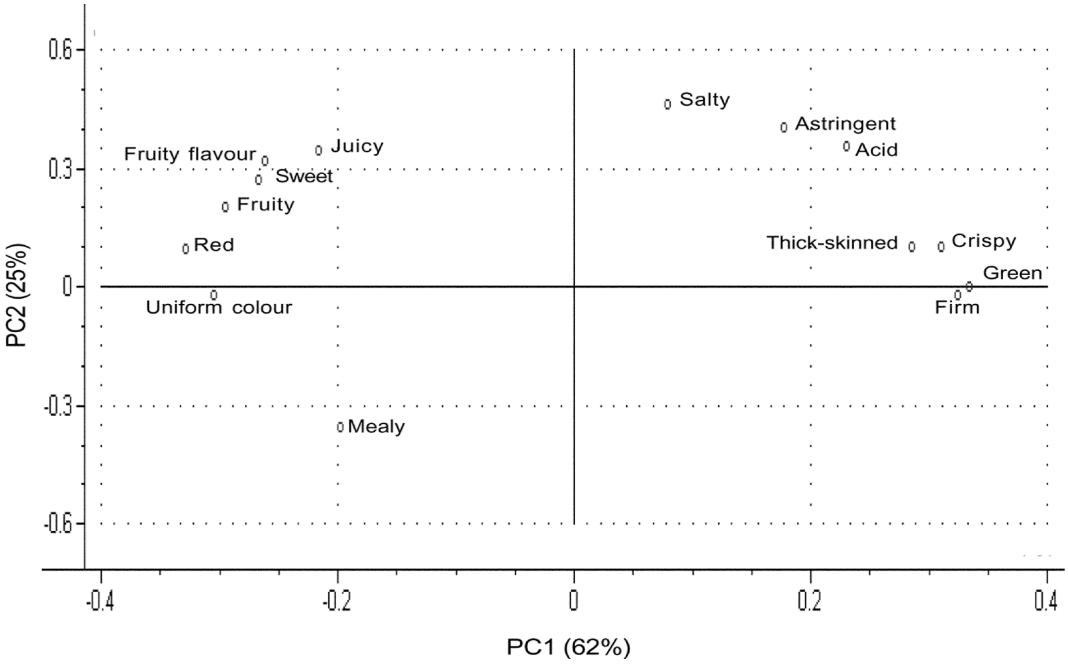


Fig. 1b - Principal Component loading plot from sensory analysis of tomato cultivars.

of the two components in discriminating among the cultivars. Tomato samples **A** and **G** on the left of the first component received high scores for the attributes "red", "fruity aroma and flavour", "sweet" and "juicy". The attributes "green", "thick-skinned", "firm" and "crispy" discriminate the cultivars **H**, **B** and **F** from the others. The cultivars **C**, **D** and **E** were mealy, uniform in colour, not very salty, astringent and acid.

Based on these results and in agreement with WATADA and AULENBACH (1979), it can be concluded that the samples were probably separated according to the degree of ripening: from green to red, from firm to juicy, from acid to sweet and from astringent to fruity.

Mean preference scores for tomato cultivars

The mean preference scores for the tomato varieties are given in Table 3. Consumers preferred the fruit of cultivar **A** (Cherry-Pachino) and disliked cultivars **F** (ES200), **H** (Sardegna) and **C** (Rita).

The problem with univariate analysis for this type of data is the implicit assumption that all subjects exhibit the same behaviour, and that a single mean

Table 3 - Mean preference scores for the eight tomato cultivars.

Cultivars	Preference Scores
A (Cherry-Pachino)	6.5^{c}(*)
E (Bravo)	5.3^{b}
G (Cherry-Licatese)	5.3^{b}
D (Furora)	5.2^{b}
B (Lobato)	4.8^{b}
C (Rita)	4.7^{ab}
H (Sardegna)	4.6^{ab}
F (ES200)	4.1^{a}

(*) Means with different superscripts are significantly different at $p<0.05$.

value is representative of all subjects.

Consumer preference for tomato: the preference map

The first two dimensions of the Internal Preference Map of the 8 tomato cultivars accounted for 49.6% of the total variance (dimension 1, 25.9%; dimension 2, 23.7%). Figure 2 shows a projection of subject scores on the two axes. The third dimension, accounting for 13.7% of the total variance, did not substantially increase the number of subjects fitted by the model. In total 61 subjects (61%) were fitted. In terms of explained variance the fit was not especially good; however, this is to be expected in consumer experiments where the heterogeneity is generally high. This is confirmed by most of the recent work on the analysis of consumer preference by Internal Preference Mapping (DALLIANT-SPINNEL et al., 1996; JAEGER et al., 1998; MONTELEONE et al., 1998; PAGLIARINI et al., 1997). It is encouraging to see that the majority of consumers (61%) projected well onto the two-dimensional product map, since the permutation test indicated that for these people more variation was explained than would be expected by chance alone.

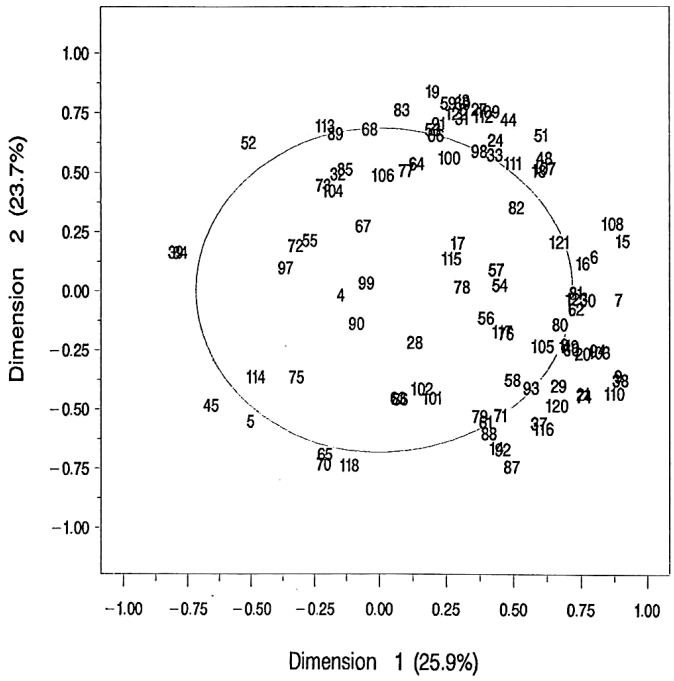


Fig. 2 - Internal Preference Mapping of tomato cultivars: score plot for all consumers.

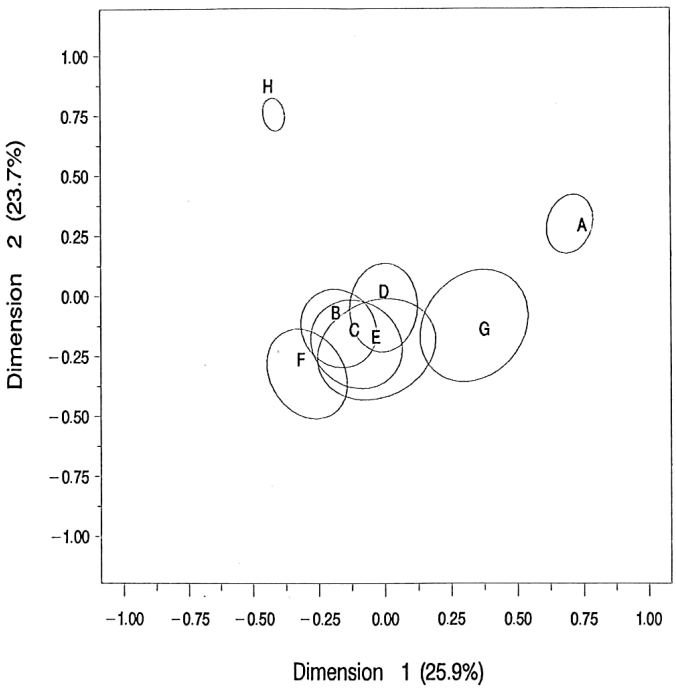


Fig. 3 - Internal Preference Mapping of tomato cultivars (all consumers). A=Cherry-Pachino; B=Lobato; C=Rita; D=Furora; E=Bravo; F=ES200; G=Cherry-Licatese; H=Sardegna.

Dimension 1 of both the consumer plot (Fig. 2) and product map (Fig. 3) indicates again that consumer preferences are clearly oriented towards cultivar **A**. Moving left to right on the first dimension, cultivars **H** and **F** were the least preferred.

Consumers were spread more widely on dimension 2, in which cultivars **H** and **A** were separated from the rest of the products. Additional interpretations can be derived by looking at the distribution of the subjects. Consumers on the bottom right of the plot were characterized by high preferences for cultivars **A** and **G**. They showed little preference for cultivar **H**. Consumers on the top right of the plot generally showed a preference for cultivar **A**, but, unlike the previous group, showed the same preference for product **H** and all the other cultivars.

In order to verify the effect of different sensory properties of products on consumer preferences, the intensity means of descriptors, determined previously, were projected onto the sample positioning map, obtained from preference scores alone (Fig. 4).

Along the first dimension of the map, consumer preference is explained by the attributes red, colour uniformity, sweet, fruity aroma and flavour and juicy. Along the second component, consumer preference is related to the attributes acid, salty and astringent. In particular, along the first preference dimension product **A** was preferred because it was red, sweet, juicy and had a fruity aroma and flavour and product **G** because it was red, mealy and had a uniform colour. Along the second preference dimension product **H** was preferred because it was acid, astringent, salty, green, thick-skinned and firm. The

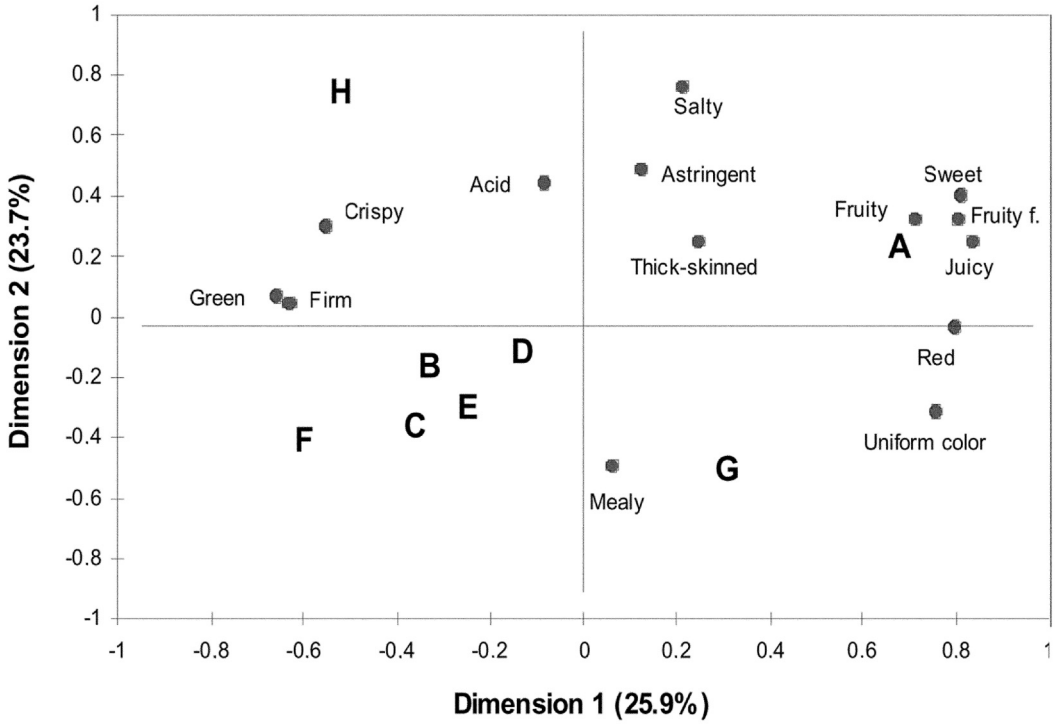


Fig. 4 - Projection of 14 sensory descriptors onto the mapping of the 8 tomato samples.

remaining 5 products (**B**, **C**, **D**, **E** and **F**), which received similar preference scores, were found to be more mealy and crispy; these products were preferred because they were not astringent, salty, sweet, fruity and juicy.

CONCLUSIONS

In this work one of the possible methodologies for selecting the sensory characteristics of fresh tomato associated with consumer preferences was presented. Sensory profiles to characterize each of the cultivars were created and subdivided into categories: two cherry cultivars (Cherry-Pachino, Cherry-Licatese), three round cultivars (Rita, Furora, Bravo), two beef-heart cultivars (Lobato, ES200) and one camone cultivar (Sardegna). Also, the preference test showed that there are at least two market segments where preferences are associated with different sensory characteristics. Although all consumers preferred the juiciest and most fragrant cultivars, at least one group of consumers also liked the Sardegna cultivar with acidity, flavour and astringency characteristics.

The results of this work should stimulate sensory research to set up methods to evaluate sensory characteristics of tomato in order to identify the most important characteristics determining consumer acceptability. Traditional statistical techniques, such as ANOVA and LSD, to evaluate data obtained from consumers, can only provide some information about preferences and are not able to describe to which sensory characteristics preferences are linked. Hence, preference mapping is a useful tool that may be used in association with traditional statistical techniques to evaluate both preferences of individual consumers or groups of consumers and the general preference trend.

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PAPER

MICROBIOLOGY AND BIOCHEMISTRY OF CACIOCAVALLO SILANO CHEESE

CARATTERIZZAZIONE MICROBIOLOGICA E BIOCHIMICA DEL CACIOCAVALLO SILANO

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ABSTRACT

The microbiological and biochemical characteristics of three batches of 60-day ripened Caciocavallo Silano cheese are reported. The sodium chloride percentage in moisture values were 6.12, 10.7 and 6.0, for batches 1, 2, and 3, respectively. Non-starter lactic acid bacteria (NSLAB), which were present at high numbers (7.0, 5.9 and 7.1 log cfu g^{-1}), were essentially comprised of Lactobacillus plantarum and Lb. paracasei subsp. paracasei, while Lb.

RIASSUNTO

Tre produzioni di Caciocavallo Silano di 60 giorni sono state caratterizzate dal punto di vista microbiologico e biochimico. La percentuale di cloruro di sodio riferita all'umidità del formaggio è risultata 6,12, 10,7 e 6,0, rispettivamente per le produzioni 1, 2, e 3. Tra i batteri lattici non-starter (NSLAB), presenti ad elevate concentrazioni (rispettivamente 7,0, 5,9 e 7,1 ufc g^{-1}), le specie prevalenti sono risultate Lactobacillus plantarum, e Lb. paracasei subsp. paracasei,

- Key words: Caciocavallo Silano cheese, *Lactobacillus,* NSLAB, Pasta Filata -

brevis was isolated from only one of the cheeses. RAPD-PCR analysis showed a wide diversity among the Lb. plantarum strains, while Lb. paracasei subsp. paracasei strains formed a homogeneous group. The cheeses had high ratios of water-soluble N/total N (27.2, 38.3 and 56.8%) and high concentrations of free amino acids (12.24, 21.69 and 22.40 mg g^{-1} cheese). The principal amino acids were leucine, lysine and phenylalanine. Urea-PAGE electrophoresis of the water-insoluble fraction and the RP-HPLC of the water-soluble fractions differentiated the cheeses. Caciocavallo Silano cheeses showed a very high degree of lipolysis (total free fatty acids were 14,725, 15,311 and 15,477 mg kg^{-1} cheeses). The principal fatty acids were butyric, caproic, capric, palmitic and oleic acids.

mentre Lb. brevis è stato isolato solo da uno dei formaggi. L'analisi RAPD-PCR ha evidenziato una notevole diversità tra i ceppi di Lb. plantarum ed una maggiore omogeneità nell'ambito di Lb. paracasei subsp. paracasei. I formaggi sono stati caratterizzati da un elevato rapporto azoto solubile in acqua/azoto totale (27,2, 38,3 e 56,8%) e da alte concentrazioni di aminoacidi liberi (12,24, 21,69 e 22,40 mg g^{-1} di formaggio), rappresentati principalmente da leucina, lisina e fenilalanina. L'elettroforesi Urea-PAGE della frazione insolubile in acqua e l'analisi RP-HPLC della frazione solubile in acqua, hanno consentito di rilevare differenze tra i formaggi. Il Caciocavallo Silano è stato caratterizzato da un elevato livello di lipolisi (acidi grassi totali, rispettivamente, 14.725, 15.311 e 15.477 mg kg^{-1} di formaggio). Acido butirrico, caproico, caprico, palmitico ed oleico sono risultati gli acidi grassi preponderanti.

INTRODUCTION

Of the 2,000 varieties of cheese produced throughout the world, 300-400 are of Italian origin (VIZZARDI and MAFFEIS, 1990). Among the Italian cheeses "Pasta Filata" cheeses are characterized by a unique, very original technology because of the stretching process, in which the acidified curds are kneaded in hot water and drawn into long threads which can be formed into various shapes. The most important "Pasta Filata" cheeses are Mozzarella, Provolone and Caciocavallo which are also differentiated into many sub-varieties depending on local areas of production (BATTISTOTTI and CORRADINI, 1993).

While some studies have been published on the biochemical and microbiological characterization of Mozzarella (CHIANESE et al., 1990; MOREA et al., 1998; APONTE et al., 1999) and Provolone (BOTTAZZI and BATTISTOTTI, 1980; BATTISTOTTI et al., 1986; RESMINI et al., 1988), similar studies on Caciocavallo cheese are not available. Different variants of this cheese are produced. Caciocavallo Silano is an oval-shaped, semi-hard cheese, weighing about 1-2.5 kg, and has a homogeneous body with very few eyes; it is produced in southern Italy (Calabria, Campania, Molise, Basilicata and Puglia) exclusively from bovine milk.

This cheese received the legal designation of "DOC" ("Denominazione d'Origine Controllata", i.e., Controlled Denomination of Origin) on May 10, 1993 and the European "PDO" (Protected Denomination of Origin) in June 1996 (SALVADORI DEL PRATO, 1998). In that year, 1100 tonnes of Caciocavallo Silano cheese

were produced (SALVADORI DEL PRATO, 1998), while 1,300 tonnes were produced in 1997.

The aim of the present work was to characterize the microbiological, physicochemical and biochemical aspects of Caciocavallo Silano cheese produced in the Puglia region.

MATERIALS AND METHODS

Cheesemaking and samples

The Caciocavallo Silano cheeses were supplied by three of the most important cheesemaking plants of the Puglia region. They were made with bovine milk having a pH of 6.6-6.7 and containing 4.9% lactose, 3.3-3.4% protein and 3.1-3.3% fat. The protocol for production is summarized in Fig. 1. Three cheeses from each of three batches (1, 2 and 3) were analysed. All samples were taken after 60 days of ripening. The weight of the cheeses was in the range 2.3-2.5 kg.

Microbiological analysis

For microbiological analysis, samples (20 g) of each cheese were dispersed in 180 mL of 2% sodium citrate solution and homogenized in a Stomacher Lab-Blender 400 (PBI International, Milan, Italy). Serial dilutions of the cheese samples were made in quarter strength Ringer's solution and plated on specific media for viable counts as follows: total mesophilic bacteria (Plate Count Agar, Difco Laboratories, Detroit, MI, USA) at 30°C for 48 h; coliforms (Violet Red Bile Lactose Agar, Difco) at 37°C for 18 h; staphylococci (Baird Parker Agar and Mannitol Salt Agar, Difco) at 30°C for 48 h; enterococci (Kanamycin Esculin Agar, Difco) at 37°C for 48 h; lactobacilli and thermophilic lactobacilli (MRS Agar, Difco) at 30°C for 72 h and at 45°C for 48 h, respectively, under microaerophilic conditions; thermophilic streptococci (M17 Agar, Difco) at 45°C for 48 h; yeasts and moulds (Wort Agar, Difco) at 30°C for 72 h. Except for coliforms and enterococci, the media for plating bacteria contained cycloheximide at a concentration of 170 mg/L^{-1}. To exclude the presence of coliforms and Staphylococcus aureus, 10 and 20 g of cheese, respectively, were homogenized and incubated in the corresponding selective liquid media for 4 days, after which an aliquot of each sample was plated for the microbiological analysis. Counts were confirmed by microscopic observation.

Isolation and identification of mesophilic lactobacilli

After incubation under microaerophilic

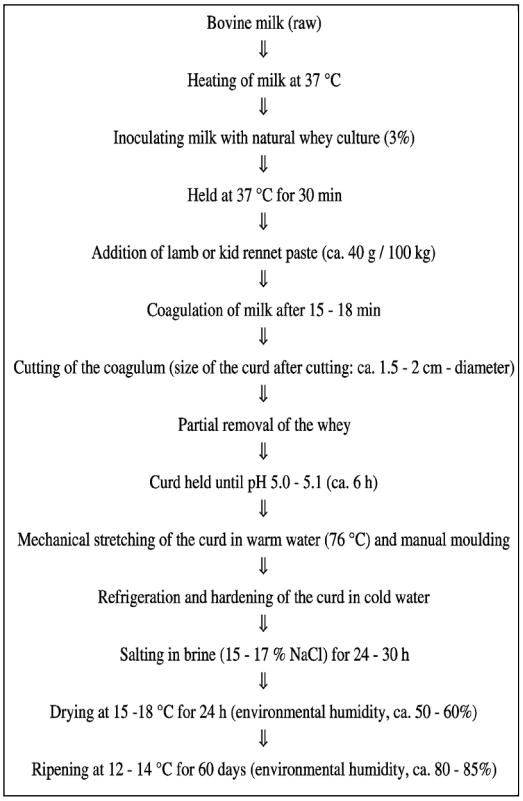


Fig. 1 - Protocol for the production for Caciocavallo Silano cheese.

conditions for 72 h at 30°C, about 10 isolated colonies were randomly selected from each of a duplicate set of countable MRS agar plates for each cheese. All isolates were checked for catalase reaction and examined microscopically. One hundred fifty three gram-positive, catalase negative, nonmotile bacterial rods were first clustered on the basis of growth at 15° and 45°C, CO_2 production from glucose, NH_3 production from arginine, and esculin hydrolysis. About 20% of the isolates belonging to each group were then used for identification; in addition to the preliminary assays, presumtively mesophilic lactobacilli were characterized for sugar fermentation by using the API 50 CHL system (bioMerieux, Marcy-l'Etoile, France) as recommended by the manufacturer, and for isomers of lactate and ethanol production by enzymatic determinations (Boehringer Mannheim, Milan, Italy).

The APILAB Plus 4.0 program (bioMerieux), in addition to the species description published by HAMMES and VOGEL (1995), were used for the identification of Lactobacillus spp.

Genotypic characterization of mesophilic lactobacilli

Identified mesophilic lactobacilli and 2 American Type Culture Collection type strains (Lb. plantarum ATCC 14917 and Lb. paracasei subsp. paracasei ATCC 25302) were genotypically characterized by RAPD-PCR analysis.

Genomic DNAs were extracted, as reported by DE LOS REYES-GAVILÁN et al. (1992), from 2 mL samples of overnight cultures grown in MRS at 30°C. The final concentration of lysozyme was 2 mg/mL. Lamda DNA (Boehringer) standards were used to estimate the level of DNA extracted.

Three primers (Life Technologies, Milan, Italy), with arbitrarily chosen sequences (P1 5' ACGCGCCCT 3', P4 5' CCGCAGCGTT 3' and P7 5' AGCAGCGTGG 3') (DE ANGELIS et al., 2001), were used singly in three series of amplifications.

The reaction mixture contained: 200 μM of each dNTP (2'-deoxynucleoside 5'-triphosphate, 1 μM primer, 1.5 mM $MgCl_2$, 1.25 U Taq DNA polymerase (Life Technologies, Milan, Italia), 2.5 μL PCR buffer, 25 ng DNA and sterile double-distilled water to 25 μL. The PCR program comprised 45 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 35°C, extension for 2 min at 72°C; the cycles were preceded by denaturation at 94°C for 4 min and followed by extension at 72°C for 5 min (ROSSI et al., 1998).

PCR products were separated by electrophoresis for 4 h at 120 V on 1.5% (w/v) agarose gel (Gibco BRL, France) and the DNA was detected by UV transillumination after staining with ethidium bromide (0.5 μg/mL). The molecular weight of the amplified DNA fragments was estimated by comparison with a 123 bp ladder DNA (Gibco BRL, France).

Reading of RAPD-PCR profiles and numerical analysis

For RAPD markers, the presence or absence of fragments was recorded as 1 or 0, respectively. Only reproducible well-marked amplified fragments were scored, faint bands being ignored. The three series of RAPD-PCR profiles were combined to obtain a unique dendrogram. Pairwise comparison of banding patterns was evaluated, calculating an index of genetic similarity using the Simple Matching coefficient (SOKAL and MICHENER, 1958). Cluster analysis was conducted on similarity estimates using the unweighted pair-group method with arithmetic average (UPGMA), from which the dendrogram representing the relationship between Lactobacillus strains was obtained. Analysis was performed using the NTSYS.PC package version 1.8 (ROHLF, 1993).

Analytical methods

Moisture, NaCl and pH were determined as described by the International Dairy Federation (IDF, 1970, 1988, 1989). Water activity (a_w) was determined at 25°C by using an Aqua Lab Decagon Device (Pullman, WA, USA). Total nitrogen (N) and water-soluble N were determined by the micro-Kjeldahl method.

Fat was determined by the Soxhlet method using diethyl ether (GOBBETTI et al., 1999).

Assessment of proteolysis

Water-insoluble and -soluble fractions of the cheeses were obtained as described by FOX (1989). Water-insoluble fractions were analysed by urea-polyacrylamide gel electrophoresis (PAGE) according to the method of ANDREWS (1983). The gels were stained using a modification of the method of BLAKESLEY and BOEZI (1977) with Comassie Blue G-90.

Peptide profiles of the water-soluble fractions were determined by RP-HPLC using a Varian System (Varian Associates Inc., CA, USA) composed by an autosampler, a ProStar solvent delivery system with three pumps, a Prostar programmable multiwavelength spectrometer, interfaced with system control and data acquisition Varian Star Workstation 5. Nucleosil RP-8 (250x4 mm, 5 μm particles, 300 Å pore size) analytical and guard (4.6x10 mm) columns (Capital HPLC Ltd., Broxburn, West Lothian, UK) were used. Elution was monitored at 214 nm and a mobile phase of two solvents, A, 0.1% (v/v) trifluoroacetic acid (TFA, sequential grade, Sigma, St Louis, MO, USA) in HPLC grade water (Milli-Q system, Waters Corp.) and B, 0.1% (v/v) TFA in acetonitrile (HPLC grade, Rathburn Chemicals Ltd., Walkerburn, Scotland) was used. A gradient starting with 100% A for 5 min and continuing with a linear gradient to 50% B (v/v) over 55 min, 50% B (v/v) for 6 min, a linear gradient to 60% B (v/v) over 4 min and 60% B (v/v) for 3 min. The column was washed with 95% B (v/v) for 5 min, followed by equilibration with 100% A for 5 min before injecting the next sample. Total free amino acids, individual amino acids and ammonia in the water-soluble fractions were determined as described by FENELON et al. (1999).

Assessment of lipolysis

Total and individual free fatty acids were determined by the method of GARCIA et al. (1990), as modified by SOUSA et al. (1997). Cheese samples (2.5 g) were ground with 5.0 g of Na_2SO_4 and 100 μL of an internal standard solution of 0.1 g pelargonic acid (C9:0) and 0.2 g margaric acid (C17:0) made up to 10 mL in a 1:1 methanol/chloroform solution stabilized with 0.05% butylated hydroxyanisole (BHA). The mixture was mixed repeatedly and free fatty acids were extracted in diethyl ether. The total volume of extract was measured and 2.0 mL were mixed with 2.0 mL of a 2 g L^{-1} solution of p-bromophenacyl-bromide in acetonitrile in a glass test tube with a sealed cap. Eighty microliters of a 5.0 g L^{-1} solution of 18-crown-6 ether were added, followed by 0.2 g of dry K_2CO_3. The mixture was mixed thoroughly and heated to 75°C for 30 min to promote derivatization. After the mixture reached room temperature, 40 μL of a 40 g L^{-1} solution of formic acid in acetonitrile were added and the mixture was reheated to 75°C for 30 min. The mixture was then filtered through a 0.45 μm acrodisk membrane and 40 μL were used for analysis. The liquid chromatography system used consisted of a Waters Alliance 2690 Separations Module and 2487 Dual λ Absorbance Detector (Waters, Milford, MA, USA). Data were collected and quantified using Millennium 32. The column (250 mm x 4.6 mm) used was a Kingsorb C18, with 5 μm packing and 90 Å pore size, with an associated guard column. The flow rate was 1.0 mL min^{-1} at a constant tempera-

ture of 33°C. A gradient elution based on a combination of water, acetonitrile and methanol was used. Absorbance of the eluate was measured at 245 nm.

RESULTS AND DISCUSSION

Microbiological characteristics

Considering the three batches of Caciocavallo Silano cheese, total mesophilic bacteria were present in the range of 5.0-6.2 log cfu g^{-1} (Table 1). There were statistically significant differences ($P \leq 0.05$) between the cheeses, particularly with respect to staphylococci, enterococci and mesophilic lactobacilli which were found at higher numbers in batches 1 and 3. In all cases, Staphylococcus aureus was not found in 20 g of cheese. The difference in the number of mesophilic lactobacilli between cheeses 1 or 3 (ca. 7.0 log cfu g^{-1}) and cheese 2 (5.9 log cfu g^{-1}) was about 1 log cycle. Lactobacillus plantarum and Lb. paracasei subsp. paracasei were the Lactobacillus species isolated most frequently from all the cheeses, while two strains of Lb. brevis were isolated from batch 1, only. Mesophilic lactobacilli have been isolated from the natural whey cultures used in the manufacture of water-buffalo Mozzarella cheese (COPPOLA et al., 1988) and Lb. plantarum, in particular, represents the most abundant strain in traditional Mozzarella cheese produced using naturally fermented whey as the inoculum (MOREA et al., 1998). Unidentified mesophilic lactobacilli have been occasionally isolated from natural starter cultures used in the manufacture of "Pasta Filata" cheeses produced in the Basilicata region (Southern Italy) (PARENTE et al., 1997). The above reported species, together with Lb. curvatus, represent the dominant mesophilic microflora of various types of cheese such as Cheddar, Fossa and Italian ewe cheeses (Fitzsimons et al., 1999; GOBBETTI et al., 1999; DE ANGELIS et al., 2001). Mesophilic lactobacilli are non-starter lactic acid bacteria (NSLAB) and, together with staphylococci and enterococci, can be considered as adventitious microflora derived from the raw material, environment and manufacturing technology. Recent studies (MCSWEENEY et al., 1993; FOX et al., 1998) suggest that NSLAB play a role during Cheddar cheese ripening.

Thermophilic lactobacilli and thermophilic streptococci were found at low, not statistically significant different numbers, in all the cheeses (3.7-4.2 and

Table 1 - Cell numbers* (log cfu g^{-1}) of the microbial groups investigated in 3 batches of Caciocavallo Silano cheese.

Microorganisms	Batch 1	Batch 2	Batch 3
Total mesophilic bacteria	6.0 ± 0.09	5.0 ± 0.19	6.2 ± 0.13
Staphylococci	5.2 ± 0.12	3.4 ± 0.06	4.6 ± 0.05
Enterococci	6.1 ± 0.07	5.0 ± 0.15	5.8 ± 0.21
Mesophilic lactobacilli	7.0 ± 0.04	5.9 ± 0.08	7.1 ± 0.12
Thermophilic lactobacilli	4.2 ± 0.06	3.7 ± 0.09	4.0 ± 0.17
Thermophilic streptococci	3.8 ± 0.11	3.3 ± 0.11	3.8 ± 0.12
Yeasts	5.5 ± 0.05	5.3 ± 0.04	5.7 ± 0.06
Coliforms	n.d.	n.d.	n.d.

* Each value is the average ± the standard deviation of three cheeses per batch.
n.d. = not detectable in 10 g cheese.

3.3-3.8 log cfu g^{-1}, respectively). Although thermophilic lactic acid bacteria were present at high numbers in the natural whey culture used as starter to inoculate the milk, in the mature cheese their number was lower than that of mesophilic lactobacilli (Table 1). A decrease in the viability of thermophilic lactic acid bacteria starters, due to autolysis of cells, has been described frequently (VALENCE et al., 1998) and the release of the intracellular enzymes plays a crucial role in the textural and flavour changes occurring during cheese ripening (EL-KHOLY et al., 1998). On the contrary, NSLAB, which are present at very low numbers in the cheese curd, became the dominant microorganisms in the cheeses at the end of the ripening period (MARTLEY and CROW, 1993; CORSETTI et al. 1998).

The rather high number of CO_2-producing yeasts found in all the cheeses (5.3-5.7 log cfu g^{-1}), could be responsible for the limited but uniform presence of eyes in the curd, which is a typical characteristic of Caciocavallo Silano cheese.

Coliforms were not detected in 10 g of cheese.

Genotypic characterization of lactobacilli

Lb. plantarum and Lb. paracasei subsp. paracasei strains were genotypically characterized by RAPD-PCR using three primers with arbitrary sequences. A dendrogram (Fig. 2), based on the analysis of RAPD profiles obtained for all the strains (the number indicates the batch from

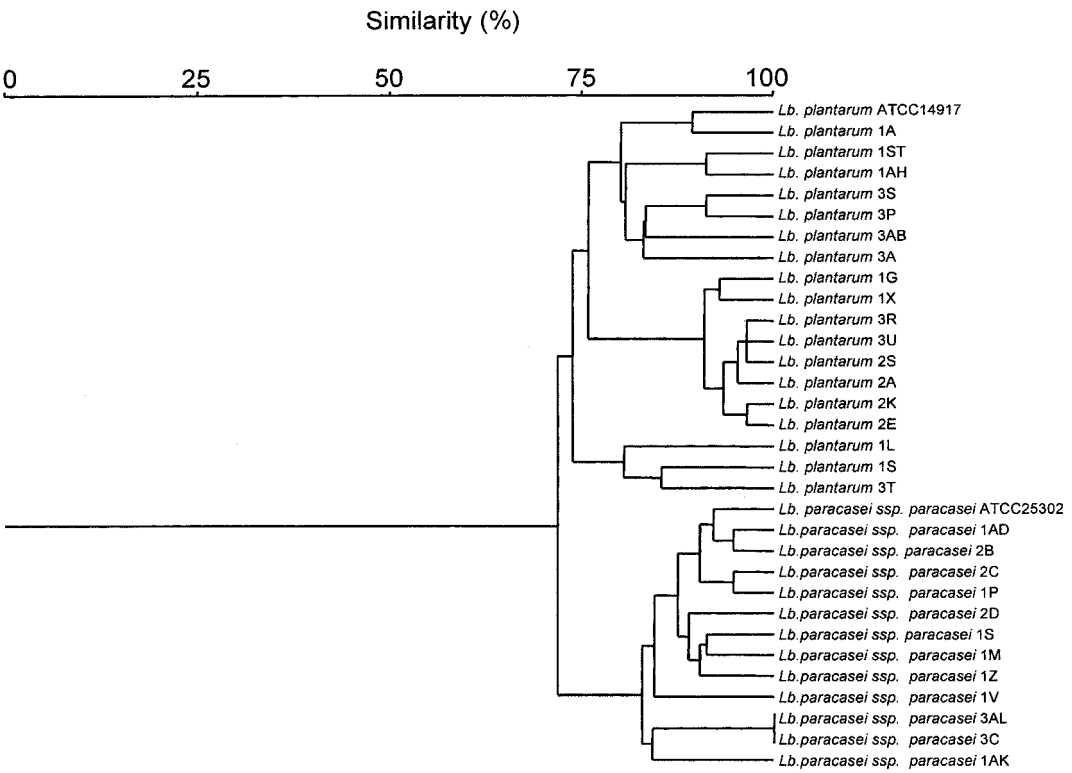


Fig. 2 - Dendrogram obtained from combined RAPD patterns with three primers of Lactobacillus plantarum and Lactobacillus paracasei subsp. paracasei strains isolated from Caciocavallo Silano cheeses. Cluster analysis was conducted on similarity estimates using the unweighted pair-group method with arithmetic average (UPGMA).

which the strains were isolated), shows their genetic similarity.

Considering a similarity level of 80%, Lb. plantarum strains grouped into three separate clusters, showing a high variability within the species (Fig. 2). While batches 1 and 3 were characterized by the presence of Lb. plantarum strains having three different RAPD profiles, only one strain, which probably represented the same isolate, was found in cheese from batch 2.

At a similarity level of about 70%, the Lb. plantarum complex joined the Lb. paracasei subsp. paracasei group; independent of the type of cheese, a very limited variability characterized the Lb. paracasei subsp. paracasei strains (Fig. 2).

The diversity of microorganisms may have repercussions on cheese ripening and flavour. As the Caciocavallo cheese characterized in the present study showed a limited number of NSLAB species, a high diversity within a species could have a possible role in differentiating the cheese during ripening.

Physicochemical characteristics

Values for protein (26.4-26.8%) and fat content (31.5-32.8%) of all the cheeses showed no statistically significant differences (Table 2).

The moisture content of the cheeses ranged from 34.7 to 41.0%. The concentration of NaCl-in-moisture (S/M) was not significantly different for batches 1 and 3 (6.1 and 6.0%, respectively) but batch 2 had a significantly ($P \leq 0.05$) higher value (10.7%). The values of a_w depended on the S/M concentration, the lowest value, 0.906, being found for cheese 2; the other samples showed a a_w of about 0.95. Cheese 2 was also characterized by the lowest value (cfu/g) for almost all the microbial groups, particularly mesophilic lactobacilli, which were about 1 log cycle lower than in the other cheeses (Table 1). It also had the lowest diversity within the Lb. plantarum species (Fig. 2).

The three batches of cheese were characterized by statistically significant differences ($P \leq 0.05$) with respect to pH (range 5.37-5.59) and NH_3 (Table 2).

Proteolysis

Quantitation and characterization of nitrogen in a water extract of cheese is a commonly used index of cheese ripening. Furthermore, the preparation of a

Table 2 - Mean values* for the gross composition of Caciocavallo Silano cheese.

	Batch		
Analytical data	1	2	3
pH	5.47 ± 0.03	5.37 ± 0.02	5.59 ± 0.02
Moisture (%)	41.0 ± 1.51	34.7 ± 0.85	38.4 ± 0.98
a_w	0.956 ± 0.003	0.906 ± 0.002	0.954 ± 0.003
NaCl in moisture (%)	6.12 ± 0.02	10.7 ± 0.03	6.00 ± 0.02
Protein (%)	26.5 ± 0.71	26.8 ± 0.90	26.4 ± 0.53
Fat (%)	31.5 ± 0.65	32.8 ± 0.69	32.7 ± 0.72
Water-soluble N/tot. N (%)	27.2 ± 0.88	38.3 ± 0.65	56.8 ± 0.87
NH_3	0.47 ± 0.02	0.57 ± 0.01	0.65 ± 0.01
Free amino acids**	12.24 ± 0.102	21.69 ± 0.084	22.40 ± 0.122

* Each value is the average ± the standard deviation of three cheeses per batch.
** Free amino acids expressed as mg leucine g^{-1} cheese.

water-soluble extract is an efficient procedure for separating the small peptides and free amino acids from proteins and larger peptides in cheese (MCSWEENEY and FOX, 1996).

The level of water-soluble N values, as a percentage of the total N (WSN/TN%), was very high in the three batches of Caciocavallo Silano cheese, varying from 27.2 to 56.8% (Table 2); batch 3, in particular, had a WSN/TN% value (56.8%) which greatly exceeded the values of pH 4.6-soluble N/TN% (comparable to the WSN/TN value for cheeses which do not show a considerable increase in pH during ripening) reported for Parmesan (32-34%) cheese (BATTISTOTTI and CORRADINI, 1993) which is characterized by a very high level of proteolysis during ripening.

Among the non-microbial enzymes, rennet and indigenous milk proteinases, especially plasmin, contribute to proteolysis in cheese (FOX et al., 1996). Although very little coagulant survives the cooking conditions applied to some cheeses, it has been stated (DI MATTEO et al., 1982) that some rennet activity survives the kneading and stretching process in Mozzarella; for bovine Mozzarella, DI MATTEO et al. (1982) reported an increase in soluble N from 550-601 mg/100 g s.s., after manufacture, to 1,050-1,201 mg/100g s.s., after 3 days of storage. Since the curd for Caciocavallo cheese undergoes similar processing, rennet activity could also be expected in this cheese. Moreover, plasmin activity could increase in rennet curd with increasing cooking temperature (FOX et al., 1996) and could also contribute to proteolysis in Caciocavallo cheese during ripening.

Urea-PAGE of the water-insoluble fraction (Fig. 3) of the Caciocavallo cheeses showed a different level of hydrolysis. Cheeses 1 and 3 had similar patterns and were characterized by a higher level of peptides of high electrophoretic mobility than sample 2. β-CN underwent very limited hydrolysis; in bacterially ripened cheeses, when animal rennets are used, β-CN is quite resistent to proteolysis throughout ripening (Fox et al., 1996). On the contrary, α_{s1}-CN was hydrolysed extensively in cheeses 1 and 3, probably due to the action of residual rennet and microbial proteinases. The lower level of hydrolysis of α_{s1}-CN in cheese 2 may be related to the higher concentration of NaCl-in-moisture (10.7%) compared to cheeses 1 and 3 (about 6%). It is reported that proteolysis of α_{s1}-CN by chymosin is stimulated by NaCl at concentrations up to 5% (FOX and WALLEY, 1971).

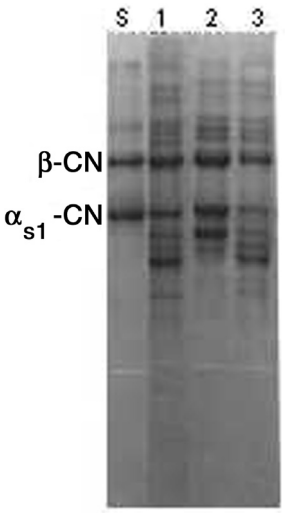


Fig. 3 - Urea-PAGE of the water-insoluble fractions of Caciocavallo Silano cheeses. Lanes: S, Na-caseinate standard; 1-3, Caciocavallo Silano cheese from batches 1, 2 and 3, respectively.

The concentration of free amino acids (as % of total protein) in Caciocavallo Silano cheese varied from 4.7%, in batch 1, to 8.3 and 8.6% in batches 2 and 3, respectively. These latter values approached the lower levels of free amino acid content reported for some Provolone cheese after 5-6 months of ripening (8.2-16.6%) and differed from that found in 6-month ripened Parmesan cheese (about 12%) and, especially, in 24-month ripened Parmesan cheese (about 23%) (BATTISTOTTI and CORRADINI, 1993).

The three Caciocavallo cheeses contained a high concentration of leucine, lysine and phenylalanine, while high values of glutamate and proline were present in samples 1 and 2, and 2 and 3, respectively (Table 3). Glutamic acid, leucine and lysine are present at high con-

Table 3 - Concentration (mg g^{-1} cheese) of individual free amino acids* in Caciocavallo Silano cheese.

	Batch		
Amino Acids	1	2	3
Cysteic acid	0.33	0.53	0.45
Aspartic acid	0.26	0.66	0.88
Threonine	0.30	0.67	0.62
Serine	0.74	1.79	1.53
Glutamic acid	1.68	3.86	1.28
Proline	0.68	2.32	2.86
Glycine	0.20	0.43	0.52
Alanine	0.32	0.69	0.99
Cysteine	0.11	0.14	0.00
Valine	1.17	1.50	2.00
Methionine	0.37	0.46	0.47
Isoleucine	0.40	1.19	1.20
Leucine	1.82	2.43	3.13
Tyrosine	0.30	0.68	0.77
Phenylalanine	1.41	1.10	1.80
Histidine	0.24	0.16	0.01
Lysine	1.39	2.56	3.16
Arginine	0.00	0.00	0.01

* Each value refers to one cheese per batch.

Table 4 - Concentration (mg kg^{-1} cheese) of individual free fatty acids* in Caciocavallo Silano cheese.

	Batch		
Fatty Acids	1	2	3
Butyric (C4)	4,251	4,356	4,541
Caproic (C6)	1,856	2,003	2,104
Caprylic (C8)	458	541	567
Capric (C10)	1,025	985	968
Lauric (C12)	862	875	845
Myristic (C14)	996	924	920
Palmitic (C16)	1,785	1,652	1,641
Stearic (C18)	256	365	345
Oleic (C18:1)	2,457	2,745	2,641
Linoleic (C 18:2)	578	687	654
Linolenic (C18:3)	201	169	251
Total free fatty acids	14,725	15,311	15,477

* Each value refers to one cheese per batch.

centrations in Provolone cheese, a "Pasta Filata" cheese similar to Caciocavallo, after 5-6 months of ripening (BATTISTOTTI and CORRADINI, 1993), while high levels of glutamic acid, leucine and phenylalanine are also characteristic of Cheddar cheese produced with an NSLAB adjunct starter (LYNCH et al., 1996).

The high degree of proteolysis characteristic of Caciocavallo cheeses 1 and 3 was confirmed by the RP-HPLC of their water-soluble fractions which contained a high number of low molecular weight peptides, mainly in the central and hydrophobic zones of the acetonitrile gradient. In spite of their similarity, chromatograms of cheeses 1 and 3 differed both with respect to peak areas and to the presence, in sample 1, of some peptides and/or amino acids in the hydrophilic zone of the acetonitrile gradient. Cheese 2 differed greatly from the others, being characterized by a very low level of peptides throughout the acetonitrile gradient. This cheese was also characterized by high NaCl content and a low a_w level (Table 2) which correlate well with the low microbial count (Table 1).

Lipolysis

All three Caciocavallo cheeses showed a high degree of lipolysis, as demonstrated by the concentration of total free fatty acids, 14,725-15,477 mg kg^{-1} of cheese (Table 4). Caciocavallo Silano cheese is produced by the use of kid or lamb rennet paste which, besides chymosin, contains a pregastric esterase, responsible for the hydrolysis of triglycerides during cheese ripening. This type of rennet paste is also used for other Italian cheeses, such as Provolone and Pecorino, which are characterized by a high concentration of free fatty acids responsible for the typical "piccante" taste (BATTISTOTTI and CORRADINI, 1993; FOX and GUINEE, 1987). In particular, for ripened Provolone, values of total free fatty acids ranging from 2,095 to 6,350 mg 100 g^{-1} of cheese have

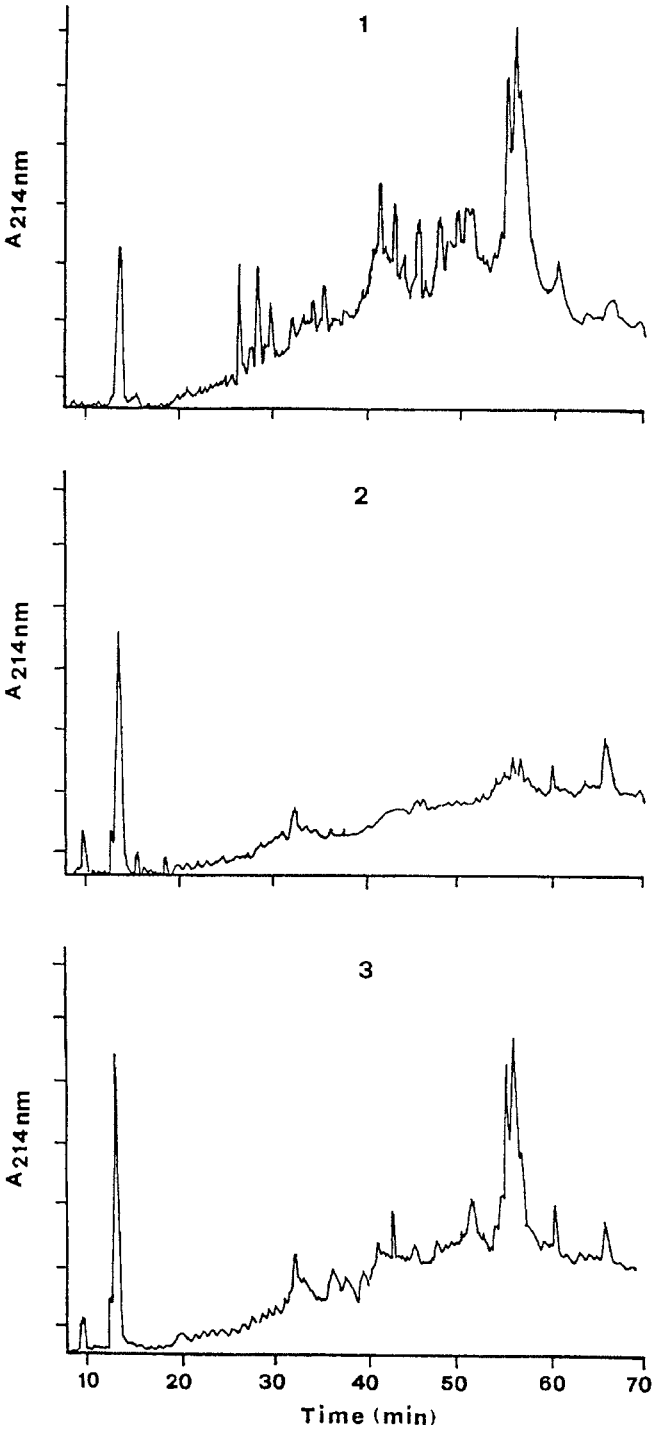


Fig. 4 - RP-HPLC of water-soluble fractions of Caciocavallo Silano cheeses from batches 1, 2 and 3, respectively.

been reported (BATTISTOTTI and CORRADINI, 1993).

Butyric (C4), caproic (C6), capric (C10), palmitic (C16) and oleic (C 18:1) acids were present at the highest concentration in all the Caciocavallo cheeses. In particular, the short chain fatty acids, are responsible for the "piccante" flavour of the Caciocavallo Silano cheeses.

Butyric acid was the only FFA related to the type of enzyme preparation used and to the desirable "piccante" flavour of Provolone cheese (LONG and HARPER, 1956). LONG and HARPER (1956) reported that the use of kid rennet paste in the production of Provolone cheese, determined after 90 days of ripening, gave a higher concentration of butyric acid (2.2 mg g^{-1} cheese) than calf rennet, alone or in combination with calf or kid lipase. The Caciocavallo cheeses analysed in this study were also characterized by a high level of butyric acid.

BATTISTOTTI et al. (1986) stated that in the sensorial evaluation of Provolone cheese, the quality must be related to the ratios of a few free fatty acids, such as C4 and C6. A similar consideration could probably be extended to Caciocavallo Silano cheese which is produced with a similar technology.

CONCLUSIONS

The aim of this study was to characterize the microbiological, physicochemical and biochemical aspects of

Caciocavallo Silano cheese produced in the Puglia region. Some differences (e. g. number of microorganisms, variable degree of proteolysis, wide variations in the concentrations of free amino acids), probably related to different processing and ripening conditions, can be found among Caciocavallo Silano cheeses.

Nevertheless, in spite of the above differences, some microbiological and biochemical characteristics seem to be common to all the cheeses: i) a high number of NSLAB, comprised mainly of two species of lactobacilli (Lb. plantarum and Lb. paracasei subsp. paracasei) and a low survival of starter lactic acid bacteria; ii) a very high degree of proteolysis, iii) a very high concentration of free amino acids, and iv) a very high degree of lipolysis, common to all the cheeses analyzed.

Further work on Caciocavallo Silano cheese will include the characterization of a higher number of cheeses from various plants as well as the complete characterization of Caciocavallo Silano cheese during ripening.

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PAPER

AROMA VALUE OF VOLATILE COMPOUNDS OF PRICKLY PEAR (OPUNTIA FICUS INDICA (L.) MILL. CACTACEAE)

VALORE AROMATICO DEI COMPONENTI VOLATILI DEL FICO D'INDIA (*OPUNTIA FICUS INDICA* (L.) MILL. *CACTACEAE*)

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ABSTRACT

Sixteen volatile compounds of prickly pear fruits (Cv. yellow, red and white) were identified by GC/MS and quantified by GC/FID. Most of their aroma values were calculated using the threshold value of standard compounds determined in water by sensory analysis. (E)-2-hexen-1-ol and hexan-1-ol amounted to about 80% of the total weight of the extracts. However, the most important aroma contributors among the identified compounds were (E,Z)-2,6-nonadien-1-ol and 2-methylbutanoic acid methyl ester, which, though present in low quantities, provided almost 97%

RIASSUNTO

Sono stati identificati e quantificati mediante GC/MS e GC/FID sedici componenti volatili estratti dalla polpa di fichi d'India appartenenti alle cultivar gialla, rossa e bianca. Sono stati quindi calcolati i valori aromatici dei singoli costituenti utilizzando i valori di soglia olfattiva di composti standard determinati in acqua mediante analisi sensoriale. L'(E)-2-esen-1-olo e l'1-esanolo costituiscono circa l'80% in peso degli aromi estratti ed identificati. Tra i componenti identificati i contributi più importanti sono dati dall'(E,Z)-2,6-nonadien-1-olo e dall'estere metilico

- Key words: aroma value; odour threshold; *Opuntia ficus indica*; prickly pear volatile compounds -

of the total aroma value owing to very low thresholds. The former aroma compound gives the typical odour of prickly pear, while the latter has a fresh fruity odour. Both compounds contributed equally to the overall aromatic value in the yellow and red fruits. The amount of 2-methylbutanoate was much higher than that of nonadienol in the white cultivar. Comparison of aroma values of the unripe and ripe fruits confirms these two compounds as the aroma quality factors at ripeness.

dell'acido 2-metilbutanoico, che forniscono circa il 97% del valore aromatico, perché possiedono valori di soglia olfattiva estremamente bassi. Il nonadienolo presenta il tipico odore di fico d'India, mentre l'estere fornisce un caratteristico odore di frutto fresco. Entrambi i componenti contribuiscono in parti uguali al valore aromatico complessivo dei fichi d'India gialli e rossi, mentre in quelli bianchi predomina il contributo dell'estere. Il confronto della distribuzione dei componenti volatili tra frutti maturi e immaturi ha messo in evidenza che il 2-metilbutanoato e il nonadienolo sono i fattori di qualità dell'aroma dei frutti maturi.

INTRODUCTION

Opuntia ficus indica (L.) Mill. (Cactaceae) is a tree species that produces edible fruits with tufts of stinging bristles scattered throughout the peel and sweet smelling flesh. Originally from tropical and subtropical regions of America, today, it is cosmopolitan. It is known as prickly pear or cactus pear in America and Australia, figue de Barbarie in France and North Africa, and fico d'India in Italy. Originally it was used as a pioneering plant to prepare soil for more profitable crops. Italian cultivars have different pulp colours: the most common is yellow, followed by the red and the white types (DAMIGELLA, 1958). It is grown primarily in Sicily (7,500 ha out of a total of 8,000 ha in Italy) with a production of about 60,000 tons/year.

In the past decade processing of the fruit into convenient foodstuffs has been investigated, by a number of researchers (EWAIDAH et al., 1992; BARBERA and INGLESE, 1993; JOUBERT, 1993; SAENZ et al., 1993; CUM et al., 1995; SÁENZ-HERNANDEZ, 1997; BARBAGALLO et al., 1998). When the fruit is homogenized and stored, however, such as in processing of juice or purée, the enzymes induce chemical changes of the volatile metabolites, and the fruit odour flattens. Stabilization of the products requires a pasteurisation temperature of about 100°C, causing the loss of volatile compounds, and giving rise to the need to restore the aroma. However, formulation of the aroma cannot be realized, at the present time, owing to insufficient chemical and sensory information. FLATH and TAKAHASHI (1978) initially investigated these compounds in Mexican prickly pear and found that alcohols represent the major class in the volatile extract. In particular, they pointed out the aromatic relevance of nonenols and nonadienols, previously recognized in cucumber and melon (KEMP et al., 1974), even though a degree of uncertainty about the double bond geometry remained. Volatile constituents in Italian cultivars of prickly pear were studied by DI CESARE et al. (1991), DI CESARE and NANI (1992) who found a different composition, depending on the variety, climatic conditions and the method utilized for juice extraction. In particular, nonenal and nonadienal

were proposed as the most significant aromatic compounds. The effect of cold storage of fruits under normal and controlled atmosphere on aroma development, as influenced by the stage of maturity at harvest, was also evaluated (DI CESARE et al., 1993). Recently, BARBAGALLO et al. (2000) analysed different classes of constituents of prickly pear, including some aromatic compounds.

Despite the copious lists of volatile compounds reported in the above papers, no sensory evaluation of single substances has been performed to individuate those responsible for the odour. The present study concerns identification and quantification of sixteen volatile compounds of prickly pear grown in Sicily (Cv. yellow, red and white) in order to determine their contribution to the aroma value. To this end the odour thresholds (in water) of standard compounds were used. For compounds whose literature values were discrepant or not available, threshold measurements were determined by a sniffing test by trained judges in an ISO-UNI sensory laboratory. The distribution of volatile compounds in unripe prickly pear (Cv. yellow) was also evaluated and compared with that of the ripe fruit.

MATERIALS AND METHODS

Yellow, red and white prickly pears were provided by "Consorzio Euragrumi" (Biancavilla, Catania, Italy). Fruits were picked in the area of Paternò (Catania, Italy) from September to December 1999. After removing the peel, the edible portion including seeds was homogenized using a blender (La Moulinette, Moulinex, Milan, Italy), and a purée was obtained. Samples were prepared from ten fruits of each cultivar and aliquots were immediately extracted. Standard compounds (Sigma-Aldrich, Milan, Italy) and extraction solvents (Merck, Milan, Italy) were GC purity grade commercial products.

Solid Phase Micro Extraction (SPME)

SPME is based on the adsorption of volatile compounds present in the head space of a food on a thin fiber covering the removable internal needle of a microsyringe. The adsorbed vapours are then desorbed by direct introduction of the microsyringe into the injector of a gas chromatograph (YANG and PEPPARD, 1994; NICOLOSI ASMUNDO et al., 1995). Preliminary experiments were conducted to determine the optimal conditions for adsorption of head space compounds. Purée (3 g) was placed in a 7 mL vial stoppered with a perforable screw plug; the vial was warmed in a thermostat at 30°C and a microsyringe (SPME Fiber Assembler, 100 μm polymethylsiloxane, Supelco, Milan, Italy) was then inserted through the plug into the head space of the vial. After 20 min at 30°C (equilibration time of adsorption) the microsyringe was removed for GC injection.

Liquid-Liquid Extraction (LLE)

Five milliliters of pentane/dichloromethane solution (2/1 v/v) containing 1-heptanol of known concentration (internal standard for the successive GC analysis) were added to 200 mL of purée. After the addition of a further 100 mL of pentane/dichloromethane, the volatile compounds were extracted from the mixture for 20 h using an LLE apparatus equipped with a vapour refrigerator at -15°C (MACCARONE et al., 1998). The organic layer was then separated and concentrated under vacuum at 20°C using a vapour refrigerator at -15°C. The residue was collected and the volume was made up to 5 mL with pentane/dichloromethane for GC analysis. Recovery runs were performed to check reliability of the LLE procedure. Extraction from model aqueous mixtures of 14 available standard compounds (comparable to the amount actually present in the purée) showed

that the recovery was almost quantitative, ranging from 91 to 97%.

Gas chromatographic analyses

Volatile compounds of SPME and LLE extracts were separated and identified using a GC/MS apparatus (Saturn III, Varian, Leìni (TO), Italy) by comparing the linear retention indexes (LRI) and the mass spectra with those of standards. Quantitative analysis was performed by GC/FID (GC-17A, Shimadzu, Milan, Italy) using the response factors previously measured from model solutions. For two compounds whose standards were not available, (Z)-2-penten-1-ol, and (Z,Z)-3,6-nonadien-1-ol, a unitary response factor was assumed. For both GC/MS and GC/FID analyses, two identical capillary columns were used (Chrompack CP-Wax 52 CB, 50 m x 0.50 mm, 2.5 μm i.d.) under the following conditions. GC/MS: Oven temperatures 45°C for 2 min, gradient 1°C/min from 45° to 70°C, gradient 2.5°C/min from 70° to 220°C; Injector 220°C; Carrier, helium at 1.0 mL/min. GC/FID: Oven temperatures 70°C for 3 min, gradient 4°C/min from 70° to 220°C; Injector 220°C; Detector 250°C; Carrier, helium at 1.2 mL/min. Samples of 0.1 μL were injected. For the SPME extracts a 3 min desorption time was used (splitless). For the liquid-liquid extracts a 1:50 split ratio was used.

The concentration of each compound is expressed as μg/kg of pulp (specific weight of pulp was 0.96±0.02 g/mL). Quantitative analysis of each extract was carried out in triplicate. The coefficient of variation in quantification of peaks did not exceed 6%.

Assignment of structure by MS

A direct comparison of LRI and mass spectra was not possible for two compounds. Their structures were then ascertained by analysis of the fragmentation patterns [Compound: Positive ion peak mass (Abundance%) Peak assignment]: (Z)- 2-Penten-1-ol: 86 (20) M; 71 (35) M – CH_3; 69 (60) M – OH; 57 (100) M – CH_2CH_3; 31 (35) CH_2OH. (Z,Z)-3,6-Nonadien-1-ol: 140 (absent) M; 122 (7) M – H_2O (C_9H_{14}); 93 (57) 122 – CH_3; 79 (55) 122 – CH_2CH_3; 67 (100) C_5H_7; 55 (37) C_4H_7; 41 (68) C_3H_5; 39 (74) C_3H_3; 31 (62) CH_2OH. The mass spectrum was similar to that of standard isomer (E,Z)-2,6-nonadien-1-ol, yielding a different base peak (41 m/z, C_3H_5) and a minor abundance of the CH_2OH fragment (11%).

Threshold test

Threshold measurement is used to determine the minimum levels for detecting odorous compounds. Samples were presented in order of increasing concentration and the judge indicated whether the designated stimulus was detectable (awareness of change from neutral background) (AMERINE et al., 1965). Tests were carried out in an ISO-UNI sensory laboratory by a trained panel of 14 students from the Agricultural Faculty of Catania University. Threshold values of five volatile compounds of prickly pear (hexanal, (E)-2-hexenal, (E)-2-hexen-1-ol, (E)-2-nonen-1-ol and (E,Z)-2,6-nonadien-1-ol) were determined using a sniffing procedure to evaluate, in different sessions, four increasing levels of each standard compound in aqueous solution. Criteria of response in determining these thresholds included the detection threshold from pure water taken as reference. The results were evaluated using a statistical table of paired comparison test (one-tailed) to designate the absolute threshold of odorous compounds (DETHMERS, 1981).

RESULTS AND DISCUSSION

Volatile compounds of prickly pears were initially investigated by head space SPME and GC/MS techniques. Comparison of LRI and mass spectra with those

of standards allowed identification of the following compounds in all cultivars: (E)-2-hexenal, (Z)-2-penten-1-ol, hexan-1-ol, (Z)-3-hexen-1-ol, (E)-2-hexen-1-ol, (E)-2-nonen-1-ol and (E,Z)-2,6-nonadien-1-ol. Trace amounts of (E)-2-nonenal and (E,Z)-2,6-nonadienal were detected in only a few samples. Successively, GC/MS of the extracts obtained by the LLE procedure showed the presence of the above compounds as well as others not revealed by SPME, namely, 2-methylbutanoic acid methyl ester, hexanal, 2-hydroxybutanone, linalool, (Z)-3-nonen-1-ol, (Z,Z)-3,6-nonadien-1-ol, salicylic acid methyl ester, hexanoic acid and octanoic acid (Fig. 1). Blank runs revealed that some peaks (marked by asterisks in Fig. 1) were due to impurities arising from the procedure of concentrating the extracts. Other minor peaks remained unknown. (E)-2-nonenal and (E,Z)-2,6-nonadienal were not detected. In this regard, FLATH and TAKAHASHI (1978) reported a very low content of 2-nonenal (0.01% of peaks total area), while 2,6-nonadienal was not listed; moreover, HATANAKA et al. (1975) showed that the enzyme alcohol dehydrogenase reduces nonenals to nonenols, and such an equilibrium is strongly favoured by the predominance of NADH over NAD^+ (BELITZ and GROSCH, 1999).

The results of the quantitative analysis of the identified compounds are reported in Table 1 as mean values obtained from three different samples of each cultivar, where the lowest and highest concentration of each compound did not exceed 10% of the mean value. The main findings are the following: i) the

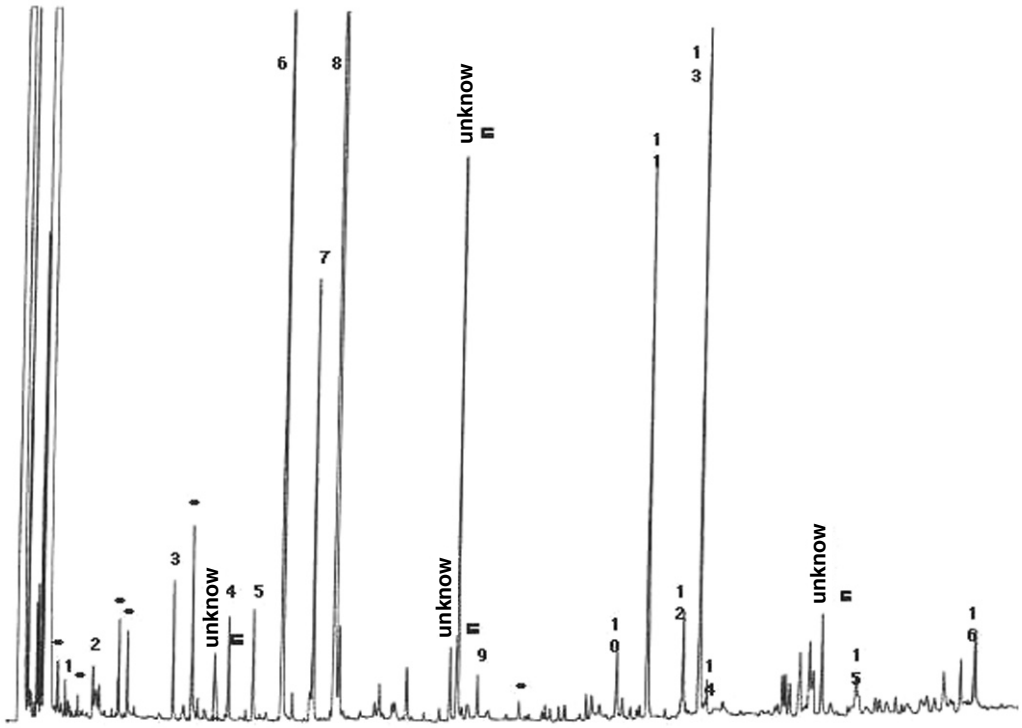


Fig. 1 - Gas chromatogram of volatile compounds of a sample of yellow prickly pear (see Table 1 for compounds corresponding to numbers).

Table 1 - Composition* of volatile compounds of prickly pear fruits in order of linear retention index (LRI).

N.	Compounds	LRI	*Cv.* Yellow		*Cv.* Red		*Cv.* White		Mean	
	(in order of LRI)		μg/kg	%	μg/kg	%	μg/kg	%	μg/kg	%
1	2-Methylbutanoic acid methyl ester	1,009	86	0.78	103	1.28	110	1.02	100	1.01
2	Hexanal	1,082	25	0.23	3	0.04	3	0.03	10	0.10
3	(E)-2-Hexenal	1,218	220	2.00	263	3.28	449	4.15	311	3.14
4	2-Hydroxybutanone	1,286	301	2.73	144	1.79	203	1.88	216	2.18
5	(Z)-2-Penten-1-ol	1,313	87	0.79	43	0.54	41	0.38	57	0.58
6	Hexan-1-ol	1,345	2,779	25.20	1,880	23.42	3,192	29.52	2,617	26.43
7	(Z)-3-Hexen-1-ol	1,378	212	1.92	162	2.02	176	1.63	183	1.85
8	(E)-2-Hexen-1-ol	1,401	6,397	58.00	4,393	54.73	6,077	56.20	5,622	56.77
9	Linalool	1,538	24	0.22	31	0.39	29	0.27	28	0.28
10	(Z)-3-Nonen-1-ol	1,676	35	0.32	52	0.65	23	0.21	37	0.37
11	(E)-2-Nonen-1-ol	1,706	294	2.67	323	4.02	168	1.55	262	2.64
12	(Z,Z)-3,6-Nonadien-1-ol	1,743	46	0.42	80	1.00	21	0.19	49	0.49
13	(E,Z)-2,6-Nonadien-1-ol	1,760	393	3.56	387	4.82	188	1.74	323	3.26
14	Salicylic acid methyl ester	1,765	19	0.17	31	0.39	54	0.50	35	0.35
15	Hexanoic acid	1,842	37	0.34	46	0.57	80	0.74	54	0.55
16	Octanoic acid	2,059	74	0.67	86	1.07	-	-		
	Total	-	11,029	100	8,027	100	10,814	100	9,903	100

* Each value is the mean of three different samples for each cultivar.

total quantity of volatiles ranged from 8,027±327.4 μg/kg in the red cultivar to 11,029±506.3 μg/kg in the yellow one; ii) the qualitative composition was comparable for all cultivars, but there were quantitative differences for individual compounds; iii) (E)-2-hexen-1-ol was the major compound in all cases (~56.8%) followed by hexan-1-ol (~26.4%), together amounting to 83% of the total weight of the compounds; iv) the amount of nonenols and nonadienols in the yellow and red fruits was higher (~800 μg/kg) than in the white ones (~400 μg/kg).

Knowledge of the composition of the volatile fraction is not sufficient to evaluate the relevance of a single compound in contributing to the aroma of prickly pear. It remained unclear which of the compounds were really significant as aroma substances, and to what extent some of them, which were present in very low concentration, affected the sensory properties. In order to estimate such contributions the "Aroma Values" (also defined "Odor Units") were calculated for each compound using the ratio between concentration in food and its odour threshold (ROTHE and THOMAS, 1963; GUADAGNI et al., 1966). Table 2 reports odour thresholds and aroma values for volatile compounds of yellow, red and white prickly pear,

except for (Z)-2-penten-1-ol, and (Z)-3-nonen-1-ol, since their threshold values and standards were not available. Fig. 2 shows the aromatic profile in terms of log Aroma Value, in which the compounds, present at a concentration greater than the threshold (ratio >1) displayed in the positive area of the plot, i.e., contribute to the aroma, whereas those having a ratio <1 (negative logarithm) do not contribute to it.

Despite their high concentrations, (E)-2-hexen-1-ol and hexan-1-ol provided negligible contributions because of high threshold values. Little contribution was given by (E)-2-hexenal, 2-hydroxybutanone, linalool and (Z,Z)-3,6-nonadien-1-ol, having low thresholds. Among the compounds identified, the most important contributors to the aroma were those having the lowest thresholds, i.e., 2-methylbutanoic acid methyl ester and (E,Z)-2,6-nonadien-1-ol (Table 2). The former aroma arises from the metabolism of isoleucine and is characterized by a fresh fruity odour (BELITZ and GROSCH, 1999). The latter arises from the peroxidation of linolenic acid (GROSCH and SCHWARZ, 1971) and has the typical odour of prickly pear, as pointed out by the judges out during the sniffing test. It is therefore an impact compound. In the yellow and red fruits both compounds contributed equally and furnished almost 97% of total aroma values, whereas in the white fruits the higher aroma value of 2-methylbutanoic acid methyl ester (67.5%) and the lower value of (E,Z)-2,6-nonadien-1-ol (28.9%) account for the different odour and acknowledged sensory freshness of the white fruit. It is important to note that 2-methylbutanoate has a chiral center, and its absolute configuration remains unknown. Nevertheless, 2-methylbutanoic acid and 2-methylbutanoates have been prevalently detected in different fruits as (S)-enantiomers (VOLKER et al., 1992).

Table 3 reports the composition and aroma values of volatile compounds extracted from a sample of unripe yellow prickly pear. 2-Methylbutanoic acid methyl ester and other minor compounds were not detected, (E,Z)-2,6-nonadien-1-ol was present in a much lower quantity than in ripe fruit, and higher levels of (E)-2-hexenal, (Z)-3-hexen-1-ol and

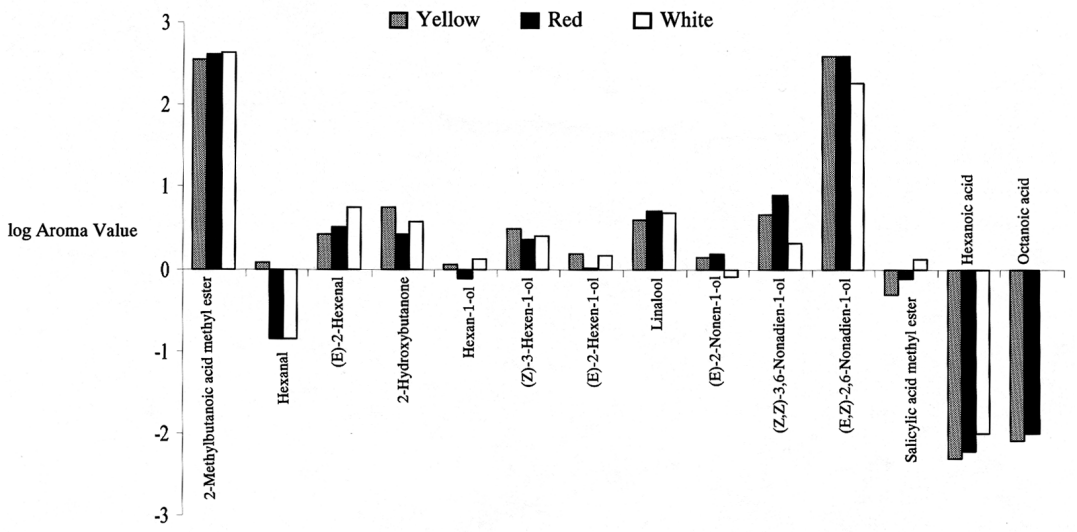


Fig. 2 - Aromatic profiles of volatile compounds in prickly pears.

Table 2 - Odor threshold in water and aroma value of compounds of prickly pear fruits.

Compounds	Threshold in water	Aroma Value					
		Yellow		Red		White	
	µg/kg		%		%		%
2-Methylbutanoic acid methyl ester	0.25[a]	344	45.11	412	49.96	440	67.53
Hexanal	21 (95%)[b]	1.19	0.16	0.14	0.02	0.14	0.02
(E)-2-Hexenal	82 (95%)[b]	2.68	0.35	3.20	0.39	5.48	0.84
2-Hydroxybutanone	55[c]	5.47	0.72	2.62	0.32	3.69	0.57
Hexan-1-ol	2,500[d]	1.11	0.15	0.75	0.09	1.28	0.20
(Z)-3-Hexen-1-ol	70[e]	3.03	0.4	2.31	0.28	2.51	0.39
(E)-2-Hexen-1-ol	4,200 (99.9%)[b]	1.52	0.20	1.05	0.13	1.45	0.22
Linalool	6[c]	4.00	0.52	5.17	0.63	4.83	0.74
(E)-2-Nonen-1-ol	209 (95%)[b]	1.41	0.18	1.55	0.19	0.80	0.12
(Z,Z)-3,6-Nonadien-1-ol	10[f]	4.6	0.60	8	0.97	2.1	0.32
(E,Z)-2,6-Nonadien-1-ol	1.0 (99.9%)[b]	393	51.54	387	46.93	188	28.85
Salicylic acid methyl ester	40[a]	0.48	0.06	0.78	0.09	1.31	0.20
Hexanoic acid	8,200[g]	0.005	0.00	0.006	0.00	0.01	0.00
Octanoic acid	8,800[g]	0.008	0.00	0.01	0.00	-	
Total	-	762.5	100	824.59	100	651.6	100

a) Belitz and Grosch (1999); b) This work. Confidence level in parentheses; c) Pyysalo *et al.* (1977); d) Buttery *et al.* (1988); e) Buttery *et al.* (1971); f) Buttery (1981); g) Salo (1970).

Table 3 - Composition and aroma value of volatile compounds in unripe yellow prickly pear.

Compounds	µg/kg	%	Aroma Value	%
Hexanal	99	0.73	4.71	3.8
(E)-2-Hexenal	1,119	8.27	13.64	11.1
2-Hydroxybutanone	88	0.65	1.60	1.3
(Z)-2-Penten-1-ol	77	0.57	-	-
Hexan-1-ol	2,314	17.11	0.93	0.8
(Z)-3-Hexen-1-ol	622	4.60	8.89	7.2
(E)-2-Hexen-1-ol	9,044	66.86	2.15	1.8
Linalool	34	0.25	5.66	4.6
(E)-2-Nonen-1-ol	45	0.33	0.22	0.2
(E,Z)-2,6-Nonadien-1-ol	85	0.63	85	69.2
Total	13,527	100	122.8	100

(E)-2-hexen-1-ol were found. The total aroma value was much poorer (122.8 odor units) than the ripe one (762.5 odor units). The relative contributions of individual compounds were also different: (E,Z)-2,6-nonadien-1-ol always predominated (69.2% of total aroma values), but (E)-2-hexenal (11.1%) and (Z)-3-hexen-1-ol (7.2%) were not negligible, accounting for the light green leafy odour of the unripe fruit. Therefore, top aroma quality of prickly pear is achieved at ripeness when 2-methylbutanoic methyl ester and (E,Z)-2,6-nonadien-1-ol are present in high levels.

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PAPER

INFLUENCE OF LOW PROTEIN DIETS SUPPLEMENTED WITH ISOLEUCINE AND VALINE ON THE QUALITY AND FUNCTIONAL PROPERTIES OF CHICKEN EGGS

EFFETTO DI DIETE A BASSO CONTENUTO PROTEICO INTEGRATE CON ISOLEUCINA E VALINA SULLA QUALITÀ E SULLE CARATTERISTICHE FUNZIONALI DI UOVA DI GALLINA

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ABSTRACT

The influence of isoleucine and valine supplementation in low protein diets having constant lysine, sulphur amino acids and tryptophan on the quality and functional properties of both fresh and cooked eggs was investigated in laying hens. A lower protein and albumen level reduced egg weight, but the dietary addition of isoleucine significantly increased this trait. Yolk weight and yolk

RIASSUNTO

Scopo della ricerca è la valutazione, sulla qualità e sulle proprietà funzionali delle uova di gallina, dell'effetto di diete a basso contenuto proteico con livelli costanti di lisina, metionina e triptofano ed integrate con isoleucina e valina. Un basso livello proteico della dieta fa diminuire il peso dell'uovo e dell'albume, tuttavia l'aggiunta di isoleucina migliora questa caratteristica. Il peso e l'indice del tuorlo non sono

- Key words: egg components and quality, functionality, isoleucine, valine -

index were unaffected by dietary treatment. The drainage of emulsion after 60 min decreased with the addition of valine. Differences among groups were small and sometimes inconsistent in relation to cake volume, dough density of cake and baking losses of meringue and Madeira cake. Cohesiveness of yolk gel was significantly higher in the control group. Hardness of yolk gel and albumen gel improved with valine supplementation.

influenzati dal trattamento dietetico. Il drenaggio dell'emulsione dopo 60 min. cala nei gruppi addizionati con valina. Le differenze fra i gruppi relativamente a volume delle torte, densità dell'impasto e perdite di cottura delle meringhe e delle Madeira sono di lieve entità. La coesività del gel di tuorlo è superiore nel controllo, mentre la durezza del gel di albume e di tuorlo migliorano con l'aggiunta di valina nella dieta della gallina.

INTRODUCTION

One of the public concerns about problems linked to the environment is pollution from animal waste. Consequently several methods for monitoring pollution have been developed and animal production systems with lower environmental impact have been studied. In Italy animal production and in particular intensive poultry production, commonly concentrated in small areas, is known to produce large amounts of waste containing high levels of nitrogen and phosphorus. Poultry are usually fed high protein diets that consequently enhance nitrogen excretion in the faeces. Several European countries are trying to limit ammonia and phosphorous emissions coming from animal farming by modifying the composition of the diet and improving nutrient utilisation. In the last decades the nitrogen intake of poultry has been reduced and the availability of industrial amino acids such as lysine and methionine has led to a reduction in nitrogen excretion without any negative effects on production (LOPEZ and LEESON, 1995). To a certain extent it is possible to reduce the protein level of the diet by adding essential amino acids with negligible detrimental effects on hen performance (SUMMERS et al., 1991, KESHAWARZ and JACKSON, 1992, LEESON et al., 1998, MELUZZI et al., 2001).

Only a few studies have been carried out on the effect of amino acid addition to the diet on egg quality. CAREY et al. (1991) observed an increase in egg weight and yolk and albumen solids by enhancing dietary methionine. Similarly, SHAFER et al. (1996, 1998) found an increase in yolk and albumen proteins as well as yolk and albumen yields by enhancing dietary methionine, whereas the functional properties of the albumen and yolk in cakes were not significantly affected. Lysine supplementation also increased yolk and albumen weights and albumen solids (PROCHASKA et al., 1996). There is no available data, however, on the combined effect of dietary protein restriction and amino acid supplementation on the quality and functional properties of eggs. These traits are quite important in countries consuming large quantities of egg products; in Italy, for example, about 40% of the eggs are used by the food industry for egg-based products (UNA, 1999).

The present study was conducted to determine the influence of isoleucine and valine supplementation in low protein diets, having constant lysine, sulphur amino acids and tryptophan, on the quality and functional properties of both fresh and cooked eggs.

MATERIALS AND METHODS

Experimental design

One hundred sixty-eight 24-week-old Hy Line Brown hens, divided into 7 groups of six replicates, were housed in conventional cages for 16 weeks. At the beginning of the trial the hens had a very homogeneous weight (1,931±103 g). Three groups received a 15% crude protein diet unsupplemented (15CP) or supplemented with isoleucine (15ILE) or with isoleucine and valine (15ILEVAL). Three groups received a 13% crude protein diet unsupplemented (13CP) or supplemented with isoleucine (13ILE) or with isoleucine and valine (13ILEVAL). The amounts of isoleucine per 100 g of feed were 780 (15ILE and 15ILEVAL) and 760 mg (13ILE and 13ILEVAL) and 670 and 570 for 15CP and 13CP. The amounts of valine per 100 g of feed were 810 (15ILE and 15ILEVAL) and 790 mg (13ILE and 13ILEVAL) and 750 and 660 for 15CP and 13CP. The remaining group (17CP) was fed a commercial diet containing 17% crude protein, 770 and 830 mg of isoleucine and valine, respectively, per 100 g of feed. The experimental diets were formulated on an isoenergetic basis (2,800 kcal/kg) and with the same starch/lipid ratio (14.5:1). Feed was provided ad libitum.

Egg quality evaluation

On a 4 week basis, eggs collected for three consecutive days were individually weighed and the eggs collected for only one day were used for determining Haugh and yolk indexes (SAUVEUR, 1988; FUNK, 1948), and yolk, albumen and shell weights.

Egg functional properties

At 4 and 16 weeks, the firmness of the yolk membrane was evaluated on 6 eggs from each group. A special tool (a perforated metal capsule) connected to an Instron Universal Testing Machine 4301 (UTM, Universal Testing Machine, Instron International Ltd, High Wycombe, England), equipped with a 100 N load cell at a crosshead speed of 1.67 mm/s was used. At the same scheduled times, the functional properties of the eggs were evaluated (three replicates/group). The foaming ability of the albumen and the stability of the foam at 30 and 60 min were evaluated according by SCHOLTYSSEK (1995). The egg yolk was used to prepare emulsions and their stability at 60 and 120 min was determined measuring the drainage as reported in SCHOLTYSSEK (1995). Yolk and albumen were separately heated to obtain coagulated samples according to WOODWARD and COTTERILL (1986) and SHAFER et al. (1998). Textural characteristics of the coagulates were evaluated by a dynamometric double compression test (50% compression) using the Instron equipment and a load cell of 100 N according to WOODWARD and COTTERILL (1986). Hardness, cohesivity and elasticity of the samples were determined according to BOURNE (1978).

Baking quality of the eggs was evaluated preparing meringue for testing the albumen, and Madeira cake for testing albumen and yolk according to the recipes of SCHOLTYSSEK et al. (1994). In the case of the Madeira cakes, 60 g of dough was put in steel rings (i.d.: 100 mm) and baked in a ventilated oven (SMEG, Alpha 30HX) at 200°C for 15 min; meringues (15 g) were baked at 60°C for 12 hours. Dough density, weight loss and volume as well as the maximum shear load of Madeira cakes were measured. For this analysis, two rectangular samples (20x50 mm x height of the baked cake) were carefully cut from the cake and the measurements were made using an Instron dynamometer equipped with a load cell of 1 kN at a crosshead speed of 3.33 mm/s.

Statistical analysis

Data concerning the functional properties tested, carried out both on fresh and cooked eggs, were submitted to one way ANOVA using the General Linear Model procedure of SAS (1985) and considering dietary treatment as fixed effect. Egg quality data were submitted to two way ANOVA with interaction, considering the of dietary treatment and treatment duration as fixed effects. Mean differences were separated by Student-Newman Keuls test.

RESULTS AND DISCUSSION

During the experimental period (16 weeks) egg production ranged from 92 to 96% and daily feed intake from 124 to 128 g/hen with no statistical significance among groups.

Table 1 shows the results concerning egg quality. As the interaction between treatment duration and diets was not statistically significant, only the results concerning the main effects are reported. A lower protein level reduced egg weight. However the addition of isoleucine and isoleucine+valine to the diet containing 15% protein significantly increased egg weight by 2.70 and 1.78 g, respectively. The same occurred for the group receiving the 13% protein diet, but the differences were less pronounced and not statistically significant. Albumen and shell weights, as observed for whole egg, were higher in eggs of groups 15ILE and 15ILEVAL and lower in groups 15CP and 13CP. Yolk

Table 1 - Effect of low protein diets supplemented with isoleucine or isoleucine and valine on egg and egg component weights, yolk index and Ha<ugh index evaluated after 4, 8, 12 and 16 weeks treatment.

	Egg weight (g)	Yolk weight (g)	Shell weight (g)	Albumen weight (g)	Yolk index	Haugh index
Dietary treatment (DT)						
17CP	60.62 ^{ABCbc}	15.30	5.68 ^{Bb}	39.64 ^{ABbc}	0.487	90.41 ^{Aa}
15CP	59.56 ^{BCc}	15.40	5.62 ^{Bb}	38.96 ^{Bbc}	0.488	91.92 ^{Aa}
15ILE	62.26 ^{Aa}	15.48	5.91 ^{Aa}	40.87 ^{Aa}	0.493	91.39 ^{Aa}
15ILEVAL	61.34 ^{ABab}	15.47	5.79 ^{ABab}	40.06 ^{ABab}	0.482	90.54 ^{Aa}
13CP	59.15 ^{Cc}	14.98	5.66 ^{Bb}	38.51 ^{Bc}	0.486	87.74 ^{Bb}
13ILE	60.23 ^{BCbc}	15.16	5.74 ^{ABb}	39.34 ^{Bbc}	0.477	89.83 ^{ABa}
13ILEVAL	59.70 ^{BCc}	15.09	5.72 ^{ABb}	38.89 ^{Bc}	0.524	91.62 ^{Aa}
Treatment duration (TD)						
4 weeks	59.04 ^{Cc}	14.43 ^{C}	5.58 ^{Bc}	39.04 ^{b}	0.506	93.00 ^{Aa}
8 weeks	59.88 ^{BCbc}	15.27 ^{B}	5.60 ^{Bc}	38.98 ^{b}	0.497	90.86 ^{Bb}
12 weeks	60.67 ^{Bb}	15.31 ^{B}	5.81 ^{Ab}	39.82 ^{ab}	0.473	89.61 ^{BCc}
16 weeks	61.93 ^{Aa}	16.03 ^{A}	5.94 ^{Aa}	39.97 ^{a}	0.487	88.51 ^{Cc}
DT	0.001	NS	0.005	0.001	NS	0.001
TD	0.001	0.001	0.001	0.01	NS	0.001
DT x TD	NS	NS	NS	NS	NS	NS
EMS[1]	18.47	2.31	0.21	11.03	0.03	31.51
DF	625	621	621	617	586	595

[1] Error mean square.
$^{A, C}$ Means in a column with no common superscript differ significantly (P<0.01).
$^{a, c}$ Means in a column with no common superscript differ significantly (P<0.05).

weight and yolk index were stable and not affected by dietary treatment. The Haugh index was also quite stable, with only group 13CP having a low value ($P<0.01$). As expected, and widely reported, egg weight and its components increased by prolonging dietary treatment namely with hen ageing, while the Haugh index decreased. The yolk index did not change with duration of treatment.

Tables 2 and 3 report data concerning the functional properties of fresh eggs after 4 and 16 weeks of treatment. The qualitative traits considered were almost stable and differences among groups were negligible at each sampling time. The drainage of emulsion after 60 min was significantly less in the 13ILEVAL group compared to 13ILE ($P<0.05$). The differences in drainage did not affect the emulsion stability.

Baking losses of meringues (about 30% of the weight) were remarkably higher than those of the Madeira cake (about 20%) but the differences among groups were not significant. Baking loss in the Madeira cake was higher only in group 17CP (Fig. 1). The volume of Madeira cake ranged from 108 to 120 mL with small but detectable differences among groups (Fig. 2). The dough density of the cake was not significantly affected by dietary treatment (Fig. 2).

Table 4 shows the results of the functional properties of processed eggs after 16 weeks of treatment. Cohesiveness of yolk gel was significantly higher in the control group ($P<0.05$). Hardness of yolk gel ranged from 29.2 to 35.4 N. In the 3 groups receiving 15% protein, a slight increase in hardness was observed when valine was added and even more so in those groups receiving 13% protein. Also hardness of albumen gel was improved by valine supplementation, particularly in group 13ILEVAL, but the differences were not statistically significant. As for the other functional properties, no sub-

Table 2 - Effect of low protein diets supplemented with isoleucine or isoleucine and valine on the functional properties of fresh eggs after 4 weeks treatment.

Treatments	Foaming ability[1]	Foam stability[2]	Foam stability[3]	Foam index[4]	Relative drainage[5]	Relative drainage[6]	Emulsion stability[7]	Firmness of yolk membrane (N)
17CP	7.44	0.59	0.45	3.27	28.33 [ab]	35.67	7.33	3.88
15CP	7.49	0.66	0.50	3.72	31.50 [ab]	38.67	7.17	3.91
15ILE	7.21	0.69	0.53	3.82	32.33 [ab]	39.00	6.67	4.14
15ILEVAL	7.86	0.67	0.51	3.99	30.50 [ab]	37.17	6.67	3.89
13CP	7.54	0.66	0.49	3.72	30.83 [ab]	37.50	6.67	4.18
13ILE	8.01	0.65	0.47	3.73	33.33 [a]	39.67	6.33	3.81
13ILEVAL	7.82	0.66	0.51	3.97	26.33 [b]	32.83	6.50	3.94
DF	14	14	14	14	14	14	14	35
EMS[8]	0.1694	0.0016	0.0014	0.1014	0.00067	0.00075	0.0002	0.3269

[1] (Weight of 100 mL albumen – weight of 100 mL foam)/ weight 100 mL foam;
[2] (Volume of albumen – drainage at 30 min)/volume of albumen;
[3] (Volume of albumen – drainage at 60 min)/volume of albumen;
[4] Foaming ability x foam stability after 60 min;
[5] (Drainage volume at 60 min x 100)/emulsion volume;
[6] (Drainage volume at 120 min x 100)/emulsion volume;
[7] Relative drainage at 120 min – Relative drainage at 60 min;
[8] Error mean square;
[a, b] Means in a column with no common superscript differ significantly ($P<0.05$).

Table 3 - Effect of low protein diets supplemented with isoleucine or isoleucine and valine on the functional properties of fresh eggs after 16 weeks treatment.

Treatments	Foaming ability[1]	Foam stability[2]	Foam stability[3]	Foam index[4]	Relative drainage[5]	Relative drainage[6]	Emulsion stability[7]	Firmness of yolk membrane (N)
17CP	8.19	0.74	0.53	4.37	24.17	29.17	5.00	3.07
15CP	8.06	0.74	0.54	4.34	23.50	29.00	5.50	3.11
15ILE	8.07	0.73	0.53	4.31	22.33	27.83	5.50	3.31
15ILEVAL	8.16	0.77	0.53	4.30	23.83	28.33	4.50	2.79
13CP	8.27	0.73	0.55	4.52	20.83	25.67	4.83	3.26
13ILE	8.03	0.75	0.53	4.28	22.00	28.17	6.17	3.23
13ILEVAL	8.19	0.76	0.53	4.60	20.83	25.67	4.83	2.89
DF	14	14	14	14	14	14	14	35
EMS[8]	0.1229	0.0017	0.0024	0.2583	0.00056	0.00054	0.00067	0.1862

[1] (Weight of 100 mL albumen – weight of 100 mL foam)/ weight 100 mL foam;
[2] (Volume of albumen – drainage at 30 min)/volume of albumen;
[3] (Volume of albumen – drainage at 60 min)/volume of albumen;
[4] Foaming ability x foam stability after 60 min;
[5] (Drainage volume at 60 min x 100)/emulsion volume;
[6] (Drainage volume at 120 min x 100)/emulsion volume;
[7] Relative drainage at 120 min – Relative drainage at 60 min;
[8] Error mean square.

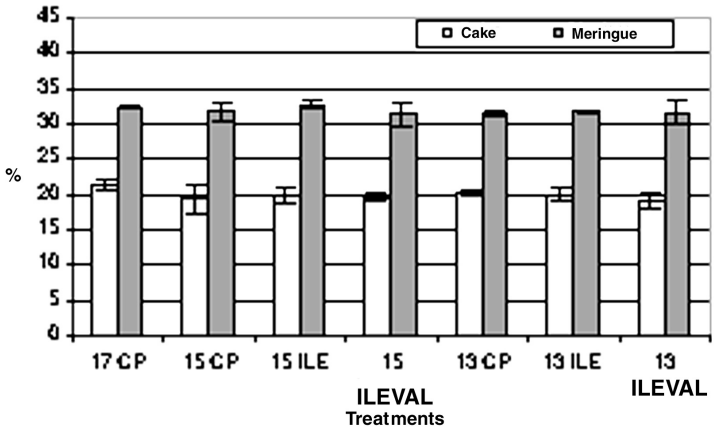


Fig. 1 - Baking loss of Madeira cake and meringue.

stantial differences emerged as a result of the dietary treatment.

The addition of less common essential amino acids such as valine and isoleucine to low protein diets gives some interesting results. Lower protein levels of hen diets decreased egg and albumen weights, but the addition of isoleucine improved these traits. However when the protein level was too low (13%) the amino acid supplementation was not able to fill this gap. These findings are partially consistent with those of SHAFER et al. (1998) who observed a concurrent increase of yolk and albumen weights by increasing dietary levels of methionine. On the contrary, in our trial, albumen and yolk components responded independently to amino acid supplementation: albumen weight was sensitive to the amino acid addition, whereas yolk weight was not affected. It might be due to a selective action of isoleucine and valine with respect to yolk and albumen whose amino acid composition is different. YAKOUT et al. (1999) observed a different effect of dietary amino acids

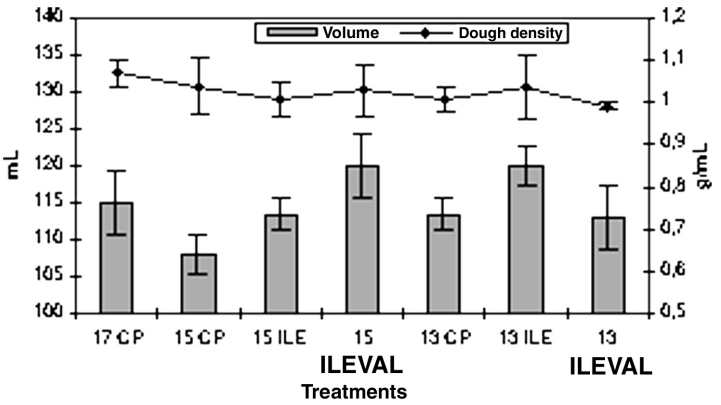


Fig. 2 - Dough density and cake volume.

on yolk percentage: it increased as lysine content increased and decreased with high sulphur amino acid concentration. However further and more and specific investigations should be carried out to verify this. A protein level of 15%, lower than that used in Italian commercial diets, is able to maintain albumen quality measured as the Haugh index, but when the protein level dropped to 13%, the Haugh index significantly decreased and, again, the addition of isoleucine and valine restored the trait to the previous values.

The functional properties of fresh eggs were hardly affected by dietary treatment and the same was so for yolk membrane strength, an important quality for mechanical separation of yolk and albumen. Even though the separate effects of the two supplemented amino acids have not been tested, valine seems to largely affect some functional properties of processed eggs. In fact the hardness of both albumen and yolk gels increased in egg products of the groups supplemented with valine. On the contrary SHAFER et al. (1998) did not find any effect of methionine supplementation on albumen or yolk hardness and springiness.

At the present time, no papers are available on the effect of isoleucine and valine on egg quality and egg functional properties, therefore it is not possible to make other comparisons. However the development of research on the use of such amino acids in hen feeding might lead to interesting findings and uses of eggs. The results reported here might be useful and advantageous for the liquid

Table 4 - Effect of low protein diets supplemented with isoleucine or isoleucine and valine on the functional properties of egg products after 16 weeks treatment.

Treatments	Fracturability of meringue (N)	Hardness of albumen gel (N)	Cohesiveness of albumen gel	Springiness of albumen gel (%)	Hardness of yolk gel (N)	Cohesiveness of yolk gel	Springiness of yolk gel (%)	Share compression force cake (kN)
17CP	572.1	14.77	0.49	91.79	31.66 ^{ab}	0.60 ^{a}	85.19	0.37
15CP	337.1	14.17	0.54	90.83	32.61 ^{ab}	0.54 ^{b}	89.60	0.34
15ILE	347.1	12.19	0.55	87.48	29.25 ^{b}	0.54 ^{b}	88.62	0.33
15ILEVAL	341.5	14.27	0.52	89.79	33.58 ^{ab}	0.52 ^{b}	94.14	0.33
13CP	426.5	12.91	0.57	85.91	32.03 ^{ab}	0.53 ^{b}	89.87	0.36
13ILE	458.6	14.45	0.54	89.38	30.37 ^{ab}	0.54 ^{b}	88.04	0.36
13ILEVAL	442.2	15.77	0.52	90.87	35.42 ^{a}	0.52 ^{b}	88.76	0.31
DF	14	14	14	14	14	14	14	14
EMS[1]	13827.92	1.9029	0.0009	0.0011	4.0009	0.0007	0.0010	0.0052

$^{a, b}$ Means in a column with no common superscript differ significantly ($P<0.05$).
[1] Error mean square.

egg industry and give suggestions for developing new egg products for target preparations.

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PAPER

INFLUENCE OF STORAGE CONDITIONS AND AGE AT SLAUGHTER ON LIPID OXIDATION AND FATTY ACID PROFILES OF APULIAN LAMB

INFLUENZA DELLE CONDIZIONI DI CONSERVAZIONE
E DELL'ETÀ ALLA MATTAZIONE SULL'OSSIDAZIONE LIPIDICA
E SUL PROFILO ACIDICO DELLA CARNE DI AGNELLI GENTILE DI PUGLIA

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ABSTRACT

Legs from 20 Apulian ram lambs were used to study the effect of storage time (6 and 8 months), storage temperature (-20° and -40°C) and age at slaughter (45 and 90 days) on fatty acid composition and lipid oxidation. These latter parameters were not markedly influenced by temperature and time of frozen storage. Saturated fatty acid content of meat was higher in animals slaughtered at 90 days ($P<0.05$), whereas unsaturated fatty acids were higher in 45-day-old animals ($P<0.05$). Meat from lambs slaughtered at 90 days of age tended to have a lower TBA value

RIASSUNTO

Il coscio di 20 agnelli di razza Gentile di Puglia è stato utilizzato per valutare l'effetto del tempo di conservazione (6 e 8 mesi), della temperatura di congelamento (-20° e -40°C) sulla composizione in acidi grassi della carne e l'ossidazione lipidica. Temperatura e tempo di congelamento hanno indotto modifiche di ridotta entità sul profilo acidico della carne e lo stato ossidativo dei grassi. Viceversa, il contenuto in acidi grassi saturi è stato più elevato nei soggetti mattati a 90 giorni ($P<0,05$), mentre è stato evidenziato un grado di insaturazione inferiore nella carne degli agnelli

- Key words: fatty acids; frozen storage; lamb; lipid oxidation; slaughter age; temperature -

(P<0.10), whereas younger lambs had a more appropriate fatty acid profile in relation to health. Meat from both age groups was suitable for long term frozen storage, even at a temperature close to that commonly used for domestic storage (-20°C).

di 45 giorni (P<0,05). Il contenuto in acido tiobarbiturico è stato solo marginalmente influenzato dall'età alla mattazione, evidenziando valori tendenzialmente più bassi nella carne dei soggetti mattati ad un'età maggiore (P<0,10). In conclusione, la carne degli agnelli mattati a 45 giorni di età è risultata migliore per quanto attiene alle caratteristiche nutrizionali, mentre entrambi i prodotti hanno mostrato un'ottima attitudine alla conservazione e al congelamento, anche ad una temperatura prossima a quella comunemente utilizzata dai consumatori (-20°C).

INTRODUCTION

Lipid oxidation during refrigerated or frozen storage is one of the major causes of quality deterioration in raw and cooked meat products. Flavour, colour, taste, texture and nutritional value (essential fatty acids and fat soluble vitamins), in particular, can be altered (JEREMIAH et al., 1990; CARLUCCI et al., 1999; LYNCH et al., 1999). Furthermore, the safety of the meat can be reduced (PEARSON et al., 1983).

Oxidation of muscle lipids results in production of primary and secondary products (hydroperoxides, free radicals, endoperoxides, malondialdehyde, epoxides, alkanes, hydrocarbons, alcohol and acids) potentially toxic to human (LADIKOS and LONGOVOIS, 1990).

The susceptibility of muscle lipids to oxidation depends on many different factors, some of them being intrinsic to the product, such as species (IGENE et al., 1979), breed (BOYLSTON et al., 1996), age, sex, diet (MONAHAN et al., 1994), type of muscle (AHN et al., 1998), proportion and degree of unsaturation (MORRISSEY et al., 1998) and free ionic iron content (KANNER et al., 1988). Others are related to technological processes, such as temperature and time of frozen storage (IGENE et al., 1979; PIKUL et al., 1985), grinding (MCNEILL et al., 1988), cooking (SALIH et al., 1989; RAMADAN, 1986; RODRIGUEZ-ESTRADA et al., 1997) and packaging (BREWER and WU, 1993), etc.

The temperature of -18°C, usually used for domestic frozen storage, even if it inhibits enzyme activity, is incapable of reducing significantly non-enzymatic chemical reactions (PEREZ VILLAREAL and HOWGATE, 1991). As a consequence, alteration of the lipid fraction of meat may continue, albeit slowly.

In Italy the current guideline concerning deep-frozen food (D.L., 1992) calls for a storage temperature of at least -18°C, but there is no restriction on storage time, leaving a wide range of discretion to the processor. Therefore, in this study, as well as the recommended value of -20°C, a temperature of -40°C, which is supposed to stop non enzymatic reactions, was also investigated. In addition, the safety of the meat was evaluated in relation to length of time of frozen storage (6 and 8 months).

The effect of age at slaughter on lipid oxidation and the fatty acid profile was also assessed on 45 and 90-day-old

lambs, since in Italy the demand for ovine meat is oriented towards young lambs: 30 to 45-day-old lambs meet about 65% of the demand, whereas heavier animals (50-90 days of age) cover another 25%.

Apulian lambs, Merinos derived indigenous animals, well adapted to the environment of southern Italy, were used. This breed can be conveniently reared in extensive systems, thus coinciding with recent European Union policies designed to de-intensify animal production and to allow sustainable development in the Mediterranean areas.

MATERIALS AND METHODS

Animals and diet

Twenty single spring born Apulian ram lambs were allocated to two slaughter times (45 vs. 90 days of age); they had the same body weight. Their mothers grazed on natural pastures located in Irsina (Matera), southern Italy. The lambs were reared naturally and remained with their mothers for the entire experiment but they were not allowed to graze. They received ad libitum alfalfa hay and commercial pellets for growing lambs. Final live weights (14.5±0.7 and 20.2±0.7, respectively, for the two age groups) were recorded immediately before slaughter.

Slaughter procedures

Lambs were stunned and exanguinated under simulated commercial conditions. Carcasses were chilled at 4°C for 48 h, weighed and subsequently dissected into commercial cuts. The legs from the right carcass sides of 10 lambs (5 slaughtered at 45 days of age and 5 slaughtered at 90 days of age) were frozen and stored at -20°C, while the legs from the left carcass sides were frozen and stored at -40°C. All cuts were stored frozen for 6 months prior to composition (percentage lean, fat and bone) and fatty acid determination. Carcasses from another 10 lambs (5 slaughtered at 45 days of age and 5 slaughtered at 90 days of age) were stored at the same temperatures (-20° and -40°C), for 8 months.

All legs were dissected into muscle, fat and bone. Muscle and adipose tissues, including subcutaneous fat, were blended together in a small food processor since Italian consumers usually perform no tissue separation before cooking.

Transesterification and fatty acid composition

Lipids were extracted according to procedures described by FOLCH et al. (1957), modified by MICHAELSEN et al. (1991). Briefly, a 5 g homogenized meat sample was blended with an extraction solvent (chloroform/methanol; 2:1, v/v) twice, filtered, placed in separator funnels and mixed with saline solution (0.88% KCl). After separating into two phases, the aqueous methanol fraction was discarded, while the lipid chloroform fraction was washed with a distilled water/methanol mixture (1:1, v/v). After further filtration and evaporation using a rotary evaporator, lipid extracts were transferred to test tubes for subsequent gas chromatographic analysis. Duplicates of 10 mL of the chloroform extract, corresponding to 100 mg of lipid, were methylated by adding 1 mL of hexane and 0.05 mL of 2N methanolic KOH (I.U.P.A.C., 1987).

Gas chromatographic analysis was performed on a Varian model Star 3400 CX instrument (Varian Medical Systems, Inc., Palo Alto, U.S.A.) equipped with a DB-23 capillary column (60 m x 0.25 mm ID, film thickness 0.25 μm). Operating conditions included: a helium flow rate of 0.9 mL/min, a FID detector set at 260°C, a split-splitless injector set at 220°C with an injection rate of 120 mL/min, an injection volume of 1 μL. The temperature programme of the column was: 4 min

at 140°C with a subsequent increase to 220°C at 4°C/min. Retention time and area of each peak were computed using Varian Star 3.4.1. software. The individual fatty acid peaks were identified by comparing retention times with those of known mixtures of standard fatty acids (FAME, Sigma Chemical, St. Louis, Mo, U.S.A) run under the same operating conditions. Fatty acids are expressed as percent of total methylated fatty acids.

Thiobarbituric acid test

Malondialdehyde (MDA) formation, as measured by 2-thiobarbituric acid (TBA), has been widely used for assessing the extent of oxidative deterioration of lipids in muscle foods (GRAY, 1978; MELTON, 1983). The TBA test was performed as described by SALIH et al. (1987), except that 5% (w/v) aqueous trichloracetic acid was used for solvent extraction on all samples. Lipid oxidation is expressed as mg MDA/kg meat.

Statistical analysis

Data were analysed by ANOVA using the GLM procedure (SAS, 1985). The main effects were age at slaughter, frozen storage temperature and time and their interactions. Where significant effects were found, the Student's t-test was used to locate differences between means.

For each age group coefficients of correlation of TBA with the major fatty acid classes were calculated. The closeness of linear relationships between variables was evaluated using the r coefficient of Pearson (SAS, 1985).

RESULTS AND DISCUSSION

As observed by BRAGHIERI et al., (1993), the leg composition of younger Apulian lambs was characterized by the lowest percentage of fat ($P<0.05$) and the highest percentage of bone ($P<0.05$), but significant differences were not observed between age groups for leanness (Table 1).

In the present study frozen storage conditions (time and temperature) and their interactions did not produce marked changes in the fatty acid profile. Only the amounts of the heptadecenoic (C17:0) and 9-heptadecenoic (C17:1) acids were affected by storage temperature. Longer frozen storage (8 vs. 6 months) resulted in a reduction of monounsaturated 9-heptadecenoic acid ($P<0.001$) and polyunsaturated isomers of linoleic acid ($P<0.05$), while the level of saturated eicosanoic acid was higher ($P<0.01$). MITEVA and BAKALIVANOVA (1987) observed that the contents of unsaturated fatty acids decreased as the length of frozen storage increased in chicken meat. Such reduction was particularly evident for linolenic and linoleic acids.

Lipid stability during frozen storage is influenced by fatty acid composition. A reduction in the unsaturated fatty acid content during frozen storage may

Table 1 - Carcass traits and leg composition (means±s.e.) of 45 and 90-day-old lambs.

	Age (days)		Significance
	45	90	
Live pre-slaughter weight (kg)	14.5±0.7	20.2±0.7	P<0.001
Carcass weight (kg)	9.3±0.4	12.4±0.4	P<0.001
Leg lean (%)	61.8±0.5	60.7±0.5	N.S.
Leg fat (%)	13.6±0.6	15.6±0.6	P<0.05
Leg bone (%)	23.5±0.4	22.2±0.4	P<0.05

be attributed to either autoxidation or β-oxidation involving tissue fatty acids. Both processes are particularly evident in poultry meat due to its comparatively high content of unsaturated fatty acids (64% vs. 47% in chicken and lamb, respectively) (MITEVA and BAKALIVANOVA, 1987).

Oxidative rancidity and autoxidation can be empirically measured using the reaction of MDA with TBA. MDA (a secondary product of lipid oxidation and prostaglandin biosynthesis) have carcinogenic (BASU and MARNETT, 1983; BOYD and MCGUIRE, 1991) and mutagenic effects on bacterial and mammalian cells, inducing both base-pair substitutions and frameshift mutations (BENAMIRA et al., 1995). The toxicity of MDA to living systems is attributed mainly to its ability to alter or cross-link with a variety of biomolecules such as proteins and enzymes (DRAPER et al., 1986). In the present experiment all products had TBA values well below threshold levels for rancidity (1-2 mg of MDA/kg of meat). However, in meat stored at -40°C, TBA values tended to be lower than in meat stored at -20°C (0.12±0.04 vs. 0.18±0.04, $P<0.20$). No effects of storage time and interactions (time x temperature, time x age at slaughter, temperature x age at slaughter and time x temperature x age at slaughter) on TBA values were observed. Failure of the samples to develop rancidity even at a higher temperature (-20°C) and for a longer time (8 months) may be due to the high fat stability as a result of a low number of double bonds. In fact, previous research on chicken meat, which is characterized by a high level of unsaturation (PIKUL et al., 1985; IGENE et al., 1979; JANTAWAT and DAWSON, 1977), indicated that different frozen storage conditions may result in increased TBA values. Meat from ruminants is known to have a high level of fat saturation. However, KOWALE et al. (1996) observed higher TBA values in mutton as a consequence of frozen storage at a higher temperature (-10°C). In addition, the meat was minced, thus presenting a much larger surface area exposed to oxidation. Despite the higher TBA values the mutton samples were not rancid. WINGER (1984) also observed that lamb meat stored at -10°C for 2 years was devoid of rancid flavours.

The total amount of saturated, unsaturated and monounsaturated fatty acids was significantly affected by age at slaughter ($P<0.05$; Table 2). In particular, results showed lower unsaturation of meat from older animals. Similar findings were also reported by PANELLA et al. (1995) in Merino suckling lambs compared with weaned lambs (12.3 and 18.6 kg of live weight, respectively). Conversely, BANSKALIEVA (1996) observed that increasing age at slaughter resulted in a slightly higher unsaturation of depot fats, which may be attributed to differences in age at slaughter and diet. KEMP et al. (1981) reported that both diet and age at slaughter significantly affected the ratio of saturated to unsaturated fatty acids in depot fats of weaned lambs.

In young unweaned ruminants, the rumen is only partially functional and dietary unsaturated fatty acids are likely to be digested and adsorbed as in non-ruminants. Older lambs are more adapted to a dry diet which requires a completely functional rumen (SAÑUDO et al., 1998). Consequently, a higher percentage of fatty acids in the meat of lambs slaughtered at 45 days of age would be expected to be unsaturated, while fatty acids in meat from 90-day-old lambs would be expected to be more saturated as a consequence of the hydrogenating action of rumen bacteria (WOOD and ENSER, 1997). A change in the fatty acid profile has also been reported to be associated with increasing levels of fatness (CASEY et al., 1988).

Different ages at slaughter (45 vs. 90 days) resulted in an increase in saturated palmitic (C16:0) and heptadecenoic (C17:0) fatty acid contents ($P<0.01$ and $P<0.001$, respectively) and a decrease

Table 2 - Fatty acid composition (%) of 45 and 90-day-old lambs (means±s.e.).

	Age (days)		Significance
	45	90	
C10:0 caprinic	0.69±0.03	0.56±0.03	P<0.01
C12:0 lauric	1.66±0.07	1.50±0.07	N.S.
C14:0 myristic	9.44±0.27	9.74±0.27	N.S.
C14:1 myristoleic	0.43±0.02	0.38±0.02	N.S.
C15:0 pentadecanoic	0.85±0.03	0.91±0.03	N.S.
C16:0 palmitic	23.42±0.30	24.93±0.30	P<0.01
C16:1ω9 palmitoleic	1.96±0.04	2.15±0.04	P<0.01
C17:0 heptadecenoic	0.84±0.02	0.95±0.02	P<0.001
C17:1 9-heptadecenoic	0.71±0.02	0.59±0.02	P<0.001
C18:0 stearic	11.74±0.25	12.08±0.25	N.S.
C18:1ω9 oleic	36.12±0.37	35.44±0.37	N.S.
C18:2ω6 linoleic	3.57±0.21	3.19±0.21	N.S.
C18:2 linoleic acids isomers	2.47±0.11	2.66±0.11	N.S.
C18:3ω3 linolenic	1.16±0.07	0.90±0.07	P<0.05
C20:0 arachidic	2.73±0.10	2.60±0.10	N.S.
C20:1ω9 9-icosenic	0.27±0.03	0.18±0.03	P<0.05
C22:0 behenic	0.77±0.12	0.47±0.12	N.S.
C22:6ω3 docosehexanoic	0.51±0.07	0.30±0.07	P<0.05
C20:4ω6 arachidonic	0.58±0.07	0.36±0.07	P<0.05
Saturated	52.16±0.48	53.74±0.48	P<0.05
Monounsaturated	40.07±0.32	39.14±0.32	P<0.05
Polyunsaturated	7.78±0.38	7.11±0.38	N.S.
P/S	0.15±0.01	0.13±0.01	P<0.20

of 9-heptadecenoic (C17:1), 9-icosenic (C20:1ω9) and docosehexanoic (C22:6ω3) fatty acid percentages (P<0.001, P<0.05 and P<0.05, respectively). Monounsaturated myristoleic (C14:1 P<0.10) and oleic (C18:1 P<0.20) acid contents tended to be less abundant in 90-day-old animals, but palmitoleic (C16:1ω9) acid was higher in older lambs (P<0.01). Meat from older lambs had lower linolenic (C18:3 P<0.01) and arachidonic (C20:4 P<0.05) fatty acids and a tendency toward lower levels of linoleic (C18:2 P<0.20) fatty acid and its isomers (P<0.20).

In agreement with SARTI et al. (1993) and STOKES and WALKER (1970), meat from younger lambs had a greater amount (P<0.01) of saturated short-chain, caprinic acid (C10:0), while the content of lauric acid (C12:0) tended to be more abundant (P<0.20), which may be attributed to differences in feeding regimen. Younger lambs were subjected to a diet based on maternal milk with a higher content of caprinic and lauric fatty acids.

At an older slaughter age, the TBA value of meat tended to be lower (0.11±0.04 vs. 0.18±0.04, P<0.10). Susceptibility to oxidation increases with the level of unsaturation, as stated previously. Therefore, the slightly lower susceptibility to oxidative changes observed for meat from older lambs may be due to the lower unsaturated fatty acid content.

TBA values tended to be positively correlated with the percentage of total unsaturated fatty acids only for 90-day-old lambs (r = 0.32; P<0.15). In both age groups no other significant correlation

between fatty acid classes and TBA was found. As expected, TBA values tended to be lower in meat from older lambs ($P<0.10$) as did the contents of linolenic and arachidonic acids. TBA values were not correlated with the content of total lipids. Similar results were reported by RHEE et al. (1988).

CONCLUSIONS

Apulian lamb fatty acid composition and susceptibility to oxidation were not influenced by storage temperature (-20° or -40°C) or time of frozen storage (6 or 8 months). This is due to the low content of unsaturated fatty acids, suggesting that Apulian lamb is suitable for long term frozen storage, even at the temperature commonly used for domestic storage (-18°C). A positive correlation was observed between TBA values and lipid unsaturation, confirming that fat stability is more affected by lipid composition than frozen storage conditions.

Lambs slaughtered at 45 days of age were characterized by a more appropriate fatty acid profile with regard to their contribution to health and nutrition. The meat from older lambs tended to be more suitable for frozen storage.

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PAPER

EFFECT OF MICROWAVE HEATING AND GAMMA IRRADIATION ON MICROBIOLOGICAL QUALITY OF SPICES AND HERBS

EFFETTO DELLE MICROONDE E DELLE RADIAZIONI IONIZZANTI SULLA QUALITÀ MICROBIOLOGICA DI SPEZIE ED ERBE AROMATICHE

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ABSTRACT

The microbiological quality of selected spices and herbs was evaluated before and after decontamination with microwave heating (100°C for 15 min) and ionizing radiation (absorbed doses of 5 and 10 kGy). Black pepper, red chili, oregano, rosemary and sage were tested. The microbiological quality of the untreated products was found to be poor, with high numbers of spore-forming aerobes and molds and frequent contamination with faecal indicators. Treatment with microwaves and irradiation gave results which varied according to the type of microorganism

RIASSUNTO

È stata valutata la qualità microbiologica di alcune spezie ed erbe aromatiche sia allo stato naturale che dopo trattamento di decontaminazione con microonde (100°C per 15 min) e radiazioni ionizzanti (dose assorbita: 5 e 10 kGy). I prodotti sottoposti a controllo sono stati: pepe nero, peperoncino, origano, rosmarino e salvia. La qualità microbiologica dei prodotti non trattati è risultata scadente, caratterizzata da elevate concentrazioni di sporigeni aerobi ed ifomiceti e da una diffusa contaminazione da indicatori fecali. I trattamenti con microonde e con radia-

- Key words: gamma irradiation, herbs, microwave decontamination, spices -

and, to a lesser extent, the particular product. Microwave heating had little effect on the most resistant forms of bacteria. After such treatment, the total viable count at 32°C was even higher in a large percentage of the samples. This could be due to the high concentration of spore-forming bacteria that were isolated in higher concentrations in the microwave-treated products than in the untreated ones. Microwave heating was more effective on the molds and faecal indicators, which, after treatment, were within the limits set by the International Commission on Microbiological Specifications for Foods (E. coli < 10^3 cfu/g). However, it was possible to reduce bacterial counts and molds to negligible levels and to eliminate the faecal indicators completely only by means of irradiation at 5 and 10 kGy.

zioni ionizzanti hanno evidenziato una diversa efficacia in rapporto al tipo di microrganismo e, in misura minore, al tipo di prodotto trattato. Le microonde hanno presentato scarsa azione nei confronti delle forme microbiche più resistenti: la carica mesofila a 32°C, dopo questo trattamento, è risultata ancora elevata in un'alta percentuale di campioni. Ciò può essere spiegato con l'elevata concentrazione di sporigeni che sono stati messi in evidenza a concentrazioni addirittura superiori nei prodotti trattati con microonde rispetto ai non trattati. Più significativa è stata l'efficacia delle microonde nei confronti delle muffe e degli indicatori fecali che sono rientrati dopo questo trattamento nei limiti posti dall'ICMSF (E. coli < 10^3 ufc/g). Soltanto con le radiazioni ionizzanti a 5 e 10 kGy è stato tuttavia possibile ridurre le cariche batteriche ed ifomicetiche a valori trascurabili e ad eliminare completamente gli indicatori fecali.

INTRODUCTION

Spices and herbs used to add aroma to foods may also help preserve them. The latter property may be due to the antioxidant characteristics of different substances present in them (e.g. phenolics, polyphenolics, oleoresins). They may also exert a bactericidal action, for example, against Campylobacter jejuni and Listeria monocytogenes (SHELEF et al., 1980; PALEARI et al., 1989; HUDSON, 1990; HEFNAWY et al., 1993). Despite these useful properties, spices and herbs can also be a source of microbial contamination. Many studies have demonstrated the presence of high numbers of spore-forming bacteria, such as Bacillus cereus (CANTONI and CASERIO, 1966; FRAZIER and WESTHOFF, 1978; KOVACS-DOMJAN, 1988). The high bacterial counts are due to the technology used to produce certain spices. Foremost among these is black pepper which is subject to fermentation, favouring the growth of Bacillus species. The presence of other bacteria such as Escherichia coli, enterococci and Salmonella might be due to poor sanitary conditions during harvesting and processing, which is not in accordance with Good Manufacturing Practices – GMP (BAXTER and HOLZAPFEL, 1982; LOCATELLI et al., 1988; LEGNANI et al., 1996).

The presence of bacteria in spices and herbs is a potential source of risk for the health of consumers. GOEPFERT et al. (1972) pointed out that the high incidence of poisoning from Bacillus cereus in Hungary was due to consumption of meat dishes seasoned with spices. In 1974, Salmonella Weltevreden, carried

by black and white pepper, caused a number of cases of poisoning in Canada (LAIDLY et al., 1974; SEVERS, 1974). In Norway between 1981 and 1982, there were a total of 126 cases of Salmonella Oranienburg infection traced to pepper added to seasoned meat sausages. Norwegian authorities traced the cause of this outbreak to two separate supplies of black pepper from Brazil, but did not specify how the contamination had taken place (GUSTAVSEN and BREEN, 1984). LEHMACHER et al. (1995) described an outbreak of salmonellosis in Germany that involved an estimated 1000 children under 14 years of age. This was traced to eating snacks seasoned with paprika contaminated by Salmonella Saintpaul, S. Rubislaw and S. Javiana.

In recent years various techniques have been used to decontaminate spices and herbs, including heated water vapor, ethylene oxide, ultrasound, ultraviolet radiation and ionizing radiation. Most experts consider ionizing radiation, in permitted doses, to be an effective means to reduce bacteria in these products, without causing any appreciable change in the color or aroma (MUNASIRI et al., 1987; NARVAIZ et al., 1989; EL-ZAWAHRY et al., 1991; ANDREWS et al., 1995; EMAN and FARAG, 1995; FARAG-ZAIED et al., 1996). A critical review of the large number of studies designed to assess the safety and nutritional quality of food processed by ionizing radiation has been published by WHO (1994; 1999). In Italy the Ministry of Health (DM, 1996) allows spices and herbs to be treated with ionizing radiation at a maximum absorbed dose of 10 kGy. However, this type of treatment, which can be carried out only in specialized centers, is expensive and difficult to perform and furthermore gives rise to concern among consumers. Another method of decontamination is microwave heating, which has been tested on various types of food, including meat, fish, milk and eggs (ROSENBERG and BOGL, 1987) as well as on spices and herbs (EMAN and FARAG, 1995; FINOT, 1995; FARAG-ZAIED et al., 1996). Microwave treatment is easier and less expensive than gamma irradiation, but may have little effect on the total viable count of spices and herbs. This is because heat production due to microwaves, caused by friction between water molecules, is reduced in commodities with low water content.

The aim of this study was to determine the microbiological quality of selected spices and herbs packaged under hygienic conditions before and after microwave heating and irradiation.

MATERIALS AND METHODS

Samples

Microbiological analysis was carried out on dried spices and herbs collected directly from the storage warehouses of a major Italian company that imports and sells these products throughout Italy. The products tested were the following: black pepper (Piper nigrum) (India), red chili (Capsicum annuum) (India), oregano (Origanum volgare) (Turkey), rosemary (Rosmarinus officinalis) (Tunisia), sage (Salvia officinalis) (Egypt). Various amounts of spices and herbs were taken from different sacks, using a sterile stainless steel sampler. They were placed in sterile glass containers and samples weighing 100 g were taken for pre- and post-treatment microbiological analyses.

Decontamination treatments

Microwave treatment was performed in a continuous plant consisting of a 2.450 Mhz wave oscillator (Daewoo KOG-3605, max 800 W – Daewoo Electronics Italia SpA, Segrate, Italy) which heated the spices and herbs to 100°C for 15 min. Irradiation was performed on samples (100 g) packed in polyethylene bags, us-

ing ^{60}Co as the radioactive source. The gamma source was located at Gamma Rad (Bologna, Italy) and gave a dose rate of 0.2 kGy/min as determined by red perspex dosimetry (Dosimeter Harwell, Swindown, UK). The absorbed doses were 5 and 10 kGy, in compliance with the Italian standards.

Microbiological analysis

Four series of samples of spices and herbs were analysed as follows: (1) 151 untreated samples (oregano, 34 samples; black pepper, 33; red chili, rosemary and sage, 28 each). (2) 94 samples treated with microwave heating (oregano, 21 samples; sage, 19; black pepper, red chili and rosemary, 18 each). (3) 25 samples (5 per product) treated with ionizing radiation at absorbed doses of 5 kGy, and (4) 25 samples (5 per product) treated with ionizing radiation at absorbed doses of 10 kGy.

The techniques used for sampling, sample preparation and culturing were those proposed by the Technical Committee on Microbiological Methods for Foods (APHA, 1992). The microbiological parameters measured were: total viable count (Plate Count Agar, Oxoid, at 32°C for 48 h); mesophilic spore-forming aerobes heating the sample at 80°C for 30 min (Tryptone Soya Agar, Oxoid, at 32°C for 48 h); faecal coliforms and Escherichia coli (Violet Red Bile Agar + MUG, Oxoid, at 44°C for 24 h); enterococci (KF Streptococcus Agar, Difco, at 36°C for 48 h) and mold count (Rose Bengal Chloramphenicol Agar, Oxoid, at 22°C for 5 days). Testing for Salmonella was carried out using the technique recommended by VASSILIADIS et al. (1976): a) pre-enrichment in Buffered Peptone Water at 36°C for 24 h; b) selective enrichment in Rappaport-Vassiliadis Enrichment Broth (Oxoid) at 44°C for 24 h; c) isolation in Brilliant Green Agar Selective Medium (Oxoid) at 36°C for 24 h.

Confirmation of E. coli was made with the test for indole; confirmation of enterococci was made by testing the catalase activity and growth in Bile Esculin Azide Agar (Difco). Suspected colonies of Salmonella grown on Brilliant Green Agar were identified using the API 20E biochemical system (Biomerieux). Mold colonies were identified by standard mycological techniques based on macroscopic and microscopic morphology. The taxonomic keys of SAMSON and VAN REENEN-HOEKSTRA (1988) were used.

Statistical analysis

Data processing was carried out using the analysis of variance (F-test, ANOVA) (DAWSON-SAUNDERS and TRAPP, 1994), after conversion of the absolute values into $\log_{10}(x+1)$.

RESULTS

Tables 1 and 2 show the percentages of positive samples and the mean log counts of the various types of microorganisms in the spices and herbs before and after the decontamination treatments. These values clearly demonstrate the high number of microbes present in the untreated spices and herbs usually on sale and/or used by food companies. The samples with the highest numbers of bacteria were black pepper and red chili. Compared to these spices the herbs (oregano, rosemary and sage) were less contaminated by bacteria but more contaminated by molds (Table 1). 73.8% of the spices and 68.9% of the herbs were contaminated by E. coli with counts (geometric means) ranging from 2.4 cfu/g (black pepper) to 11.0 cfu/g (red chili) (Table 2). Despite this widespread contamination, samples positive for E. coli complied with the guidelines proposed for dried vegetables by the International Commission on Microbiological Specifi-

Table 1 - Effect of microwave heating and irradiation on the level of contamination of the spices and herbs with heterotrophic bacteria, spore-forming aerobes and molds.

Spices and herbs	Total viable counts			Spore-forming aerobes			Molds		
	% of positive samples*	means (log cfu/g)	SD (log cfu/g)	% of positive samples*	means (log cfu/g)	SD (log cfu/g)	% of positive samples*	means (log cfu/g)	SD (log cfu/g)
BLACK PEPPER									
Untreated	**100**	6.83	0.46	**100**	6.51	0.82	**90.9**	2.92	1.24
Microwave Treated	**100**	6.71	0.37	**100**	6.80	0.16	**100**	1.02	0.62
Irradiated - 5kGy	**60**	1.07	0.98	**40**	0.95	1.10	**20**	0.31	0.63
Irradiated - 10kGy	**0**	0	0	**0**	0	0	**0**	0	0
RED CHILI									
Untreated	**100**	5.99	0.61	**100**	5.83	1.15	**96.4**	3.97	1.08
Microwave Treated	**100**	4.72	1.19	**100**	6.84	0.27	**100**	1.22	0.74
Irradiated - 5kGy	**20**	0.26	0.52	**80**	1.08	0.28	**80**	1.42	0.11
Irradiated - 10kGy	**0**	0	0	**40**	0.77	0.91	**20**	0	0
OREGANO									
Untreated	**100**	5.43	0.43	**100**	2.48	0.93	**100**	4.72	0.53
Microwave Treated	**100**	4.20	0.64	**100**	3.04	0.46	**100**	0.95	0.21
Irradiated - 5kGy	**40**	0.45	0.54	**0**	0	0	**80**	0.78	0.16
Irradiated - 10kGy	**0**	0	0	**0**	0	0	**0**	0	0
ROSEMARY									
Untreated	**100**	4.76	0.66	**100**	2.72	0.78	**100**	4.72	0.67
Microwave Treated	**100**	3.20	0.50	**100**	3.25	0.21	**77.8**	0.94	0.77
Irradiated - 5kGy	**40**	0.82	0.95	**100**	1.54	0.58	**40**	0.80	0.48
Irradiated - 10kGy	**40**	0.63	0.75	**80**	1.37	0.34	**40**	0.46	0.12
SAGE									
Untreated	**100**	5.53	0.40	**100**	3.40	0.36	**100**	4.79	0.19
Microwave Treated	**100**	4.05	0.45	**100**	3.75	0.26	**100**	1.03	0.51
Irradiated - 5kGy	**20**	0.12	0.24	**60**	1.14	0.45	**20**	0.33	0.29
Irradiated - 10kGy	**40**	0.63	0.75	**20**	0.26	0.52	**0**	0	0

* limit of detection: 1 cfu/g.

cations for Foods (ICMSF, 1986). Only two samples of red chili (7.1%) exceeded the maximum ICMSF level of 10^3 cfu/g and none of the samples were found to be contaminated by Salmonella. Enterococci, which are not included among the ICMSF microbial indicators, were isolated from 83.4% of the untreated samples at counts varying from 6.4 cfu/g for rosemary to 144 cfu/g for red chili.

After microwave treatment, the samples of spices and herbs all fell within the ICMSF limits for E. coli, which was isolated from 44.7% of the treated samples at counts varying from 1 to 9 cfu/g (geometric mean: 2.6 cfu/g). Enterococci, however, were found in 85.1% of the microwave treated samples with counts ranging from 1 to 320 cfu/g (geometric mean: 13.5 cfu/g). It can be seen from Table 2 that enterococci were isolated more frequently and at higher concentrations than E. coli in all types of products whether untreated or treated with microwave heating.

Microwave treatment was less effective

Table 2 - Effect of microwave heating and irradiation on the level of contamination of the spices and herbs with faecal coliforms, Escherichia coli and enterococci.

Spices and herbs	Faecal coliforms			*Escherichia coli*			Enterococci		
	% of positive samples*	means (log cfu/g)	SD (log cfu/g)	% of positive samples*	means (log cfu/g)	SD (log cfu/g)	% of positive samples*	means (log cfu/g)	SD (log cfu/g)
BLACK PEPPER									
Untreated	**90.9**	3.11	1.71	**66.6**	0.38	0.47	**84.8**	1.98	1.49
Microwave Treated	**94.4**	0.66	0.42	**33.3**	0.18	0.29	**100.0**	0.91	0.74
RED CHILI									
Untreated	**72.7**	4.14	2.06	**82.1**	1.04	1.04	**82.1**	2.16	1.84
Microwave Treated	**94.4**	0.59	0.21	**66.6**	0.36	0.30	**94.4**	0.78	0.23
OREGANO									
Untreated	**88.2**	4.35	1.49	**61.8**	0.41	0.29	**76.4**	1.53	1.17
Microwave Treated	**71.4**	0.55	0.24	**33.3**	0.18	0.22	**71.4**	0.57	0.27
ROSEMARY									
Untreated	**89.3**	3.73	0.92	**65.5**	0.41	0.29	**89.3**	0.81	0.26
Microwave Treated	**72.2**	0.75	0.72	**44.4**	0.22	0.21	**84.2**	0.65	0.18
SAGE									
Untreated	**92.8**	4.59	0.93	**82.1**	1.01	0.90	**85.7**	1.50	1.26
Microwave Treated	**78.9**	0.87	0.83	**47.4**	0.20	0.23	**77.7**	0.82	0.63

* limit of detection: 1 cfu/g.

as far as the various indices examined are concerned. The samples treated with microwave heating showed, on average, a high reduction in the counts of faecal coliforms (from 78.8% in black pepper to 87.4% in oregano), of molds (from 65.1% in black pepper to 80.1% in rosemary) and of E. coli (from 52.6% in black pepper to 80.2% in sage) and a more limited reduction of enterococci (from 19.8% in rosemary to 63.9% in red chili) and of the total viable count (from 1.8% in black pepper to 32.8% in rosemary). The small effect on the total viable count is probably due to the fact that it includes spore-forming bacteria which to some extent are resistant to microwave treatment. This is supported by the high counts of sporeformers found in the treated samples, at levels that were on average higher than those found in the untreated samples (statistically significant differences – $p<0.001$). It could be hypothesized that exposure to 100°C for 15 minutes caused a thermal shock which favored the germination of spores and thus an increase in the counts of these microorganisms. Irradiation, even at the lower dose (5 kGy), showed a much greater efficiency than microwave heating, completely eliminating the faecal coliforms, E. coli and enterococci. Only in the case of molds were there no significant differences between the two types of treatment; differences were, however, seen for the total viable counts and the sporeformers ($p<0.001$). No significant differences in efficiency were observed between the two doses of irradiation.

The prevalent species of molds isolated in the untreated samples was Aspergillus niger (Table 3). Black pepper had the highest number of mold species, 9 out of the 12 identified overall. The species

Table 3 - Fungal isolates from untreated and treated spice and herb samples.

Molds		Spices			Herbs		
		Untreated	Microwave Treated	Irradiated 5kGy	Untreated	Microwave Treated	Irradiated 5kGy
Alternaria alternata	No of isolates identified	0	0	0	30	0	0
	% of positive samples	**0**	**0**	**0**	**15.5**	**0**	**0**
Aspergillus glaucus	No of isolates identified	31	12	0	9	0	0
	% of positive samples	**11.5**	**11.1**	**0**	**3.3**	**0**	**0**
Aspergillus niger	No of isolates identified	221	66	4	279	48	9
	% of positive samples	**82.1**	**55.5**	**10.0**	**90.0**	**48.3**	**13.3**
Aspergillus tamari	No of isolates identified	0	0	0	15	0	0
	% of positive samples	**0**	**0**	**0**	**3.3**	**0**	**0**
Aspergillus terreus	No of isolates identified	25	8	0	0	0	0
	% of positive samples	**18.0**	**8.3**	**0**	**0**	**0**	**0**
Aspergillus ustus	No of isolates identified	16	0	0	0	0	0
	% of positive samples	**11.5**	**0**	**0**	**0**	**0**	**0**
Aureobasidium pullulans	No of isolates identified	8	0	0	0	0	0
	% of positive samples	**4.9**	**0**	**0**	**0**	**0**	**0**
Cladosporium spp.	No of isolates identified	7	0	0	4	0	3
	% of positive samples	**4.9**	**0**	**0**	**3.3**	**0**	**13.3**
Epicoccum nigrum	No of isolates identified	0	0	0	10	0	0
	% of positive samples	**0**	**0**	**0**	**3.3**	**0**	**0**
Paecilomyces niveus	No of isolates identified	15	0	0	0	0	0
	% of positive samples	**11.5**	**0**	**0**	**0**	**0**	**0**
Penicillium spp.	No of isolates identified	170	150	2	51	48	0
	% of positive samples	**36.1**	**47.2**	**10.0**	**15.5**	**25.9**	**0**
Rhizopus spp.	No of isolates identified	42	13	8	25	23	5
	% of positive samples	**16.4**	**25.0**	**50.0**	**14.4**	**31.0**	**13.3**

remaining after microwave treatment were Aspergillus spp. (46.3% of isolates, of which 26.8% was A. niger), Penicillium spp. (36.6%) and Rhizopus spp. (9.8%). Irradiation, on the other hand, reduced the molds to only a few units.

DISCUSSION

The results of the investigation show that spices and herbs need to undergo decontamination treatment, since in their natural state they contain high levels of heterotrophic bacteria and molds, as well as frequent contamination with faecal indicators. The spices (black pepper and red chili) were found to be more contaminated by bacteria, while the herbs (oregano, rosemary, sage) were more affected by molds. The differences are first of all due to the processing technology applied to the commodities but also to differences in the chemical and physical characteristics of the raw materials. The greater contamination

from molds in the herbs could, for example, depend on their higher a_w, while the lower bacterial contamination could be the result of their antibacterial properties (SHELEF et al., 1980). Poor sanitary practices during harvesting and processing, not in accordance with GMP, may be the cause of the contamination of spices and herbs with E. coli and enterococci. The high levels of enterococci may also be the result of a greater resistance of these microorganisms to the drying process. Since the low water content of the products in question reduces the survival of less resistant bacteria such as E. coli, we believe that the microbiological evaluation of spices and herbs should also include enterococci.

Both techniques used for the decontamination of spices and herbs (microwave heating and irradiation) produced an acceptable level of microbiological safety. Microwave heating was particularly effective against molds and brought about a decrease in the faecal indicators to within safety levels. The only parameter not reduced satisfactorily was the total viable plate count, probably on account of the ineffectiveness of the microwave treatment against spore-forming bacteria. Only irradiation, even at an absorbed dose of 5 kGy, was found to reduce the bacteria and molds to negligible levels and completely eliminate indicators of faecal contamination, including enterococci.

Apart from considerations on the effectiveness of eliminating potentially harmful bacteria, the choice of treatment for decontaminating spices and herbs should also take into account the cost of the treatment and the technical manageability of the decontamination systems. Furthermore, their organoleptic characteristics should be sufficiently maintained for use in the food industry. The organoleptic characteristics are preserved better during irradiation than with microwave treatment due to the sensitivity of many of the essential oils to heat (MUNASIRI et al., 1987; NARVAIZ et al., 1989; ANDREWS et al., 1995; HOWARD et al., 1995; ANTONELLI et al., 1998). Because of the fundamental importance of maintaining the basic quality of the spices and herbs, the decontamination treatments must not be increased in such a way that could impair the aroma of the products, for example by prolonging the time of exposure to microwave heating. Preventative measures should therefore be directed above all to the importers of the raw materials, with a view to improving the microbiological quality of their production and storage in the countries of origin. In addition, food manufacturers should take specific hygienic precautions in their factories, paying particular attention to the overall cleaning of the equipment and warehouses, the creation of suitable microclimatic conditions and disinfecting procedures. If these basic conditions are respected, microwave heating may also guarantee the microbiological safety of spices and therefore be preferred to ionizing radiation, which is a more expensive treatment and furthermore gives rise to concern among consumers.

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FLAIR-FLOW EUROPE



PROTECTION FOR VULNERABLE INTESTINES

FFE 389/01/HP2

Did you know that in the human gut there are more than 10^{14} bacteria, representing more than 400 species? In healthy humans, the endogenous (resident) and exogenous (transient) bacteria are kept in careful balance.

Previous work by participants in this completed FAIR project has examined models of the gut showing the minimal conditions necessary to inhibit the introduction and growth of the potentially pathogenic bacteria Clostridium difficile and Clostridium perfringens. Mechanisms for inhibition of growth of C. perfringens and C. difficile were further explored during this project. The results obtained demonstrated that this inhibition can be due to the production of an antimicrobial substance, called ruminococcin, or competitive inhibition (exclusion of pathogens by normal flora bacteria).

Using the C. difficile gut model, a number of potential factors involved in colonisation were identified. From this, genes were cloned and proteins obtained which could be administered to patients orally to stimulate local immunity in the intestine. This would inhibit the attachment of the pathogenic bacteria to the intestinal epithelial cells and thus prevent pathological progression.

The development of an orally administered vaccine would be useful for patients (especially hospital fed elderly), whose protective gut bacteria had been destroyed, for example, after taking a course of antibiotic treatment. Intestinal infection leading to diarrhoea resulting from C. difficile represents a major public health problem, being responsible for 15-25% of cases of post-antibiotic diarrhoea and at least 95% of cases of pseudomembranous colitis. Since it has been estimated that the cost of each episode of C. difficile associated disease is over 3,000 Euros, there is also an economic burden for European health service providers.

The next stage for the researchers is to collaborate with European industries that are capable of carrying out clinical trials using newly developed vaccines.

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MONITORING THE GOOD GUYS IN THE GUT

FFE 390/01/HP3

Across Europe, people are being inundated with functional food products purported to have health-promoting effects emanating from their effects on large bowel microflora. Amongst other roles, intestinal microflora prevent the build-up of harmful pathogens (such as Campylobacter pylori and Escherichia coli). Conversely, microflora can also have undesirable activities leading to the formation of potentially harmful compounds. Current methods of assessing composition of gut microflora are inadequate and hamper the identification of factors controlling the gut environment, making it difficult to evaluate the effectiveness of such functional food products.

The goal of this 3-year FAIR project was to develop and apply innovative methods for monitoring human gut microflora in different age groups in response to dietary interventions, including determining the efficacy of components of functional foods in reducing bacterial gastroenteritis.

Findings from the project suggest that although some issues need further research, it is now feasible to investigate the effect of functional foods on human gut microflora. It was also observed that great diversity exists in the flora of the adult gut, especially in old age.

Experiments with probiotics (microbial cultures which are purported to balance intestinal flora) and prebiotics (compounds that support the growth of colonies of certain bacteria in the gut) demonstrated that the growth of certain bacteria could be stimulated by using synbiotics (components that include both probiotics and prebiotics), but results were not dramatic.

One major observation was that growth of C. jejuni and E. coli was inhibited by a prebiotic mixture, thus having implications for the development of symptoms of food poisoning.

These new methods offer the opportunity to analyse gut microflora more efficiently and therefore evaluate more effectively the roles of dietary interventions and functional foods.

Project No: FAIR-CT97-3035

(http://www.dife.de/dife/studien/blaut/develop.htm)

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WASTE MANAGEMENT IN OLIVE OIL PRODUCTION

FFE 393/01/CG3

The objective of this finished shared cost project was to create a comprehensive package of treatments for waste from the two-phase olive oil extraction and to disseminate the results by establishing a catalogue and database for processing alternatives and the economic impact.

Mediterranean countries produce 95% of the total world production of olive oil estimated to 2,4 million tons per year, the largest producers being Spain (950,000 tons), Italy (450,000 tons) and Greece (430,000 tons). The environmental impact of the production is considerable because of utilization of some 12 million tons of water and production of 8 million tons of sludge.

Pressing of the olives has been replaced in many modern olive oil plants by decanter centrifuges separating the oil from the solids (alperujo, a by-product similar to sludge we have to dispose of), followed by a physical cleaning of the olive oil by removing fine solids and water.

The project group focused on optimising the oil recovery, minimizing the water consumption and finding the most optimal treatments and handling of the alperujo sludge, considering animal feeding, composting, fluidised bed drying, combustion and other solutions.

Project No: FAIR3-CT96-1420 (IMPROLIVE)

http://www.umc.es/info/improliv

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VACUUM INFUSION: A NEW TECHNOLOGY TO IMPROVE THE TEXTURE OF PROCESSED FRUIT & VEGETABLE

FFE 394/01/SME4

The objective of this FAIR project was to demonstrate the industrial viability of the vacuum infusion technique in the processing of fruits, vegetables, berries, mushrooms, etc.

The concept of the vacuum infusion technique consists in the removal of air from within the vegetables by vacuum treatment for 1-2 min at 0.1-0.2 bar, followed by their infusion (or impregnation) in a solution containing hydrocolloids, calcium or other solutes. This improves the texture, appearance and other quality parameters.

The vegetables tested during the project were (whole or sliced) apples, peaches,

strawberries, pears, mushrooms, cucumbers, pumpkins, etc. The infusion substances were pectin, alginate, gelatine, gums, ascorbic acid, citric and malic acids as well as calcium and sugars. A newly developed mobile pilot plant was used for all experiments in each of the participating countries: France, Sweden, Finland and Portugal.

In particular, the vacuum infusion technology has shown excellent results and quality improvement of thawed or pasteurised berries and fruits for e.g. yoghurt, marmalade and bakery fillings by improvement of firmness and reduction of drip loss.

The technology used as a pre-treatment before freezing or pasteurisation is claimed to improve the sensory characteristics. (Because of the novel products produced by this technology the legal situation of the products must be clarified). As the Vacuum Infusion is considered as a new technology, a legal authorization needs to be further obtained for the commercialization of the products.

Project No: FAIR-CT98-3814.

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DETECTION OF MOULD CONTAMINATION

FFE 396/01/SME6

Fungal contamination of foods and feeds results in considerable economic losses, because of direct damage to crops, discolouration, off-odours, taints and flavours, reduced yields and loss of nutritive value. More importantly, fungal contamination of foods is associated with mycotoxin production, which has toxic effects on ingestion, in both humans and animals. For that reason, development of fast and reliable detection methods is very important in production and processing of foods.

The goal of this finalized project is to develop and validate rapid and convenient broad-specificity assays for the detection of general fungal contamination of cereals.

The scientists developed and validated 5 different methods:

- Broad-spectrum immunoassay using antibodies raised against the soluble fraction of the mycelium from several individual fungi as well as from mixed cultures. Both monoclonal and polyclonal antibodies were produced and evaluated in assays. A monoclonal was selected and fully validated for assay of barley extracts. The assay correlated well with traditional methods.
- Immunoassay for the steroid ergosterol, which is an index of the contamination by fungi. Several antibodies were produced following immunization with a range of ergosterol-protein conjugates and preliminary evidence was obtained that some of these antibodies could be used in an immunoassay.
- Near Infra-Red Spectroscopy (NIR/NIT) to determine the fungal load in cereals by predicting the ergosterol content. Establishing a predictive model, it was shown that NIR/NIT could accurately predict the ergosterol content of contaminated barley.
- Detection of volatiles emanating from mouldy grains, using an "electronic nose".

The scientists found a suitable combination of electronic sensors that could be used for predicting ergosterol content of grains.

- DNA probes for detection of specific fungi were developed and evaluated. To facilitate the routine use, the probes were incorporated into a simple membrane-based detection system involving reverse hybridisation. Six fungal assays were shown to work on contaminated barley samples simultaneously.

Project No: FAIR-CT96-1120 (MOULDETECT).

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FISH OIL BENEFITS WITHOUT A FISHY FLAVOUR

FFE 397/01/HP4

It is well documented that the incidence of heart disease is low amongst populations who eat large quantities of oily fish such as trout and salmon. In particular, the effect appears to be mediated by the fish oils, which contain long-chain polyunsaturated fatty acids (n-3 PUFAs) such as eicosapentaenoic acid (EPA) and docosohexaenoic acid (DHA). As well as their role in reducing risk of coronary thrombosis and atherosclerosis, some n-3 PUFAs are important in early infant development, such as for the development of neural and retinal tissue.

Fish consumption per capita (particularly oily fish) is low in many countries, and taking fish oil as a dietary supplement is not very appealing. Previous attempts to incorporate fish oils into foods has had limited success due to the unappealing "fishy" flavour in the food product.

The aims of this completed FAIR project were threefold:

- to develop a dried ingredient delivery system by which fish oils could be incorporated into foods;
- to assess bioavailability of the n-3 PUFA from developed food products;
- to determine the lowest amount of n-3 PUFA needed for a positive health effect (dose response study).

A process was successfully developed whereby n-3 PUFAs were incorporated into a range of products – bread, biscuits, soup and an infant milk formula – whose taste was acceptable.

Bioavailability studies, carried out as adult human feeding trials, showed that the new delivery system of incorporating n-3 PUFAs in the diet via dried ingredients incorporated into specific foods was as effective as fish oil capsules in raising n-3 PUFA in the diet and subsequently in plasma. This suggests that an alternative to increasing intake of fish oil by supplements or natural oily fish can be offered.

Results from the dose-response study showed that 0.9 g/day was the minimum dose of n-3 PUFA to be effective in lowering triacylglyceride levels. This dose caused no enhancement of LDL cholesterol (LDL-C) oxidation and produced little or no change in plasma total cholesterol or LDL-C levels.

There was a clear dose-response relationship between the low levels of n-3 PUFA supplemented and subsequent increases in both LDL n-3 PUFA composition and platelet phospholipid EPA levels. A dose of only 0.9g/day reduced plasma triacylglycerol concentration by 0.24 mmol/L, corresponding to a theoretical 8-18% reduction in cardiovascular risk. Similarly, a low dose of 0.3-0.9 g of n-3 PUFA significantly increased HDL cholesterol (HDL-C) levels (so called "good cholesterol"). There is a strong inverse relationship between HDL-C and coronary heart disease (CHD) mortality. In this study, the observed increase in HDL-C represents a theoretical 11-16% decrease in CHD risk.

Whereas previous studies have shown that n-3 PUFAs have an anti-thrombotic cardio-protective effect, however, this study failed to demonstrate any effect on indices of coagulation and fibrinolysis (such as plasma fibrinogen and factor VII) at low dose levels.

In conclusion, these results illustrate health benefits when fish oil is consumed via the ingestion of palatable foods supplemented with dried ingredients derived from fish oil providing low doses of n-3 PUFAs.

Project No: FAIR CT95-0085 (NUTRIFISH).

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NUTRACEUTICALS – LONG CHAIN FATTY ACIDS AND FOLATE

FFE 403/01/SME7

The functional foods market is still increasing in Europe and is now said to be worth EURO 9,6 bn/year. Among the many healthy ingredients used in functional foods are the long chain n-3 polyunsaturated fatty acids, mainly from fish oils, which may influence positively the cardiovascular function. However, the documentation in intervention studies has not been conclusive and more studies are needed. Another important class of functional foods ingredients is the B-vitamins, in particular folate, also thought to influence cardiovascular health as well as the development of infants.

This 4-year project, which started in 2000, aims to undertake a comprehensive, in-depth evaluation of the beneficial roles and interactions of long chain n-3 polyunsaturated fatty acids (n-3 LC-PUFA) and a folate derivative (5-methyltetrahydrofolate) in cardiovascular (CV) health and infant development. The n-3 LC-PUFA are important for the development of the brain, nervous tissue and the retina and folate is essential for the prevention of neural tube defects. Many studies have also shown a decrease of homocystein – a well known marker of CV diseases – as a result of increased folate intake. The study will investigate effects on CV disease-related biomarkers and on the development of infants when their mothers are supplemented with n-3 LC PUFA and folate during pregnancy.

Beverages, yoghurt, baby food and a maternal nutrition formula fortified with n-3 LC-PUFA and / or folate will be developed. These foods will provide a propor-

tion of recommended intakes per serving. This presupposes stable, bioavailable and (for n-3 LC-PUFA) highly concentrated forms of nutraceuticals. The project will explore the feasibility of using newly developed oils, which are rich in n-3 LC PUFA, to generate beneficial alterations in biomarkers of CV disease and inflammation. The existence of a synergistic effect on CV health when n-3 LC PUFA and folate are combined will also be examined. Flair-Flow 4 will disseminate the results of this project as they become available.

Project No: QLK1-1999-00888 (NUHEAL)
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ADDING VALUE TO ONION WASTE

FFE 404/01/SME8

Over 450,000 tonnes of onion waste are produced annually in Europe mainly from the UK, Holland, Italy and Spain. The waste is not particularly suitable as fodder and disposal commonly involves landfill which costs between 15 and 45 ECU per tonne. There is considerable industrial pressure to identify alternative means of disposal. Converting the waste into useful products could be an appropriate route. This project is addressing the problem by extracting and processing the waste for commercially valuable food ingredients, including onion oil or onion juice for flavouring purposes, fructo-oligosaccharides, pectic polysaccharides and low-lignin-dietary fibre for supplementation of texturally sensitive foods.

Flair-Flow previously disseminated information on this project's first year results (see F-FE 307/98). According to the final report the main achievements could be summarized as follows:

- Brown and white peeling waste could be separated with greater than 95% efficiency.

- Extraction by alcohol-water mixtures of flavours and saccharides was feasible on an industrial scale and membrane technique (pervaporation) was a suitable approach for production of onion flavours with fresh onion odour. The flavour quality depended on the membrane type, extraction solvents and the conditions. Use of cell-wall-degrading enzymes and steam distillation increased considerably the level of soluble fibre. Purification of the extracted fructo-oligosaccharides (FOS) was not considered to be commercially feasible on its own because of cheaper sources of FOS.

- Brown onion fibres from the outermost layers of onion are difficult to modify either biochemically (cell-wall degrading enzymes) or physically (extrusion) because of the high degree of calcium cross-linking of the poorly branched pectin polymers. In addition, extrusion resulted in a very dark product which did not lend itself to food use. However, a method was developed for decolourising the waste, producing a nearly white fibre with increased solubility.

- Combinations of processes including milling, extrusion, autoclaving, chemical and biochemical (enzymic) modification could modify the functionality of white

onion dietary fibres (DF). In particular, the water holding capacity and the solubility was studied.

- Pectins and pectic polysaccharides from onion waste have poor gelling characteristics and demonstrate a low-viscosity. Their film forming property is under investigation.

Some of the products separated and processed from the onion waste were used to successfully supplement pizzas, bread, vegetable drinks and dairy products. Of particular interest is the potential to produce new, low-calorie, non-protein, non-fat and non-starch food-grade instant thickening agents.

Project No: FAIR-CT-96-1184 (EU-ONIONS)

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MEASURING FRESH FISH QUALITY

FFE 405/01/SME9

Estimation of fresh fish quality has great importance for prize settings, quality and safety of fresh fish. Formerly, quality was estimated subjectively by dealers and specialists based on the EU-scheme (Council decision No 103/76) but now this EU Craft project has developed a much more systematic and objective measuring system.

The objective of the project was to adapt an objective sensory Quality Index Method (QIM) for raw material of various species of fish and to develop further the instructions for its practical use in fish auctions and by quality inspectors.

The developed QIM system is based on the significant sensory parameters for raw fish and a score system from 0 to 3 demerit (index) points is used for each of the freshness parameters: appearance (skin, stiffness), eyes (cornea, form, colour of pupil) and gills (colour, smell). It has been shown, that the total score is linearly related to the storage time in ice. The index can also be used to predict the remaining shelf life of the specie in question.

Until now, the project group has developed the QIM system for 12 different species: cod, haddock, pollock, ocean perch, salmon, trout, herring, shrimp, dab, plaice, turbot and sole.

Based on the QIM, the group developed a software package (WiseFresh) for easy and fast monitoring of the quality index. The software also contains pictures and illustrations and can be delivered in English, Dutch, Danish and Icelandic. The QIM and the software have been described in more detail on www.qim-eurofish.com.

Following this Craft project, a strategic alliance (QIM Eurofish) has been build between the three institutes involved, The Netherlands Institute for Fisheries Research, The Danish Institute for Fisheries Research and the Icelandic Fisheries Laboratories. The objective of this alliance is to further develop the quality system and to promote and implement the sound use of QIM as a versatile quality tool

within fisheries distribution or production chains in Europe. The alliance is described on www.qim-eurofish.com.

Project No: FAIR-CT97-9063

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INFANT FEEDING PRACTICES AND INTESTINAL DEVELOPMENT

FFE 407/01/HP8

Health professionals would agree that breast milk is the preferred source of nutrition for all babies. In certain circumstances, however, breast-feeding is not possible and the infant has to be fed with formula milks.

Infancy is a crucial time for gut development and maturation, and breast-fed infants have marked differences in their gut microflora to formula-fed infants. This has been suggested to be a contributory factor in the increased incidence of gastrointestinal infections reported among formula-fed infants. Breast-fed babies also appear to develop a gut microflora environment that leads to increased resistance to pathogens, reducing predisposition to disease in early life and into adulthood. To date, formula milks have not been able to achieve these desirable effects, and this suggests that formula milks could be improved to contain certain ingredients that could lead to a similar gut microflora to breast-fed infants.

A second critical period of gut microflora colonisation is during weaning, but little is known about the changes occurring in gut microflora during this time, nor the implications this may have in later life.

The MEDIGUT project has tried to overcome the ethical and practical problem of studying the intestinal microflora in early life by developing an in vitro model of the infant gut microflora. Such a model allows the mechanisms behind gut maturation and development to be explored. This, then, helps to identify and develop foods that promote health both in early life and in adulthood. The models, based on the flora of breast-fed infants and weaning infants, will be used to monitor changes in gut bacteria populations and activities. As a result of this project, much information on the development of infant gut flora, showing the differences between breast- and formula-fed infants has been obtained and the models can be used to assess and develop functional food components that will provide formula-fed infants with benefits to gut-health that breast-fed infants enjoy.

Project No: FAIR-CT97-3181 (MEDIGUT)

http://www.gla.ac.uk/departments/humannutrition/medigut/medigut.html

Contact Person: Dr Christine Edwards, Department of Human Nutrition, Yorkhill Hospitals, Glasgow G3 8SJ, UK, Tel.: + 44 (0)141 2010711, Fax: + 44 (0)141 2019275, e-mail: cae1n@clinmed.gla.ac.uk

HEALTHY AGEING IN EUROPE

FFE 408/01/HP9

All over Europe, people are living longer. As a result of this, the population of Europe is on the brink of a major shift, with the number of elderly people becoming an increasing fraction. This shift is likely to have many public health and social implications because elderly people are more susceptible to degenerative and infectious diseases. Not only are such diseases unpleasant for the individuals concerned, but they carry tremendous financial implications for health services. Strategies are therefore needed to improve and maintain the quality of life of the ageing population.

A new EU-funded project "Crownalife", so called because there is emphasis on the preservation of the period of independence recognised as "the crown of life", has been set up to tackle this issue. The focus of the study is on preventive nutrition opportunities and the potential of functional foods to derive health benefits, thus improving quality of life for the elderly population. In particular, the project aims to explore strategies to restore and maintain a balanced intestinal environment. In order to do this, partners in the project will assess age-related changes in the structure and function of the intestinal flora in countries across Europe. Using this information, the potential roles of functional food in restoring and maintaining a healthy intestinal flora in the elderly will be evaluated.

The outcome of this study will include: the development of nutritional guidelines and recommendations adapted for the elderly population; the design and provision of food supplements adapted for gut health and the design of a new generation of functional foods, based on new concepts and technologies, to improve health in old age. The project is part of a concerted action entitled PROEUHEALTH and more details can be obtained from the co-ordinator.

Project No: QLK1-2000-00067 (CROWNALIFE)

http://www.vtt.fi/virtual/proeuhealth/consumerplatform/project4/index.htm

Contact Person: Prof. Joël Doré, INRA Domaine de Vilvert, 78352 Jouy-en-Josas Cedex, France, Tel.: +33 1 34652438; Fax: +33 1 34652462, e-mail: dore@jouy.inra.fr

WHAT'S GOING ON IN YOUR BABY'S BELLY?

FFE 409/01/CG7

The intestine of each of us hosts millions of bacteria. This bacteria population is what we call the "intestinal flora". The main function of the intestinal flora is to degrade the food we eat, and its activity is highly complex.

The digestive tube of newborn babies becomes colonised by bacteria from their first hours of life. Before birth, it is sterile, and does not host any bacteria. Through a mechanism still unknown, the intestine of the newborn baby will progressively select (i.e. retain) a limited number of bacteria genera and species, to reach a certain balance in the space of several hours or days. One of the reasons for the mystery surrounding these mechanisms is the difficulty of studying events taking place in the human intestine, for evident practical and ethical reasons.

But it is very important to study the intestinal flora. For example, researchers

think that this flora has a role in the activation of the immune defence in newborn babies. A European project set off, with questions like this in mind, to try and overcome the practical and ethical constraints which usually arise when carrying out this type of study. The idea was to set up "model" systems which researchers could easily use, which would mimic what actually happens in a real intestine.

Scientists who took part in this project agreed upon a two fold approach: a continuous microorganism model culture on one hand; on the other a "rat" model, associated with a human microbial flora. The goal of this work was to make sure that using this system would give a good representation of the reality in a human intestine. Thanks to these models, researchers are able to continue their investigations to better understand the essential "balance-keeping" role of intestinal flora for the good functioning of the human body and for its protection against infectious diseases.

Project No: FAIR-CT97-3181 (MEDIGUT)
http://www.gla.ac.uk/departments/humannutrition/medigut/medigut.html
Contact Person: Dr Christine Edwards, Department of Human Nutrition, Yorkhill Hospitals, Glasgow G3 8SJ, UK, Tel.: +44 (0) 141 2010711, Fax: +44 (0) 141 2019275, e-mail: cae1n@clinmed.gla.ac.uk

A SWEETER FUTURE...THE MILKY WAY!

FFE 410/01/CG8

Lactose is the sugar naturally present in milk. During the manufacture of cheese, yoghurt or other milk products, the water that milk contains must be removed in order to thicken the product. This concentration may be done in different ways, but it always represents a significant loss of lactose. Whereas milk products are highly regarded by consumers and therefore easy to sell, lactose presents hardly any commercial value. Therefore, food manufacturers do not know what to do with it and very often they dispose of this sugar in their effluents to the rivers. The disposal of a sugar in a flow of water may pose ecosystem balance problems if its concentration is too high, even in the case of a natural product, such as lactose. One of the reasons that makes it difficult to find a use for lactose is the fact that many consumers are not able to digest it: we say that they suffer from an intolerance to lactose. Therefore, lactose valorisation is a very important question which arises in all European countries and large other parts of the world.

The objective of this project was to find new uses for lactose. As it exists in great amounts, and is therefore inexpensive, researchers thought of using it as the base molecule to be transformed into various products through biological methods, preferentially by enzymes, which are the biocatalysts present in every living organism. They got interested in techniques which allow the splitting of lactose by means of the enzyme β-galactosidase into two other sugars: glucose and galactose. The difficulties consisted in choosing the right enzyme source, and mainly in defining accurately the physico-chemical conditions of the operation. In this regard, they put in evidence the importance of temperature, pH (acidity), and of the initial concentration of lactose. Several types of reactors, where the enzymatic reaction takes place, were tested. One of them was developed to industrial scale and the product is now used in ice cream manufacture. As side products so called galacto-

oligosaccharides (GOS) were formed; their exact structure depends on the type of β-glycosidase applied in the transformation reaction. GOS are used as prebiotic preparations in "novel food" which stimulate the growth of health-promoting benigne bifido- and lactobacteria in the gut. Glucose and galactose were also transformed enzymatically to gluconic acid and D-tagatose, respectively. The latter is a non-cariogenic sweetening substance of low caloric content and well suited for diabetics. Researchers taking part in this project think this product could have a future, as its manufacturing cost would not be very high. Also lactobionic acid, which has a buttermilk-like taste, was successfully synthesized by novel enzymes. Its calcium salt may serve for prevention of osteoporosis. Additionally several other promising compounds were prepared from lactose in the framework of this project.

Project No: FAIR-CT-96-1048 Enzymatic Lactose Valorization

Project Co-ordinator: Prof. Klaus D. Kulbe, University of Agriculture/Institute of Food Technology, Biochemical Engineering Division, Muthgasse 18, 1190 Vienna, Austria, Tel.: +43 1 36006 6250/6270; e-mail: sek@edv2.boku.ac.at

IMPROVING QUALITY OF HARD CHEESES

FFE 412/01/SME10

The production of hard cheeses in Europe which undergo a propionic acid fermentation is about 600.000 tons and includes brands or types like Emmental, Gruyère, Comté, Maasdamer, Italien hard cheeses, etc. Two types of bacteria are involved in the fermentation, Propionibacterium (PAB) and lactic acid bacteria (LAB, especially Lactobacillus helveticus and Lactobacillus delbrueckii subsp. Lactis).

The objective of this terminated FAIR project was to improve the quality of the hard cheeses by controlling the fermentation. Particularly, the interactions between the propionibacteria and the lactic acid bacteria are crucial for flavour, texture and appearance and more than 100 different pairs of strains were tested.

Some major results were:

- Building a new model simulating the growth of the PAB and LAB bacteria in hard cheeses;
- Linking the lipolytic activity in ripened Emmentaler cheese to aroma development;
- The lipolytic activity from PAB strains varied by a factor 1 to 10;
- Strains of PAB and LAB were genetically characterized;
- Main pathways for the metabolism of lactate by propionibacteria were investigated;
- Accumulation of trehalose and glycine-betaine by different strains could be important in their resistance to stresses in cheese;
- A rapid and alternative conductimetric method were developed to test the interactions between the PAB/LAB pairs.

Project No: FAIR-CT96-1024

Project Co-ordinator: Dr. Jean-René Kerjean, ITFF (Institut Technique Français des Fromages), BP 6224 - 35062 Rennes, France, e-mail: itff.rennes@wanadoo.fr

FOOD COLLOIDS, BIOPOLYMERS & MATERIALS
14-17 April 2002
Wageningen, the Netherlands

This conference is the 9 th in a series of biennial conferences dedicated to food colloids. It will be concerned with fundamental, applied and processing aspects of food colloids in general. Particular emphasis will be placed on the following subjects:

1. Gel Formation and Gel Properties;
2. Formation and Stability of Emulsions and Foams;
3. Microstructure and Microrheology;
4. Phase Separation;
5. Molecular Interactions and Self Assembly;
6. Crystals and Aggregates.

This conference will be made up of invited speakers, oral presentations and posters. Authors are invited to submit Abstracts of proposed contributions by September 7th, 2001, preferentially via e-mail to Foodcolloids2002@phys.fdsci.wau.nl.

More information can be found at: http://www.foodsciences.nl/FoodColloids2002 or contact: Dr. M.A. Bos and Dr. T. van Vliet, Wageningen Centre for Food Sciences, Wageningen University, Food Physics Group, P.O. Box 8129, 6700 EV Wageningen, The Netherlands, Tel.: +31 317485541, Fax: +31 317483669, E-mail: FoodColloids2002@phys.fdsci.wau.nl

Announcement

8th SEMINAR OF THE EUROPEAN NUTRITION LEADERSHIP PROGRAMME
7-14 March 2002
Luxembourg

The aim of this programme is to assist the development of future leaders in the field of human nutrition in Europe. Emphasis will be given to understanding the qualities and skills of leaders, team building, communication of nutrition information in a broader context, and to understanding the role of nutrition science in society.

The programme is designed for final PhD students and post-doctoral fellows in human nutrition sciences, studying or working in Europe. Preference will be given to candidates under the age of 35 years. From the applications received, thirty candidates will be selected by an international committee to attend each seminar.

Deadline for applications: 15 November 2001.

Registration fee: 750 Euro.

The ENLP organization can provide a small number of grants up to a maximum of 500 Euro. Those interested should send, together with their application form, a letter justifying their

application for such a grant together with a budget.

Further information and application forms: Mrs Lous Duym-Brookman Division of Human Nutrition and Epidemiology, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, the Netherlands, Tel.: +31 317483054, Fax: +31 317483 342, E-mail: lous.duym@staff.nutepi.wau.nl Internet: www.ftns.wau.nl/nutepi/enlp

Conference Announcement

8th INTERNATIONAL WORKING CONFERENCE ON STORED PRODUCT PROTECTION
22 to 26 July 2002
York, North County, United Kingdom

The Conference brings together research scientists, consultants, extension workers and industrialists, involved in the safe storage of the world's durable food commodities such as grains, legumes, pulses, nuts, beverage crops and animal feedstuffs. The meeting will showcase work on the pests and diseases which may cause spoilage, adverse health effects and loss of the crop, and discuss new techniques aimed at safe, effective and environmentally friendly management of stored commodities. The objectives are to exchange information from widely differing agricultural and economic scenarios, ranging from farmer scale in the tropics to bulk scale in Australia, North America and Europe, and to devise solutions to storage problems that are most appropriate and cost effective.

More information can be found at: Organized by: Central Science laboratory - E-mail: alerts@conferencealerts.com; contact: Helen Terre Blanche; E-mail enquiries: iwcspp@icscs.co.uk - Website: www.york.icscs.co.uk/iwcspp2002

BOOKS

THE FUNCTIONAL FOODS REVOLUTION: HEALTHY PEOPLE, HEALTHY PROFITS?

By Michael Heasman and Julian Mellentin, Directors of the Centre for Food & Health Studies, London

ISBN: Pb 185383 688 5.

Price: £ 16.95/US$22 (excluding p&p).

Published: 2001, in paperback

Publisher: by Earthscan, London (GB).

Order from: Lavis Marketing - Tel. +44 1865 7677575 - Fax +44 1865 750079 - e-mail: new-nutrition@lavismarketing.co.uk

The book tells the story of how the world's major food companies are looking to use their nutritional and scientific expertise in pursuit of the health benefits of food, and unleash products and marketing campaigns to tell consumers how to beat disease and keep well through food. It describes the trend to re-position everyday foodstuffs on the basis of their health promoting properties as "leveraging hidden nutrition assets". This is one of seven business strategies, some that work and others that don't, identified by the authors as driving the functional foods revolution.

Following the lead in Japan where the functional foods revolution started, food companies have introduced hundreds of new products claiming specific health benefits. Often targeted at a distinct medical condition, such as lowering "harmful" cholesterol, these "hi-tech" products have now been overtaken by companies using new insights from nutrition science, to re-launch and market everyday foods from breakfast cereals to fruit juices emphasizing the health benefits of their food components. This "low-tech" model of functional foods is the one now driving the bigger market for foods with health benefits.

Two of the other key issues in determining the success or failure of most functional brands – pricing and marketing communications are also treated.

The authors document why functional foods have become so important to the future of the food industry, the global marketing and business strategies being adopted and the major players involved. They also look at the broader business environment that is emerging, particularly the policy and regulatory context, from health claims to consumer communications.

IDF Publications

QUALITY MANAGEMENT FOR SMALL & MEDIUM-SIZED DAIRY PROCESSORS

A total of 50 persons attended the workshop in Zimbabwe. Session l is devoted to milk production. Information is provided as to how good quality raw milk can be provided at farm level to ensure the manufacture of good quality dairy products. Session 2 targets milk reception, milk handling and transport, storage and processing. Session 3 provides interesting perspectives on Quality Control and Laboratory Analysis; giv-

ing interesting insights on what takes place in different countries as well as detailed information on how to achieve excellence in this area. Session 4 deals with Quality Management Systems. Emphasis is placed on the vital need for self-regulation in producing safe food, and the benefits of organised systems of achieving quality management. Session 5 is devoted to the educational needs of establishing quality management systems. Session 6 addresses Product Safety. Session 7 concentrates on Sensory Education, providing new insight into how products can be evaluated more objectively by sensory evaluation techniques. Session 8 concentrates on reaping the benefits of quality management in the fields of laboratory services, marketing, and achieving customer satisfaction, and managing change in a dairy enterprise.

67 pp. - English only.
Bulletin N. 365/2001 - 55 Euro (2219 BEF)

FUNCTIONAL REQUIREMENTS FOR MILKING MACHINES REFERRING TO THE ISO STANDARDS 3918, 5707 AND 6690

Proper functioning of the milking machine is essential in achieving proper milking performance and maintaining udder health and milk quality. The 1996 version of ISO Standards 3918, 5707 and 6690 contains various performance requirements that should be followed in order to milk cows adequately. This paper aims to help the field personnel to interpret the results of milking machine testing. For each functional requirement, this paper helps to explain the consequences when a performance requirement is not fulfilled. It is also of assistance in determining the causes and solutions for a particular problem. Although the functional requirements come from ISO Standards, the interpretation is that of the IDF Group of Experts A32.

16 pp.

GUIDE TO THE USE OF ISO STANDARDS FOR MILKING MACHINE INSTALLATIONS AND ADDITIONAL DEFINITIONS

The expert members of the IDF Group A32 – Milking machines – have found a number of errors and in some cases unclear formulations in the ISO Standards for milking machines installations: ISO 3918 Vocabulary; ISO 5707 Construction and performance, and ISO 6690 Mechanical tests. This paper aims to assist the users of the three ISO Standards in interpreting them for practical application. The first part presents the existing formulation of the errors and the proposed changes are described. The second part introduces some "Additional definitions to ISO 3918".

6 pp.

MINIMUM COMPETENCY STANDARDS IN DAIRY CURRICULA

Originally intended as a basis for the establishment of a list of dairy education institutions in IDF member countries, the minimum competency standards published here have been developed to satisfy a need for criteria for use in industry and educational bodies. These minima suggest areas of study relevant to the particular roles appropriate to the required level of employment and recognize that course structure will vary de-

pending on the expertise and the philosophy of the establishment providing the educational/training programme and in concert with local industry requirements. Two main categories of dairy personnel are encompassed in these guidelines: the Dairy Operator (considered to be engaged mainly in direct dairy product manufacture or in basic laboratory analytical work), and the Dairy Specialist (expected to fulfil various supervisory, technical or managerial positions).

2 pp.

VARIABILITY OF THE INFLUENCE OF β-LACTOGLOBULIN PHENOTYPES ON MILK COMPOSITION

IDF work on genetic variants of milk proteins was initiated in 1992. This gave rise to two seminars and a paper on milk protein variant genes. The items covered were: Implications of genetic polymorphism of milk proteins on production and processing of milk; Milk protein polymorphism; Differential expression of milk protein variant genes in heterozygous animals. The brief review presented here is a technical update of the above topics.

2 pp.

CHEMICAL-PHYSICAL CONFIRMATION TESTS ("HIGHER" VALIDATION LEVEL) FOR THE DETECTION OF RESIDUES OF ANTIMICROBIALS IN MILK

By M. Pedersen, G. Suhren

Numerous methods have been developed for the detection of residues of antiinfectives in milk, which are more or less often used in the national food control programmes. It became obvious that compared to the substances for which MRLs or comparable values are fixed, methods with a "higher" validation level are still missing, as for many β-lactam antibiotics, macrolides, aminoglycosides, the quinolones and, with the exception of chloramphenicol, for all substances of the group "various".

7 pp.

FIRST EXPERIENCES WITH AUTOMATIC FLOW CYTOMETRIC DETERMINATION OF TOTAL BACTERIAL COUNT IN RAW MILK

By G. Suhren, H.-G. Walte

The use in everyday practice in dairy laboratories of automatic flow cytometric determination of total bacterial count in raw milk has grown dramatically. This report examines the principle and the practice of the method and the comparison with the traditional plate count method for total bacterial count.

The report has already appeared in Kieler Milchuirtschaftliche Forschungsberichte 50(3): 249-274 (1998).

13 pp.

RECOMMENDATIONS FOR THE HYGIENIC DESIGN OF STORAGE TANKS FOR MILK & MILK PRODUCTS

IDF Group B36 – Hygienic design of equipment used in dairy plants – was set the objective of producing IDF recommendations on hygienic design and construction equipment for use in storage, processing, handling and packaging

of dairy products, as well as the instrumentation to permit the equipment to function in a hygienic manner to protect public health.

This paper includes:

(a) Scope of work and definitions;

(b) Materials used in dairy processing equipment;

(c) Fabrication and design criteria for dairy processing equipment, to deal with design details of, for example, seals and gaskets, depth of packets, radiuses in corners, etc.

5 pp.

Bulletin N. 358/2000- 53 pp. in total - 1500 BEF (37,18 Euro)

41, Square Vergote - 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
E-mail: ijfs@unipg.it or paolofan@unipg.it

REFERENCE EXAMPLES

EXAMPLES of use in a Reference list are given below. The bold-faced parenthetical type of citation above the example is indicated ONLY for information and is NOT to be included in the reference list.

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