ISSN 1120-1770

ITALIAN JOURNAL OF FOOD SCIENCE

Rivista italiana di scienza degli alimenti

IJFS

Volume XVII
Number 3
2005

Spedizione in ab. post. comma 26 - art. 2 - legge 549/95 n. 3/2005 - Torino

CHIRIOTTI EDITORI

ITALIAN JOURNAL OF FOOD SCIENCE

(RIVISTA ITALIANA DI SCIENZA DEGLI ALIMENTI)

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

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Publisher:
Alberto Chiriotti
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Tel. +39 0121 393127 - Telefax +39 0121 794480
E-mail: info@chiriottieditori.it - URL: www.chiriottieditori.it

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

Impact Factor: 0.387 published in the 2004 Journal of Citation Reports, Institute for Scientific Information

Subscription Rate: 2005: Volume XVII

Ordinary	€	120.00
Supporting	€	500.00

IJFS is abstracted/indexed in: Chemical Abstracts Service (USA); Foods Adlibra Publ. (USA); Gialine - Ensia (F); Institut Information Sci. Acad. Sciences (Russia); Institute for Scientific Information; CurrentContents®/AB&ES; SciSearch® (USA-GB); Int. Food Information Service - IFIS (D); Int. Food Information Service - IFIS (UK); EBSCO Publishing.

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REVIEW

REHYDRATION OF DRIED FRUIT PIECES IN AQUEOUS SUGAR SOLUTIONS: A REVIEW ON MASS TRANSFER AND FINAL PRODUCT CHARACTERISTICS

REIDRATAZIONE DI FRUTTA IN PEZZI ESSICCATA IN SOLUZIONI ACQUOSE ZUCCHERINE: TRASFERIMENTO DI MATERIA E CARATTERISTICHE DEL PRODOTTO FINALE

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ABSTRACT

The results from different papers on the rehydration of air-dried and freeze-dried fruit pieces in aqueous sugar solutions are reviewed. The effect of the extrinsic rehydration variables on the mass transfer between fruit and solution and on the physico-chemical characteristics of the rehydrated products are also reported and discussed. By varying the concentration of solutions used as rehydration medium and the nature of the solute, it is possible to ad-

RIASSUNTO

La presente rassegna riepiloga i risultati di diversi studi sulla reidratazione di frutta in pezzi essiccata o liofilizzata in soluzioni acquose zuccherine. Vengono inoltre riportati e discussi gli effetti delle variabili estrinseche di reidratazione sui trasferimenti di massa tra la frutta e la soluzione e sulle caratteristiche chimico-fisiche dei prodotti reidratati. Variando la concentrazione della soluzione utilizzata per ricostituire il frutto essiccato o la natura dei soluti è possi-

- Key words: chemico-physical properties, dried fruit, firmness, rehydration, rehydration agent, rehydration capacity, rehydration rate, sugar solution -

just the water and soluble solids transfer in order to obtain products with the desired water activity, freezing point, freezable water content and firmness. Rehydration in aqueous sugar solution could thus improve the stability, technological functionality and sensory quality of fruit pieces used as an ingredient in food to be consumed at room, chilling or sub-freezing temperatures.

bile modulare il trasferimento di acqua e soluti al fine di ottenere prodotti con caratteristiche desiderate di attività dell'acqua, punto di congelamento, acqua congelabile e consistenza. Il processo di reidratazione in soluzioni zuccherine può quindi permettere di migliorare la stabilità, la funzionalità tecnologica e la qualità sensoriale di frutta in pezzi inclusa come ingrediente in alimenti da consumare a temperatura ambiente, refrigerata o di congelamento.

INTRODUCTION

Fruit pieces are widely used in food formulations such as pastries, confectionery products, ice-cream, frozen desserts and yoghurt. Maintenance of their natural colour, flavour and satisfactory texture is essential for consumer acceptance (DALL'AGLIO *et al.*, 1986; MALTINI *et al.*, 1993; MASTROCOLA *et al.*, 1995; TORREGGIANI and BERTOLO, 2001). Fruit ingredients should also have well-defined functional properties in order to be compatible with the food system without affecting shelf-life (MALTINI *et al.*, 1993; MASTROCOLA *et al.*, 1995; 1996). The compatibility of the fruit with other components of a formulated food depends basically on the equilibrium of the respective water activity (a_w) values. In fact, by adjusting a_w, it is possible to avoid or limit both mass transfer among different food components (LEVINE and SLADE, 1986; MALTINI *et al.*, 1993) and phase separation phenomena (TORREGGIANI *et al.*, 1991; GIANGIACOMO *et al.*, 1994, MASTROCOLA *et al.*, 1996).

Fruit pieces with medium to low a_w values can be obtained by individual or combined dehydration techniques (PONTING *et al.*, 1966; ROGACHEV and KISLENKO, 1973; DIXON *et al.*, 1976; LERICI *et al.*, 1977, 1985; HAWKES and FLINK, 1978; ERBA *et al.*, 1994; TULASIDAS, 1995; TAPIA *et al.*, 1999; FENG *et al.*, 1999a,b; MASKAN, 2000; FITO *et al.*, 2001; DALLA ROSA *et al.*, 2001, TAIWO *et al.*, 2002a). Moreover, in order to obtain an uptake of soluble solids and further reduce water activity, a direct osmotic pre-treatment prior to dehydration of the fruit can be adopted (PONTING *et al.*, 1966; DIXON *et al.*, 1976; LERICI *et al.*, 1977, 1983; HAWKES and FLINK, 1978; MASTROCOLA *et al.*, 1988; TORREGGIANI, 1993; TORREGGIANI and BERTOLO, 2001; ERLE and SCHUBERT, 2001; TAIWO *et al.*, 2002a,b). The osmotic dehydration process, also referred to as the "dewatering and impregnation soaking" (DIS) process, involves counter-current water and solute transfer (LERICI *et al.*, 1985; RAOULT-WACK *et al.*, 1991a,b), where the latter permits compositional changes to be achieved that affect product stability parameters (a_w and viscosity) as well as the physical and sensorial properties of the product (DALLA ROSA *et al.*, 1995; TORREGGIANI and BERTOLO, 2001).

In the case of strawberries, it is very difficult to obtain fruit pieces with low levels of water activity by partial drying. Some researchers have recently investigated the use of air-drying, freeze drying, microwave drying and osmotic dehydration of strawberries (TOMASICCHIO *et al.*, 1986; GARROTE and BERTONE, 1989; DALLA ROSA *et al.*, 1989; 1995;

GARROTE *et al.*, 1992; YANG and LE MAGUER, 1992; ALVAREZ *et al.*, 1995; HAMMAMI and RENE, 1997; VENKATACHALAPATHY and RAGHAVAN, 1999; ERLE and SCHUBERT, 2001; SHISHEHGARHA *et al.*, 2002). Generally, the results showed a disruption of the cell membranes in heat-treated fruit and cell wall alteration during the osmotic treatments. Therefore most of these dried fruit pieces do not meet the specific needs of the food industry. Hence it would be valuable to use a standard shelf-stable product, such as dried or freeze-dried fruit pieces, which can be further rehydrated under appropriate conditions to obtain specific a_w values and firmness.

The adjustment of product a_w and firmness can be obtained by using a sugar solution as the rehydration medium, because it minimizes firmness and colour changes after the drying and rehydration process (MASTROCOLA *et al.*, 1995; 1997; 1998; 1999; PITTIA *et al.*, 2004).

Almost all the studies reported in the literature on rehydration of fruit only consider rehydration in water (OLIVEIRA and ILLICANU, 1999). A large part of the data refer either to water uptake kinetics and rehydration process variables (OLIVEIRA and ILLICANU, 1999), or to the effect of drying and pre-drying treatments on water uptake and changes in structural properties during and after rehydration (SITKIEWICZ and JAKUBCZYK, 1998; PAPPAS *et al.*, 1999; KROKIDA *et al.*, 1999; RASTOGI *et al.*, 2000; PROTHON *et al.*, 2001; KROKIDA and MAROULIS, 2001; FUNEBO *et al.*, 2002; TAIWO *et al.*, 2002a,b). Apart from firmness, there has been limited interest in the quality characteristics of rehydrated fruit products which could be important for their acceptability in formulated food systems.

In the present paper a brief presentation of the factors that affect the rehydration process is given. The effect of extrinsic factors on the kinetics of the rehydration process of dried fruit pieces in aqueous solutions and on the physico-chemical and quality characteristics of the rehydrated products are also analysed and discussed.

REHYDRATION OF DRIED FRUIT PIECES

Rehydration is essentially a sorption process, however, because of the non-homogeneity of the dried plant tissues and the complexity of the main physical and biochemical processes involved, it is difficult to describe it as a merely diffusion process (OLIVEIRA and ILLICANU, 1999).

Rehydration is influenced by forces acting within the plant tissues (intrinsic factors) or by the interaction between the matrix and immersion medium (extrinsic factors). Some intrinsic factors include: size and geometry, chemical composition of the product, product formulation, pre-drying treatments, drying techniques and conditions (which could affect product a_w, moisture content, shrinkage and porosity and could cause case-hardening), and post-drying procedures. Some extrinsic factors include: composition of the immersion media, temperature and hydrodynamic conditions.

An extensive review of intrinsic factors that affect rehydration has already been presented by OLIVEIRA and ILLICANU (1999). The results of several studies on the effects of extrinsic factors on rehydration kinetics and quality changes of dried plant tissues rehydrated in aqueous solutions will be reported and discussed in this review.

Role of extrinsic factors on rehydration of dried fruit in aqueous solutions

When assessing rehydration, it is important to consider two aspects: the rehydration rate and rehydration capacity.

The rehydration rate usually decreases with time of immersion. Low initial rehydration rates are required for products that need short immersion times so that the product will remain crisp before consumption (OLIVEIRA and ILLICANU, 1999). Rehydration capacity refers to the maximum amount of water that the product is able to uptake upon immersion; it is also reported as water content (or uptake) at equilibrium (PELEG, 1988; OLIVEIRA and ILLINCANU, 1999). Rehydration capacity can be measured after equilibrium is reached or calculated by data modeling although it is frequently measured after a given immersion time (OLIVEIRA and ILLICANU, 1999; TAIWO *et al.*, 2002a,b).

The effect of the concentration of the immersion medium on the rehydration kinetics of air-dried apple cubes immersed in glucose solution is reported in Fig. 1. The shapes of the rehydration curves indicate that the mass transfer between the dehydrated apple pieces and sugar solutions takes place mainly during the initial period of rehydration (5 min). When a dried material is immersed in a solution, diffusion phenomena take place. In the first phases of the process, the rehydration of dehydrated fruit pieces in aqueous sugar solution involves mostly diffusion and capillary imbibition effects (MASTROCOLA *et al.*, 1997; 1998; MARABI and SAGUY, 2004). Eventually, when vegetative cell walls become partially rehydrated, they act like semi-permeable membranes (LERICI *et al.*, 1985; LE MAGUER, 1988; MASTROCOLA *et al.*, 1998) that are permeable to water and carry out a selective action towards soluble solids, depending on their molecular weights. Under these conditions, mass transfer is regulated by osmotic phenomena, where the driving force is the difference in the osmotic potentials between the cells and the solution. The mass transfer in cellular tissues during osmotic treatments is not explained by diffusion models (MARCOTTE *et al.*, 1991; MARCOTTE and LE MAGUER, 1992) because there is more than one important flow (LERICI *et al.*, 1985) and shrinkage also occurs (LE MAGUER, 1988).

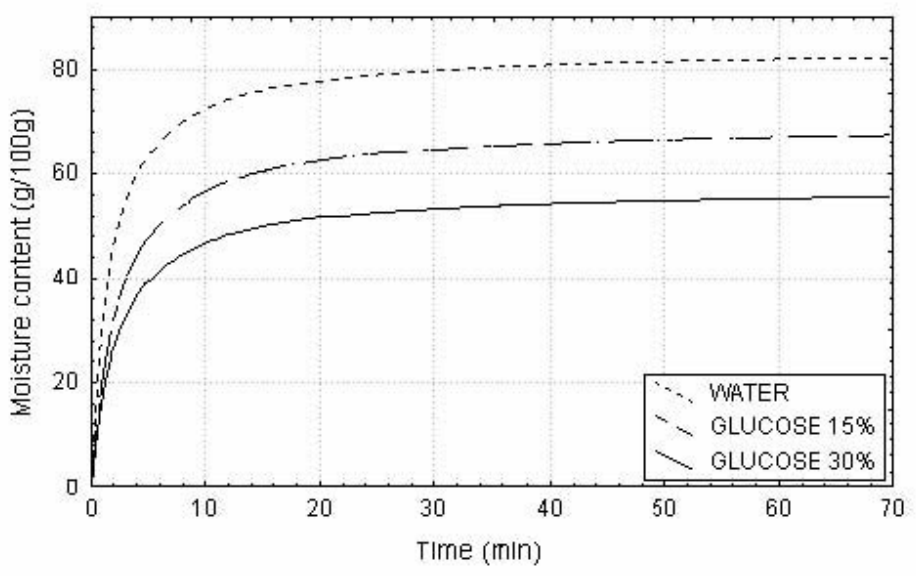


Fig. 1 - Rehydration kinetics of air-dried apple cubes in aqueous solution at different concentrations (elaborated from MASTROCOLA *et al.*, 1998).

An increase in the syrup concentration during air-dried apple rehydration in glucose solution resulted in a decrease of both rehydration capacity (MASTROCOLA *et al.*, 1995; 1997; 1998) and initial rehydration rate (Fig. 1) because of the increase in the osmotic potential of the solution (MATSUURA and SOURRIRAJAN, 1986). The rehydration capacity in an aqueous glucose solution is almost linearly affected by concentration in a range from 0 to 30% (MASTROCOLA *et al.*, 1997; 1998) and the same behaviour was observed for the osmotic potential of the glucose solution (MATSUURA and SOURRIRAJAN, 1986). The solute concentration also has a limiting effect on water gain during rehydration of beans and carrots in diluted salt solutions; this was attributed to the water binding effect of solutes (CURRY *et al.*, 1976; DEL VALLE *et al.*, 1992).

MASTROCOLA *et al.* (1995; 1997; 1998) observed the loss of solids in dried and freeze-dried fruit during rehydration time when diluted glucose solutions were used, while there was an uptake of solids when solutions with more than 15% glucose were used. The extent of changes in the solid content was almost linearly correlated with the concentration of the sugar solution independent of the type of fruit and dehydration process used (air or freeze-drying). During the so-called "osmotic dehydration", the transfer of solutes from the concentrated solution into the food occurs counter-current to the water flux (dewatering), while during rehydration in aqueous sugar solutions, the solutes flow in different directions depending on the concentration of the solution. If the fluxes are in the same direction, the dried fruit is subjected to an impregnation process.

The interaction between the medium concentration and matrix could further affect the mass transfer during the rehydration of dried products. The high porosity of freeze-dried products accounts for a higher rehydration rate of these products (OLIVEIRA and ILLICANU, 1999; MARABI and SAGUY, 2004), but could be responsible for a lower rehydration capacity (MASTROCOLA *et al.*, 1995; 1996; 1997; 1998; OLIVEIRA and ILLICANU, 1999). In the study of MARABI and SAGUY (2004) on rehydration of air- and freeze-dried carrots in aqueous solutions, they suggested that, on the basis of the effective moisture diffusivity data, only air-dried samples follow a Fickian diffusion and that there is a critical porosity value above which the water uptake mechanism changes from a Fickian diffusion to imbibition into a porous medium. Capillary imbibition plays an important role at the early stages of the rehydration of freeze-dried food particulates, leading to almost instantaneous water uptake (WEERTS *et al.*, 2003; MARABI and SAGUY, 2004). During apple cube rehydration in sugar solution, the limiting effect of the sugar concentration on water uptake was much higher in air-dried samples than in freeze-dried samples (MASTROCOLA *et al.*, 1998). This could be attributed to the different water uptake mechanisms in the two different products. The lower rehydration capacity observed in freeze-dried food could be due to the effect of particle size distribution in dry food particulates (PEPPAS and BRANNON-PEPPAS, 1994).

As observed for water uptake, the changes in soluble solids is also affected by the matrix porosity; the freeze-dried apple cubes showed less total solid loss than the air-dried cubes during rehydration in water (MASTROCOLA *et al.*, 1998). Analogue results were reported by MARABI *et al.* (2004a) for total organic carbon during air- and freeze-dried carrot rehydration. During air-drying, soluble solids migrate to the surface of the fruit pieces before the water evaporates, leading to the formation of a barrier that causes a greater loss of solids during rehydration (MASTROCOLA *et al.*, 1998). During the rehydration of apple cubes

in sugar solutions, the change in solids due to the increased concentration of the rehydration agent was much higher in freeze-dried samples than in air dried ones which could be due to sample porosity (MASTROCOLA *et al.*, 1998).

The rehydration process, similar to the osmotic dehydration process is affected not only by the solution concentration but also by the nature of the solutes. MASTROCOLA *et al.* (1998) found that rehydration of freeze-dried apples in a 30% HFCS (high fructose corn syrup) solution permitted a higher rehydration rate and capacity to be obtained than rehydration in a 30% glucose solution (Fig. 2). This is most likely due to the higher water activity of the 30% HFCS solution and thus to its lower osmotic pressure. There is less uptake of solids during rehydration with HFCS solutions than with glucose solution (MASTROCOLA *et al.*, 1997; 1998). The use of high-molecular-weight solutes to minimize solid uptake has been widely investigated in studies on osmotic dehydration (LERICI *et al.*, 1985; SAUREL *et al.*, 1994; LAZARIDES *et al.*, 1995). Its limiting effect on solid uptake is primarily related to the reduced diffusivities of solutes that occur when their molecular weight increases (Stokes-Einstein law). Moreover the cell wall network reduces the apparent diffusivity when solute molecular weight increases (SAUREL *et al.*, 1994). The effect of the nature of the solutes on mass transfer during the rehydration of dried fruit is dramatically affected by the internal structure of the dried materials. The use of HFCS rather than glucose had a positive effect on the rehydration rate and capacity which was more evident in freeze-dried products (Fig. 2) than in air-dried products (Fig. 3). The lower porosity of air-dried fruit pieces could, in fact, limit the diffusion phenomena of the high-molecular-weight HFCS, thus minimizing the effect of the nature of the solute on the

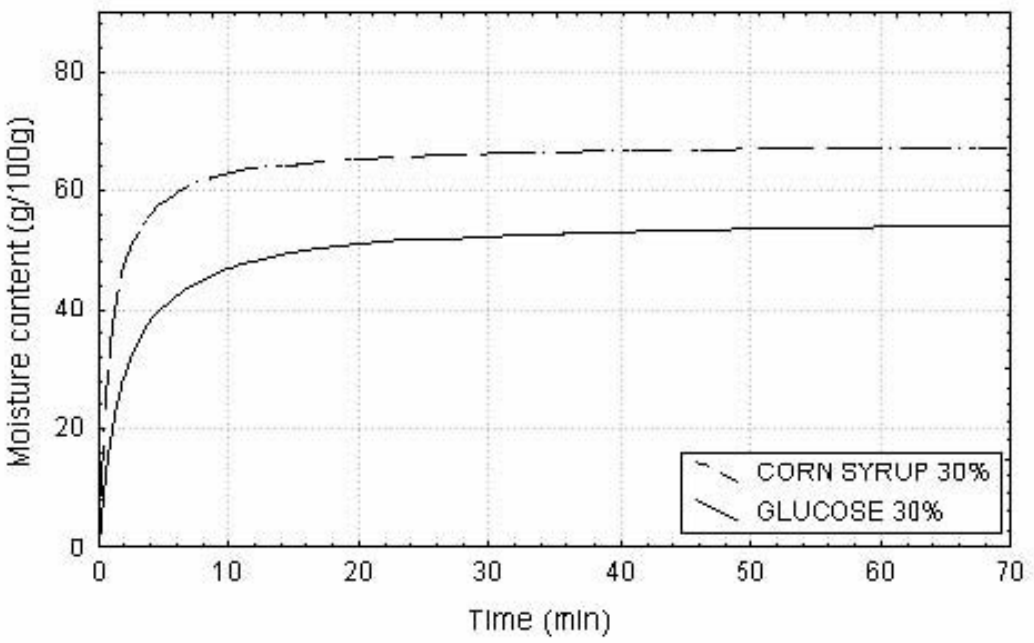


Fig. 2 - Rehydration kinetics of freeze-dried apple cubes in aqueous solution with different solutes (elaborated from MASTROCOLA *et al.*, 1998).

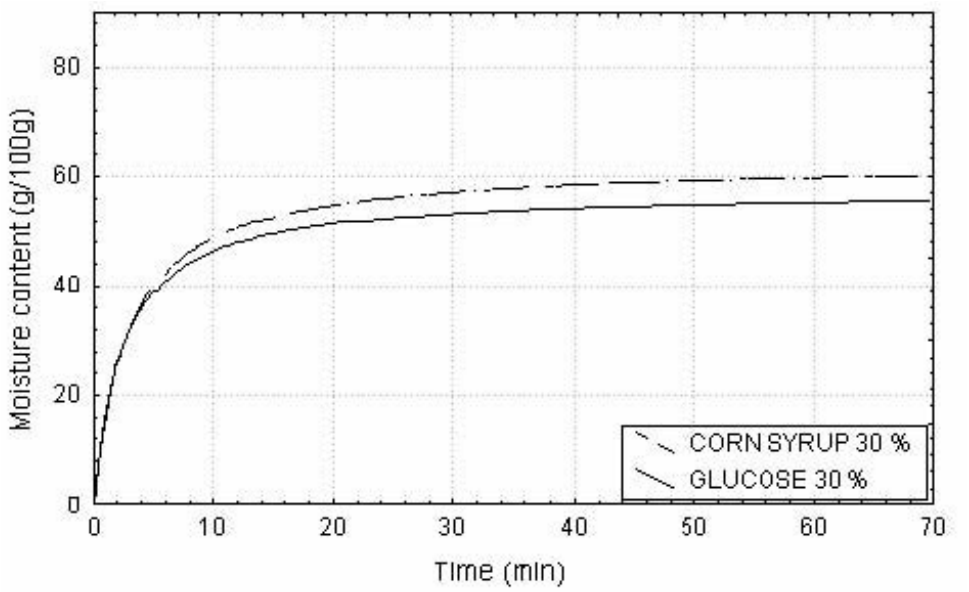


Fig. 3 - Rehydration kinetics of air-dried apple cubes in aqueous solution with different solutes (elaborated from MASTROCOLA *et al.*, 1998).

initial rate of water uptake. The uptake of solids in air-dried fruit is less than in freeze-dried fruit pieces due to their lower porosity which hinders the diffusion phenomena (MASTROCOLA *et al.*, 1997; 1998). Soluble solids mass transfer phenomena could also be limited by the formation of a superficial concentrated layer of high-molecular-weight polysaccharides whose penetration is hindered by the low porosity of the air-dried fruit. Similar results have been observed during osmotic treatment of model gels and vegetable products in highly viscous media such as concentrated sugar solutions (LENART and FLINK, 1984a,b; RAOULT-WACK *et al.*, 1991b,c).

Higher temperatures increase the rehydration rate in water but do not usually have a significant effect on rehydration capacity. The effect of temperature on rehydration rate is both due to the decreased viscosity of the immersion medium and to the negative effect of temperature on the structure of the food material. The overall effect of various temperatures is typically described by an Arrhenius-type relationship (OLIVEIRA and ILLICANU, 1999). BARBANTI *et al.* (1998) investigated the effect of temperature (20°-80°C) during the rehydration of air-dried apples in sucrose solutions at different concentrations (0-30%); they reported a limited increase in the rehydration capacity (after 1-10 min) with increasing temperature. The increase in rehydration capacity was more evident with longer rehydration times. Even if temperature does not influence the rehydration capacity, higher temperatures dramatically decrease the total solids due to solid loss (BARBANTI *et al.*, 1998).

Stirring or agitation of the immersion media improves the rehydration rate but since the major resistance to water transfer lies within the food material, agitation is not expected to play a major role except for highly viscous immersion media (OLIVEIRA and ILLICANU, 1999). This consideration was confirmed by MARABI *et al.* (2004b) in a study carried out on rehydration of air- and freeze-

dried carrots in aqueous solutions. The results showed that the effect of agitation speed on effective moisture diffusivity was negligible and became significant only when the viscosity was increased by using a 3% starch solution. In another study carried out by BARBANTI *et al.* (1998) the agitation speed (0-250 r.p.m.) did not influence the rehydration capacity over time (1-10 min) of air-dried apples in sucrose solutions from 0 to 30% with kinematic viscosities ranging from 1 to $2.83x10^{-6}m^2s^{-1}$. Under the same experimental conditions, the agitation speed did not significantly increase the loss of solids (BARBANTI *et al.*, 1998).

CHARACTERISTICS OF REHYDRATED FRUIT PIECES

In addition to rehydration rate and capacity, the other parameters that are thought to characterise the rehydration process are the very characteristics of the rehydrated fruit pieces: stability, firmness, size, shape and colour (MASTROCOLA *et al.*, 1995; 1997; 1998; TAIWO *et al.*, 2002b; PITTIA *et al.*, 2003).

In order to meet the specific needs of the producers of formulated foods, it would be helpful to have a standard high quality dried product which could be further diversified by rehydration. The characteristics of dried-fruit pieces can be adjusted by choosing a proper rehydration medium (MASTROCOLA *et al.*, 1995; 1997; 1998).

In the opinion of many authors (MALTINI *et al.*, 1993; LEVINE and SLADE, 1986; MASTROCOLA *et al.*, 1995; 1997; 1998), the "compatibility" of the fruit with other components of a formulated food is highly dependent on the equilibrium between its water activity (a_w) value and that of the base-food in which it is contained. The control of this parameter prevents or reduces the diffusion of moisture between ingredients with different levels of water content (MALTINI *et al.*, 1993).

The water activity values of rehydrated products reported in the literature were highly correlated with the soluble solids content, independent of time and rehydration media (Table 1). This result could be of considerable interest. For a given a_w value that is suitable for the inclusion of fruit pieces in a formulated food, it is possible to calculate the corresponding soluble solids content and determine the sugar solution concentration and rehydration time.

Freezing point values showed the same trend as the a_w values. Data on the freezing point could be helpful when rehydrated fruit pieces are mixed with frozen products such as ice-cream, sherbet and frozen desserts (FENNEMA, 1981). In these cases, fruit ingredients which do not form ice at a given temperature should be chosen, that is, products with an a_w value lower or equal to the equilibrium a_w at that temperature, or fruit with water activities higher than the equilibrium in which a definite amount of ice will separate according to the freezing behaviour (MALTINI *et al.*, 1993). Moreover, when this kind of product is included in ice-cream, which is generally consumed at an average temperature of -15°C, it is of interest to know the amount of frozen water in fruit pieces at this temperature. In the case of rehydrated fruit pieces (apple and strawberries), even if their freezing temperature is higher than that at which the ice-cream is consumed, for soluble solids contents higher than 50 g/100 g of water, these products have a very small quantity of freezable water (MASTROCOLA *et al.*, 1997; 1998).

Texture is another important quality characteristic of rehydrated fruit pieces (SITKIEWICKZ and JAKUBCZYK, 1998; KROKIDA *et al.*, 1999; KROKIDA and MAROULIS, 2001; PROTHON *et al.*, 2001; FUNEBO *et al.*, 2002; TAIWO *et al.*, 2002a,b) that basically depends on the swelling and plasticizing properties of pectin and cellulose when hydrated. According to some authors, at room temperature, the

Table 1 - Linear regression of the variations of a_w: water activity; FP: freezing point (°C); FW: frozen water at -15°C (%); F-15: firmness at -15°C (N); F: firmness at room temperature (N) as functions of soluble solids content (SSw – g/100 g water) in fruit pieces rehydrated in sugar solutions. Data from MASTROCOLA *et al.* (1997, 1998).

Product	Observed characteristic	Linear regression equations (Y = observed characteristic)	Correlation coefficients (r)
Air- dried apples	a_w	Y = -0.00076SSw + 0.983	0.984*
	FP	Y = -0.0802SSw – 1.68	0.982*
	FW-15	Y = -0.666SSw + 68.25	0.980*
	F-15	Y = -40.678SSw + 4,748	0.976*
	F	Y = 2.653SSw + 343.1	0.955*
Freeze-dried apples	a_w	Y = -0.00075SSw + 0.981	0.979*
	FP	Y = -0.0811SSw – 1.88	0.974*
	FW-15	Y = -0.854SSw + 78.044	0.972*
	F-15 (SSw below 40 g/100 g water)	Y = -126.007SSw + 5637	0.936*
	F	Y = 1.123SSw + 137.7	0.940*
Freeze- dried strawberries	a_w	Y = -0.0093SSw + 0.992	0.993*
	FP	Y = -0.01022SSw – 0.98	0.990*
	FW-15 (SSw below 40 g/100 g water)	Y = -0.775SSw + 75.9	0.996*
	F-15	Y = -101.83SSw + 5,119	0.935*

*Significant at $p \leq 0.001$.

texture depends directly on the total insoluble solids content of the food (MALTINI *et al.*, 1993), or is well correlated with the total dry matter content (MASTROCOLA *et al.*, 1995; 1997; 1998). When rehydrated fruit pieces are kept at freezing temperatures, the texture is strongly influenced by frozen water and, only when the water content is low, is the texture influenced by the compositional characteristics of the solids and by their hydration level. In the case of freeze-dried apple and strawberry pieces, the hardness remained quite constant (300-400 N) for soluble solids content over 40 g/100 g of water (MASTROCOLA *et al.*, 1997; 1998) and it dramatically increased when the soluble solids decreased (Table 1). In view of these results, freeze-dried rehydrated apple and strawberry (kept at -15°C) have a small amount of ice crystals which will not influence the firmness of the product if the soluble solids content is higher than 40 g/100 g of water. These soluble solids contents can be achieved by rehydrating fruit pieces using syrups with a sugar content higher than 20% (MASTROCOLA *et al.*, 1997; 1998). In the case of air-dried apples, it is not possible to separate the hardness effect due to the partially hydrated fruit tissues from that derived from the presence of ice crystals.

The regression equations reported in Table 1 also show the positive and linear effect of solute concentration on hardness (measured at room temperature) of air-dried and freeze-dried apple cubes after rehydration in sugar solution. The air-dried products showed a more evident increase of firmness with an increased soluble solids content. The rehydration in sugar solutions could thus balance the decrease of firmness of ap-

ple cubes after drying and rehydration in water. The latter is due to the structural damage that occurs during drying and the hysteresis phenomenon which takes place during rehydration (KROKIDA and MAROULIS, 2001).

Another important quality characteristic of rehydrated fruit pieces is colour (TAIWO *et al.*, 2002a,b; PITTIA *et al.*, 2004). During rehydration and 24h of storage, freeze-dried apple cubes rehydrated in a 30% HFCS solution showed a better colour maintenance than those rehydrated in water (PITTIA *et al.*, 2004). The a_w depleting effect of the solute could, in fact, limit the enzymatic browning reactions which take place during rehydration of freeze-dried fruit. Colour changes during rehydration in water were observed in freeze dried apples but not in osmotically dehydrated apples (TAIWO *et al.*, 2002b). In fact the enzymatic activity could be conserved by using freeze-drying methods that are aimed at obtaining material with uninjured biochemical pathways (LEWICKI *et al.*, 2001).

CONCLUSIONS

This review shows that it is possible to adjust the water uptake and the soluble solids balance of rehydrated fruit pieces by controlling the length of the rehydration process and the type and concentration of the rehydration media. Thus it is possible to obtain products with different composition and functional properties from the same dehydrated fruit for specific uses in different formulated foods (bakery products, ice-cream, yoghurt). Dehydrated fruit pieces can be considered as semi-manufactured products whose quality characteristics can be modified, if necessary, by rehydration in an appropriate medium. The rehydration process can improve stability, technological functionality and sensory properties, depending on the soluble solids content. In this context, the high quality of the final product could balance the cost of a drying-rehydration process, particularly when these products are used in high value formulated food.

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Revised paper received January 27, 2005 Accepted May 30, 2005

PAPER

TRAINING OF A SENSORY PANEL FOR QUANTITATIVE DESCRIPTIVE ANALYSIS OF LAMB MEAT

L'ADDESTRAMENTO DI UN PANEL SENSORIALE PER L'ANALISI DESCRITTIVA QUANTITATIVA DELLA CARNE D'AGNELLO

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ABSTRACT

The training and application of a sensory panel for the Quantitative Descriptive Analysis of lamb meat are described. A proper vocabulary has been developed and validated by reference samples obtained from different kinds of meat cooked under different conditions or mixed with specific ingredients that mimic possible lamb meat sensory characteristics. The final list contains 14 attributes for odour (3), flavour (7) and texture (4). Data have been checked to assess panel conso-

RIASSUNTO

Il lavoro descrive l'addestramento e l'applicazione di un panel di giudici sensoriali per l'analisi descrittiva quantitativa della carne d'agnello. I descrittori sono stati definiti e validati attraverso campioni di riferimento ottenuti da diversi tagli di carne cotti in condizioni diversificate oppure addizionati con opportuni ingredienti per riprodurre specifiche caratteristiche sensoriali della carne d'agnello. La scheda finale comprende 14 descrittori per odore (3), flavour (7) e texture (4). I dati sono stati

- Key words: lamb meat, panel training, sensory descriptive analysis -

nance, reproducibility and discrimination ability by providing accurate and reliable definitions of the sensory characteristics of lamb meat and their correlation with production/management systems.

verificati in termini di accordo tra giudici, riproducibilità e capacità discriminante fornendo una misura accurata delle caratteristiche sensoriali e permettendo la sua correlazione con i principali parametri produttivi.

INTRODUCTION

The sensory quality of meat is influenced by several factors that act before and during eating and which are often mutually interacting. Many of them are related to the production and management system of the animals such as breed, age at weaning, diet, sex, body weight and age at slaughter (ARSENOS *et al.*, 2002; GEAY *et al.*, 2001; PIASENTIER *et al.*, 1999, 2001; PRIOLO *et al.*, 2002; ROUSSET-AKRIM *et al.*, 1997; SANUDO *et al.*, 1996, 1997, 1998; WOOD *et al.*, 1999; VERGARA and GALLEGO 1999, 2000; VERGARA *et al.*, 1999). Others are related to meat processing and to storage conditions (CARLUCCI *et al.*, 1999). Finally, differences in cooking techniques greatly influence the perceived quality of the meat; Maillard reactions are related, for instance, to the surface temperature of meat during cooking and greatly affect odour, flavour and colour (WHITFIELD, 1992). The temperature profile through the meat section and the final core temperature influence the change of protein structure, humidity losses and, therefore, texture, odour and flavour. An in-depth study can be found in the paper by BEJERHOLM and AASLYNG (2003).

Descriptive analysis by a panel of trained judges (also called assessors) is probably the best way to objectively assess and compare the sensory properties of food products. An extensive review on traditional and new methods of descriptive sensory analysis can be found in MURRAY *et al.* (2001). The conventional profiling (Quantitative Descriptive Analysis - STONE *et al.*, 1974) is probably the most widely used and most consolidated descriptive technique.

Selection and training of judges based on literature references and normative indications (JELLINEK 1985; ISO 8686/1; ISO 8586/2) are necessary to obtain reproducible assessments with good discriminatory ability and high sensitivity and, consequently, to have reliable and useful results.

Subsequently, the panel develops a descriptive vocabulary that is suitable for the product under analysis and comes to a general agreement on it (STONE and SIDEL, 1985). Reference standard products must be selected for vocabulary validation in order to develop and check the ability of assessors to recognise, describe and correctly measure each attribute (RAINEY, 1986). This is a necessary step because it assures homogeneity in the use of the vocabulary and allows data obtained by different panels or in different times to be compared. In the case of meat, the literature provides very few useful indications about standardised references (for texture, tenderness, juiciness and flavour) (CROSS *et al.*, 1978; BETT, 1993; GORRAIZ *et al.*, 2000; BYRNE *et al.*, 2001). In most cases reference products have to be developed by drawing from the experience acquired with other food products (e.g. dairy product reference, LAVANCHY *et al.*, 1993).

Since reliable panel performance is essential, if sound results are to be obtained, a further critical step is the sta-

tistical validation of the data. Numerous approaches have been proposed to evaluate the training performance: in terms of panel agreement, e.g. by the egg shell plot techniques (HIRST and NAES, 1994) or by the percentages of variance accounted for (DIJKSTERHUIS, 1995), discriminative ability and reproducibility, e.g. by ANOVA to study the effect of sample, assessor, and replicates (NAES and LANGSRUD, 1998; CARLUCCI *et al.*, 1999).

Here we describe the procedures followed for the training and application of a sensory panel suitable for the quantitative descriptive analysis of lamb meat. With this work we hope to establish some consistent procedures for defining a vocabulary for the sensory analysis of lamb meat and other meat types, in general.

This work was conducted within the framework of the project FAIR III CT96 1768 "Identifying and changing the qualities and composition of meat from different European sheep types which meets regional consumer expectations" supported by the European Union and which involved the main lamb-producing countries in Europe: France, Greece, Italy, Spain, the United Kingdom and Iceland (FISHER *et al.*, 1999; DRANSFIELD *et al.*, 2000; SANUDO *et al.*, 2000, 2003; ENSER *et al.*, 1999).

MATERIALS AND METHODS

Descriptive panel and vocabulary development

Twelve assessors (7 males and 5 females) were selected from those trained in a previous course (GALLERANI *et al.*, 2000), for the detection, recognition and assessment of tastes, odours, flavours, and physical characteristics, as well as for the use of classification scales.

An initial list of possible sensory attributes was generated during group assessment of different lamb meats, which were intentionally selected with the aim of covering the expected variability of the actual samples (STONE and SIDEL, 1985). This list includes a series of sensory terms which was previously defined with the other partners of the above-mentioned project and was used by all the panels involved: the texture attributes "tenderness" and "juiciness", the odours "lamb" and "abnormal" (to be evaluated, separately, for lean and fat) and the flavours "liver", "sheep meat", "rancid", "fatty" and "dairy". These attributes were proposed by the panel leader, while new attributes were suggested by judges during the training phase. All of these attributes were extensively examined during group discussions in order to reach a consensus about their meaning, establish an evaluation procedure and chose suitable reference standards (STONE *et al.*, 1974).

Reference and training samples were prepared by adding lamb and sheep extract, liver, fat or oxidised oil at different concentrations to several types of beef and sheep meat. In Table 1 we summarise the tests presented to the judges for texture and flavour descriptors. As a first step, the evaluation of these training samples was performed by all the judges together and subsequently, the same samples were evaluated individually by each judge. The judges were trained to rate the intensity on a 100 mm unstructured line anchored at each extreme (LAND and SHEPHERD 1984). Data were analysed to determine between-sample and between-judge statistical differences and the total correlation of each assessor with the panel.

The established evaluation protocol, including 14 attributes (3 for odour, 7 for flavour and 4 for texture, Table 2), was validated during a preliminary session performed on 4 real samples of lamb meat. Each sample was replicated 3 times.

Table 1 - Individual tests used during the preliminary phase (vocabulary development) and results achieved by the panel in terms of discriminatory ability (results of one way-ANOVA).

Descriptor/ Test N.	Samples	Observations (F and p calculated using ANOVA)
Tenderness/1	6 samples: 2 lamb meat types (suckling lamb and adult sheep LL meat) grilled to three different internal temperatures (Ti=67°, 70°, 75°C)	The panel discriminates the two meat typologies and recognises the temperature effect (F=36.8; p=0.00)
Tenderness/2	6 samples: 5 different cuts of beef (belly, haunch, roast beef, shoulder, under-shoulder) and one cut of adult sheep (LL) grilled to the same internal temperature (Ti=72°C)	The panel discriminates the samples (F=9.77; p=0.00)
Tenderness/3	3 samples of three different sheep meat types (suckling and light lamb and sheep LL) grilled to the same internal temperature (Ti=72°C)	The panel discriminates the samples (the suckling lamb was found different from the others) (F=7.25; p=0.00)
Tenderness/4	3 samples of minced meat, mixed with dried bread and added with three different water concentrations (0, 8, 21%)	The panel discriminates the samples (F=10.01; p=0.01)
Juiciness/1	6 samples: as Tenderness / 2 Test	The panel discriminates the three meat types and the temperature effect can be seen (F=7.26; p=0.00)
Juiciness/2	6 samples: as Tenderness / 1 Test	The panel discriminates the three meat types and the temperature effect can be seen (F=15.25; p=0.00)
Fibrosity/1	6 samples: as Tenderness / 2 Test	The panel discriminates only one sample (haunch) from the others (F=7.75; p=0.00)
Chewiness/1	6 samples: as Tenderness / 2 Test	The panel discriminates the samples (F=13.4; p=0.00)
Chewiness/2	3 samples of minced meat, mixed with dry (stale) bread and added with three different water concentrations (0, 8, 21%)	The panel discriminates the samples containing 0% water from the ones containing 21% water (F=6.88; p=0.00)
Lamb odour/1	4 samples of minced lamb meat added with *lamb extract** at 4 different final concentrations (0, 5, 7, 10%)	The panel discriminates between the samples at 0 and 5% and the ones at 7 and 10% concentration (F=7.4; p=0.00)
Lamb odour/2	3 samples of three different sheep meat types (suckling and light lamb and sheep LL) grilled to the same internal temperature (Ti=72°C)	The panel discriminates the suckling lamb from the other two types (F=4.44; p=0.02)
Liver flavour/1	4 samples of minced lamb meat added with *liver extract** at 4 different final concentrations (0, 0.25, 0.5, 1%)	The panel partially discriminates and recognises the different concentration levels (F=5.46; p=0.00)
Liver flavour/2	3 samples of minced lamb meat added with *liver extract** at 3 different final concentrations (2, 4, 8%)	The panel discriminates the highest concentration level from the others (F=32.54; p=0.00)

Descriptor/ Test N.	Samples	Observations (F and p calculated using ANOVA)
Sheep meat flavour/1	3 samples of minced lamb meat added with *sheep extract** at 3 different final concentrations (2.5, 5, 10%)	The panel discriminates the highest concentration level from the others (F=6.43; p=0.01)
Sheep meat flavour/2	3 samples of sheep meat: sheep/haunch, sheep/LL, suckling lamb/LL	The panel discriminates between the sheep meat types and the lamb meat (F=8.14; p=0.00)
Lamb flavour/1	3 samples of three different sheep meat types (suckling and light lamb and sheep LL) grilled to the same internal temperature (Ti=72°C)	The panel does not discriminate the samples (F=1.72; p=0.20)
Fat flavour/1	4 samples of lamb meat added with fat at 4 different final concentrations (0, 8, 12, 20%)	The panel partially discriminates the different fat concentration levels (F=11.33; p=0.00)
Milk flavour/1	4 samples of lamb meat added with powdered milk** at 4 different final concentrations (0, 5, 8, 14%)	The panel discriminates between the samples at 0 and 5% and the ones at 8 and 14% concentration (F=45.28; p=0.00)
Oxidised flavour/1	4 samples of lamb meat added with oxidised oil*** at 4 different final concentrations (0, 2, 5, 11%)	The panel discriminates between the samples at 0 and 2% and the ones at of 5 and 11% concentration (F=15.48; p=0.00)

NOTE - Extract preparation: * Sheep/Lamb/Liver: 200 g, respectively, of the fattest parts from lamb *L. thoracis*, calf liver and fattest parts from sheep *L. lomborum*, were minced and put in sealed plastic bags. The bags were first left in a water bath at 70°C for 4 hours and then frozen. Afterwards the bags were thawed in the microwave to 70°C. Finally they were hot filtered through a fine sieve. The extracts were kept at -20°C and thawed in the microwave before use. ** Powdered Milk: freeze-dried whole milk. *** Oxidised oil: oxidised almond oil (Temperature=50°C, artificial light for ten days).

Meat samples

The lamb meat subjected to sensory profiling came from the 6 countries involved in the EU project FAIR CT96-1768: France, Greece, Italy, Spain, United Kingdom and Iceland. Each country provided two lamb types to be analysed, as part of the first year FAIR project work. The 12 different types were chosen as being representative of the European lamb meat market and of various production methods and carcass characteristics. Details regarding breed, sex, age, carcass weight, and feeding adopted during the finishing period of the samples are reported in Table 3.

Each meat type was obtained from homogeneous groups of animals consisting of at least 120 lambs under controlled conditions. Lambs were slaughtered according to the welfare codes of practice adopted by the EU-licensed abattoirs in the country of origin and carcasses were kept at room temperature for six hours to avoid cold-shortening. At 24 hours post-mortem, sections of *Longissimus lumborum* (*LL*) were removed from the chilled carcasses, vacuum-packed, aged for 6 days at 2°-4°C, frozen at -20°C and then stored until sensory analysis. Eighteen samples for each lamb type (the *LL* of 18 different animals) were analysed by the panel during 18 evaluation sessions.

Sensory evaluation

The panel evaluated the different lamb meat types using

Table 2 - List of sensory attributes identified during vocabulary development.

Descriptor	Definition	Acronym
ODOUR	Procedure: separate the fat from lean and smell first the fat, then the lean	
Lamb in fat	Typical odour of cooked lamb meat	OF_lam
Sheep in fat	Typical odour of cooked sheep meat	OF_she
Abnormal in fat	Extraneous odours (all different odours from those above)	OF_abn
Lamb in lean	Typical odour of cooked lamb meat	OL_lam
Sheep in lean	Typical odour of cooked sheep meat	OL_she
Abnormal in lean	Extraneous odours (all different odours from those above)	OL_abn
FLAVOUR	Procedure: chew the lean and perceive the sensation during breathing out	
Sheep	Typical flavour of cooked sheep meat	F_she
Milk	Flavour associated with milk powder	F_mil
Liver	Flavour associated with liver	F_liv
Oxidised	Flavour associated with rancid fat	F_oxi
Fat	Flavour associated with cooked fat	F_fat
Fish flavour	Flavour associated with cooked fish	F_fis
Abnormal	Extraneous flavour (all different flavours from those above)	F_abn
TEXTURE	Procedure: perceive the sensation during chewing	
Tenderness	Force needed to bite the meat sample (in inverse relationship)	Ten
Juiciness	Humidity perceived during chewing	Jui
Fibrosity	Fibres (number and firmness of fibres) perceived during chewing	Fib
Chewiness	Energy to cut up the meat (until having the bolus ready to swallow)	Che

the vocabulary developed in the preliminary phase and reported in Table 2. Each attribute was rated on a 100 mm unstructured line anchored at each end.

Eighteen sessions were organised over a three-month period. During each session, the panel evaluated the 12 lamb types. In all, each judge evaluated 216 samples: 18 samples (the LL of 18 different animals) of each meat type (12). In order to reduce judge fatigue, each session was divided into two "sitting" periods of 20 minutes each, separated by a 15-minute rest time. During each sitting, 6 samples were given to the judges: one every three minutes. A balanced-block design for a 6-product test, 12 judges, 18 replicates was applied with the aim of minimising the time-order effect (MUIR and HUNTER, 1992).

In order to standardise meat preparation and serving, samples were thawed at 2°-3°C for 24 hours, cut in 2x2 cm blocks using the middle part of the still-frozen slices and then kept at room temperature for 3 hours before cooking. The 6 samples to be served in a given sitting period were roasted together on steel grills in an electric oven preheated to 120°C (1700 W of power, 15 cm grill distance from the heating elements of the oven). Meat core temperature was measured using a thermocouple probe placed in the centre of a reference meat block. Samples on the grill were turned over once the inner meat temperature had reached 50°-52°C and removed from the oven once they had reached 75°C. Cooked meat samples were wrapped in aluminium foil, labelled with 3 digit codes and kept at 60°C (by an air heater) until the assay.

Table 3 - The lamb types submitted to sensory profiling.

Lamb type code	Country	Genotype	Sex	Age (weeks)	Hot carcass (kg)	Feeding (finishing period)
1	United Kingdom	Suffolk Mule	Castrated male	16	18.1	Grass and clover pasture
2	United Kingdom	Welsh Mountain	Male	24	15.6	Perennial ryegrass pasture
3	Spain	Rasa Aragonesa	Male	11	10.3	Concentrates + straw
4	Spain	Churra 'Lechazo de Castilla y Leon'	Male	5	5.6	Milk
5	France	Texel, Ile-de-France, Charolais and crosses	Female	28	16.7	Lowland permanent pasture
6	France	Lacaune	Female	14	15.7	Concentrates + straw
7	Greece	Karagouniko	Male	7	8.5	Milk
8	Greece	Karagouniko	Male	18	15.7	Concentrates + lucerne hay
9	Iceland	Icelandic	Male	18	17.3	Natural highland pasture
10	Iceland	Icelandic	Female	18	16.9	Natural highland pasture
11	Italy	Bergamasca	Castrated male	60	31.2	Natural lowland pasture + crop residues
12	Italy	Appenninica	Male	10	11.3	Milk + concentrates

Data analysis

Results obtained during the vocabulary definition phase and the following analysis of actual samples were analysed separately by various statistical methods: a) Analysis of Variance (ANOVA) was applied to study the effects of samples and judges, b) correlation between each judge and the group mean without the assessor for each attribute (correlation coefficients) and the overall total correlation (for all attributes) was used to assess the agreement among the judges, c) Principal Component Analysis (PCA) performed on the data matrix of the attribute allowed the panel agreement on the meaning of each attribute to be assesed (DIJKSTERHUIS, 1995), d) Generalised Procrustes Analysis (GPA) on judges' scores provided a consensus configuration that minimises the differences among judges in the evaluation (DIJKSTERHUIS and GOWER, 1991; ARNOLD and WILLIAMS, 1985). All of these analyses were performed using Senstools, vers. 3.1 (OP&P Product Research BV, The Netherlands).

Moreover, discriminant Partial Least Square Analysis (dPLS) and Linear Discriminate Analysis (LDA) based on the work of BEEBE and KOWALSKI (1987) was applied to test the potentiality of sensory profiling for classifying samples belonging to different groups. This analysis was performed using the software Win-Das (John Wiley & Sons, Ltd: Chichester, UK) according to the method of KEMSLEY (1998).

RESULTS AND DISCUSSION

Vocabulary development and panel performance

In the preliminary phase of vocabulary development, the data from each test (Table 1) were submitted to one-way ANOVA: the results (F and p val-

ues) are shown in the last column on the right.

The panel was able to reliably separate the reference samples proposed in all the tests, with the exception of the test on "lamb" flavour (p=0.20).

Using the same set of data, the correlation of each assessor with the panel mean was evaluated for each attribute and globally (the overall total correlation over all attributes). There was a good level of agreement among the judges (correlation coefficient > 0.5). The highest values were obtained for the following descriptors: "tenderness", "chewiness", "fibrosity" and the "liver", "fat", "milk" and "oxidised" flavours.

Afterwards the panel agreement was verified on the complete data set of lamb samples (the 12 lamb types of Table 3 replicated in 18 sessions). The values of the judges' correlation coefficients were high for 13 out of 17 descriptors, while "juiciness" and the "oxidised", "liver" and "abnormal" flavours did not reach the threshold of 0.5. The highest correlation values (r > 0.9) were obtained for the texture descriptors, with the exception of the "juiciness" attribute. Among the odour attributes, the highest correlation coefficients were achieved for "lamb" (evaluated in both lean and fat), and "sheep meat", while "milk" and "fish" had the best correlation among flavours.

These results were confirmed by the percentage of variance explained by the first component of the PCA performed on the data matrix of each attribute (DIJKSTERHUIS, 1995). As shown in Fig. 1 the variance accounted for by the first PCA component (VAF%) was always more than 40%, with the exception of the "juiciness" attribute.

As for individual judge performance, all assessors showed a satisfactory overall correlation with the panel mean: the highest correlation value was obtained by judge 19 (r=0.8) and the lowest by judges 5 and 10 (r=0.6).

To check the reproducibility of the panel, we analysed the data collected in the preliminary session with four different lamb samples replicated three times. The 2-way ANOVA, on both the fac-

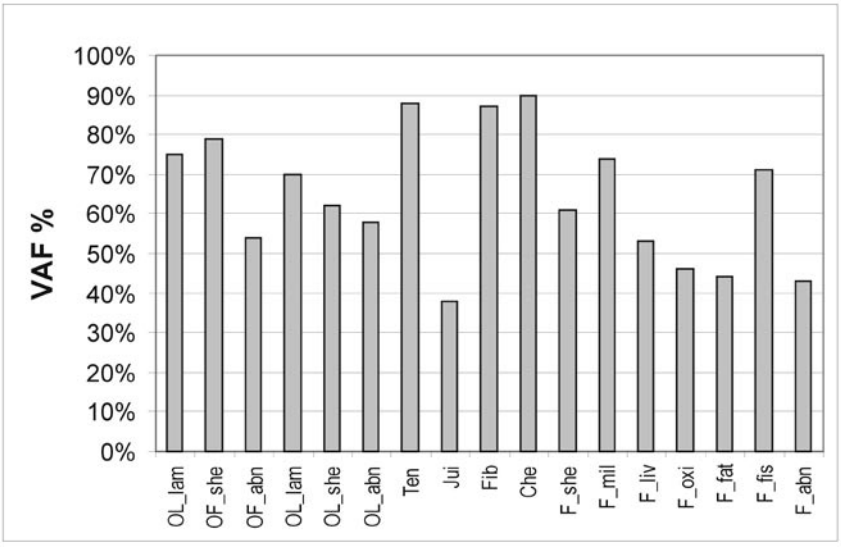


Fig. 1 - The "variance accounted for" in the 1st PCA dimension (VAF) evaluated on the data matrix of each attribute. The "percentage of variance explained" measures the agreement among the judges with respect to the meaning of the attribute. See Table 2 for meaning of abbreviations of attributes.

tors judge/replicate and product/replicate, indicated no significant differences among replicates in either case.

Fig. 2 shows the consensus space as described by GPA (first 2 dimensions account for 58% of total variance) for the three replicates of each sample. The axes of the ellipses in Fig. 2 show the standard deviations for each dimension. The three replicates were quite close for each sample which is further indication of good panel reproducibility.

Sensory profiles of lamb types

One-way ANOVA on sensory data of lamb samples showed significant differences among lamb types for all the attributes tested. Only "juiciness" and "oxidised" flavour had F values close to the lower significance levels (F=3.74 and 3.18, respectively), as expected on the basis of panel performance obtained for these attributes during the training phase. In particular three texture descriptors ("chewiness", "fibrosity" and "tenderness"), two odours ("lamb" and "sheep") and three flavours ("sheep", "fish" and "milk") are particularly relevant for sample discrimination.

In order to achieve a multidimensional description of these differences, GPA was applied on the sensory data, by averaging the replicates. In Fig. 3 the products

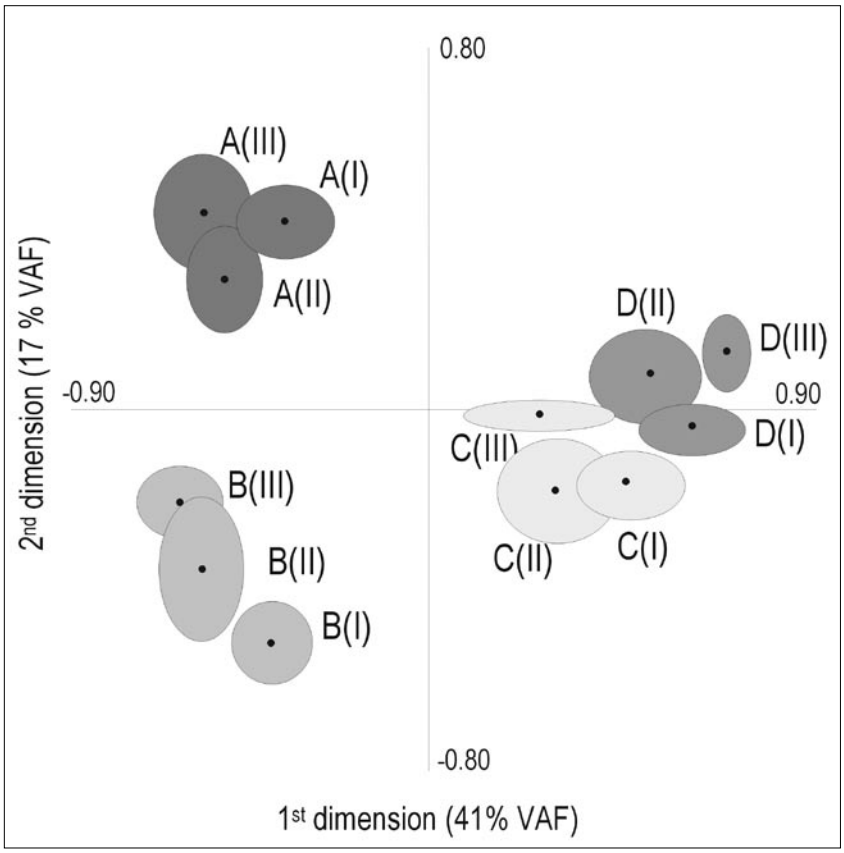


Fig. 2 - Sensory profiles of the samples analysed during the preliminary session to check panel performance computed by Generalised Procrustes Analysis: 1st dimension (41% VAF) x 2nd dimension (17% VAF). Four lamb meat type samples (coded by A, B, C, D) replicated three times (coded by I, II, III).

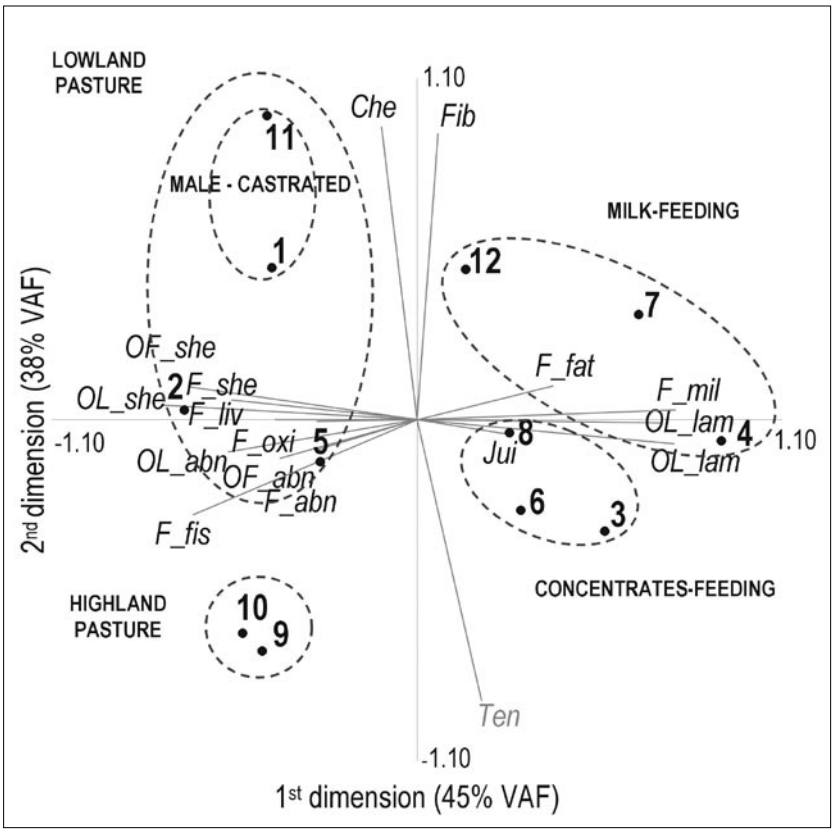


Fig. 3 - Sensory profiles of the lamb meat types performed by Generalised Procrustes Analysis (1st dimension (45% VAF) x 2nd dimension (38% VAF)). The lamb types are coded with numbers from 1 to 12, as indicated in Table 3, and the attributes, the segments starting from the axis origin, are marked with the abbreviations of Table 2.

and attributes are positioned in the consensus space defined by the first two dimensions which account for about 83% of the total variance (1st component: 45%; 2nd component: 38%).

The first dimension, highly correlated with the odour and flavour attributes, clearly separates the samples according to animal age and diet. The very young exclusively milk-fed animals (Spanish type 4 and Greek type 7), the Italian 10-week old lambs fed with milk and a concentrate supplement (sample 12) and the young animals fed with hay or straw and concentrates (Spanish type 3, French type 6 and Greek type 8) are on the right part of the plot (1st component positive). The heavier animals grazing on both highland pasture (Icelandic types 9 and 10) and lowland pasture (types 1 and 2 from the United Kingdom, type 5 from France and type 11 from Italy) are located on the opposite side (negative 1st component).

The first group (positive abscissa) is characterised by "lamb" odour in both fat and lean and also by "milk" flavour; other odours and flavours are important for the second group (negative abscissa): "sheep" and "liver", as well as extraneous flavours/odours ("oxidised" or "abnormal").

The 2nd dimension is highly correlated with texture attributes and introduces a further clustering. The two Icelandic lamb types 9 and 10, belonging to the Icelandic breed, are well-separated along the 2nd dimension axis from types 11 and 1 of the castrated lambs of the Italian-type obtained from Bergamasca and the English type Suffolk Mule, respectively. These last lamb types (samples 11 and 1) are more fibrous, less tender and less chewable. Otherwise, the Icelandic lamb meats (samples 9 and 10) are characterised by a higher tenderness and a "fish" flavour, as are the English meat types (samples 1 and 2).

To eventually propose and adopt a protocol for sensory quality/origin assessment of lamb meat in a practical context, it is important to evaluate whether the panel is able to correctly identify a sample with a certain defined characteristic. This is indeed the case and to show this let us consider, for example, the two groups that are well separated along the first GPA component: "pasture" (lowland and highland pasture) vs. "non-pasture" (milk/concentrate feeding). A more powerful analysis to develop a discriminating model for the sample can be obtained following the work of HOLLAND *et al.* (1998). In this case a modified Partial Least Square (discriminant Partial Least Square: dPLS) calibration on dummy variables associated with the groups provides a tool for identifying the relevance of each attribute to be useful to separate the groups. Moreover, this is the basis for the discriminant analysis used to assign each sample to the proper group. The number of samples that are assigned to the correct group gives a confidence value for the proposed classification method. The result of a dPLS analysis on sensory data of the "pasture" samples vs. "non-pasture" samples is shown in Fig. 4. Each point corresponds to a different sample (18 samples for each of the 12 kinds of lamb meat). Only a few samples are not

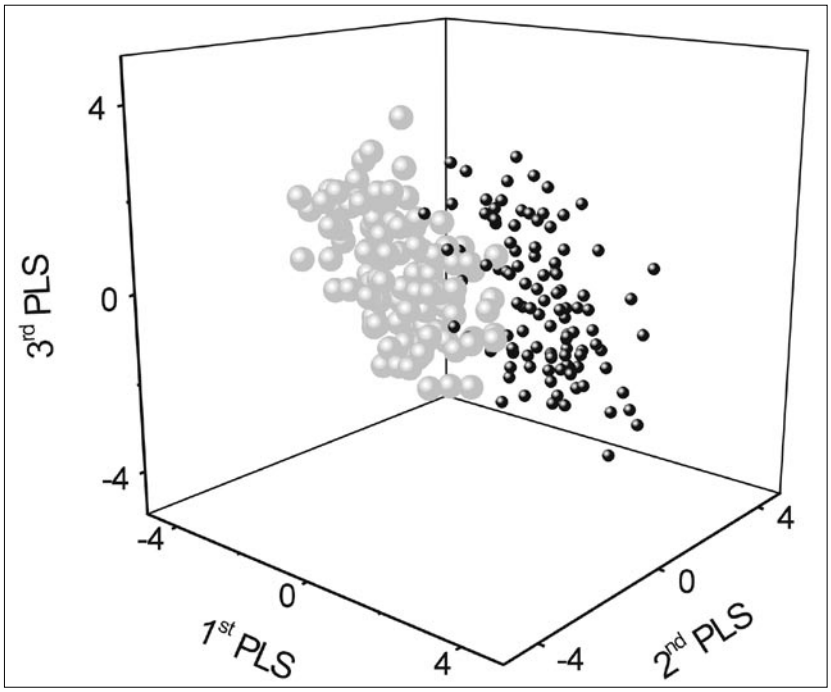


Fig. 4 - Discriminant Partial Least Squares analysis on all samples evaluated by the panel: 108 "pasture" samples (larger grey points) and 108 "non-pasture" samples (smaller black points).

correctly classified by linear discriminant analysis on these data (each point is associated with the closest group measuring the distance as Mahalanobis distances from the group centre). Using the 1st and 2nd PLS loadings, 5 "non-pasture" samples (95% correct re-assignment percentage) and 11 "pasture" samples (90% correct re-assignment percentage) are not assigned correctly. A useful by-product of this analysis is that the 1st loading of dPLS analysis (Fig. 5) give an indication about the relative contribution of each variable to the discrimination. Here the "lamb" odour perceived in both fat and lean and the "milk" and "fat" flavours (negative 1st PLS loading) characterise the samples with a negative 1st component ("non-pasture" samples, grey samples in Fig. 4), texture attributes do not seem to be relevant and the remaining ones characterise the other group. This analysis confirms the results of the GPA analysis (Fig. 3) and, at the same time, provides a way to classify unknown samples. Moreover the relative importance of the single attributes is evident and can be the basis for developing a quality control procedure based on the descriptive analysis of a reduced set of variables or on instrumental analysis tailored to the most relevant attributes.

CONCLUSIONS

A complete protocol for training and selecting a panel of sensory judges for the evaluation of lamb meat samples is described. The procedures have been checked and show good discriminating power, reliability and accuracy. Even though the development and testing of the panel has been mainly considered, the data suggest a significant correlation between sensory attributes and produc-

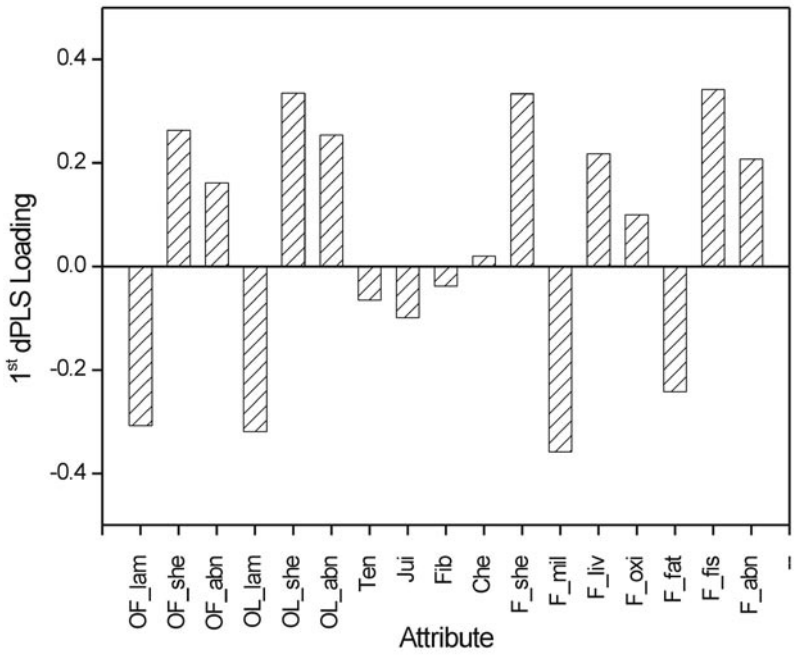


Fig. 5 - 1st loading of discriminant Partial Least Squares analysis shown in Fig. 4. Positive values characterise "pasture" samples (positive abscissa in Fig. 4) and negative values characterise "non-pasture" samples (negative abscissa in Fig. 4).

tion/management parameters. The developed methodology allows the most representative lamb meat types from the six countries participating in the EU project (FAIR III CT96 1768) to be evaluated. The results indicate the presence of a relationship between the sensory characterisation of the meat and the main management factors including breed, age and diet, confirming the panel performance and the usefulness of the approach. The texture attributes "tenderness", "fibrosity" and "chewiness" and the odour/flavour attributes "lamb", "milk", "sheep", "liver", "fat" and "fish" are the principal sources of variation.

Furthermore, discriminant analysis on sensory data provides a tool for classifying unknown samples and selecting a reduced set of attributes that can eventually be used to develop an easier/cheaper protocol for quality control in a practical context.

In conclusion, the main results of this work, are: i) the availability of a list of relevant attributes and reference products for evaluating lamb meat sensory characteristics, ii) a procedure for training and testing a sensory panel using these attributes, iii) the possibility of using this tool to identify the effect of the main production parameters (breed, sex, age, feeding) and, finally, iv) the development of predictive models to classify unknown samples on the basis of sensory data.

ACKNOWLEDGEMENTS

Research partly funded by the ECC FAIR III CT96 1768 Project.

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Revised paper received March 21, 2005 Accepted May 6, 2005

PAPER

MICROBIOLOGICAL QUALITY OF VEGETABLE PROTEINS DURING THE PREPARATION OF A MEAT ANALOG

QUALITÀ MICROBIOLOGICA DELLE PROTEINE DI ORIGINE VEGETALE DURANTE LA PREPARAZIONE DI UN PRODOTTO ANALOGO DELLA CARNE

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ABSTRACT

A canned vegetable protein product (or meat analog) consisting of wheat gluten, textured soy protein, hydrogenated vegetable fat and seasonings was analyzed to study the characteristics of the raw materials, estimate the microbial bioburden of the canned product prior to retorting and evaluate processing stages critical in the control of microbial growth. Two hundred and eighty-eight lots of this product were analyzed within a 24-month period. The highest aerobic mesophilic counts were

RIASSUNTO

Gli obiettivi di questo lavoro erano quelli di studiare le caratteristiche delle materie prime (proteine di soia strutturate e glutine di frumento) di un prodotto in scatola di origine vegetale, analogo della carne, valutare la carica microbica del prodotto inscatolato prima del processo di sterilizzazione e valutare i punti critici di processo, per il controllo della crescita microbica. Lotti (288) di questo prodotto sono stati analizzati durante un periodo di 24 mesi. I più alti livelli di flora batterica aerobica mesofi-

- Key words: Bioburden, canned food, meat analog, vegetable protein product -

4.96 log CFU/g for soy protein raw material, 6.76 log CFU/g for ground soy protein and 7.15 log CFU/g for canned meat analog before retorting at 121°C. The high bioburden level of textured soy protein and exposure of this raw material to conditions that promoted growth during processing caused an increase in the pre-processing bioburden. However, the sterility tests of the final product demonstrated the efficacy of the retorting process.

la riscontrati sono stati 4,96 log UFC/g per le proteine di soia grezze, 6,76 log UFC/g per la proteina di soia macinata e 7,15 log UFC/g per il prodotto analogo della carne inscatolato prima del trattamento di sterilizzazione a 121°C. L'alta carica microbica della proteina di soia e l'esposizione di questa materia prima a condizioni che hanno promosso la crescita microbica durante il processo di lavorazione hanno causato un incremento della carica microbica prima della sterilizzazione. Comunque, i test di sterilità condotti sul prodotto finito hanno dimostrato l'efficacia del processo di sterilizzazione.

INTRODUCTION

Vegetarianism is receiving more attention than ever before from both the general public and the food industry. There is a strong and growing demand for food that is natural, convenient and contains few additives. Although there has been a marked increase in the sale of vegetarian food, the safety of these products should be fully evaluated (MESSINA and MESSINA, 1997). In the United States, the "meat analog (and substitute)" category has achieved double-digit growth annually for the last five years (RAJARAM and SABATÉ, 2000). Scientific evidence points to a correlation between a vegetarian diet and reduced risk of such chronic diseases as obesity, coronary disease, hypertension, diabetes, and some types of cancer. Historically, vegetarian diets have met nutritional needs in many cultures, but other reasons for being vegetarian include religious beliefs, economic status, environmental and ecological concerns, and personal health.

Meat analogs are products processed from vegetable protein to resemble meat in texture and flavor. Meat analog products can be obtained via the following procedures: (i) spinning isolated soy protein into a "tow" of fibers, followed by shaping and flavoring to make meat-like products; (ii) processing formulated soy flour through an extruder into different sizes and shapes to give chewable products with the desired flavor, and (iii) by using heat and pressure during continuous extrusion of soy protein formula of small chunks which, when hydrated, present a chewable texture and a taste similar to meat. The principal advantage of this third method is the use of low cost soy flour in the preparation rather than the more expensive isolated protein (SMITH and CIRCLE, 1972).

Meat analogs are made from natural sources, such as soybeans, peanuts, wheat, and corn. The biological quality of the protein in the vegetable protein product should be at least 80 percent casein, which is determined by using the Protein Efficiency Ratio (PER) scale. The meat analog should contain at least 18 percent protein of the hydrated weight (w/w) after preparation (USDA, 1991).

Wheat gluten alone, when measured against the standard casein reference

(PER = 2.5) in rat bioassays, rates rather low on the PER scale, from 0.7 to 1.0 or 28 to 40% of the casein value. Vegetable proteins, in general, rate low on the PER scale because they frequently lack one or more essential amino acids. Blends of different vegetable proteins often result in higher PER values. For a wheat gluten/soy protein ratio of 30:70, a PER value of 2.4 is achieved, or roughly three times the rating of wheat gluten alone (AAOCC, 1964).

The canned vegetable protein product in this study consisted of wheat gluten, textured soy protein, hydrogenated vegetable fat, garlic, onion, salt, meat flavoring, caramel color and seasonings; it was processed to resemble ground meat in appearance and flavor. It is a ready-to-eat food, readily available in Brazilian supermarkets and is used in many vegetarian recipes. It is also used as a protein supplement, especially for children and adult lunch programs, at public institutions. The preservation of this canned product is assured by controlling the sanitary quality of the raw materials and the extent of heat processing.

The objectives of this paper were to study the characteristics of the raw materials (textured soy protein and wheat gluten), estimate the microbial bioburden of the canned product prior to retorting and to identify the critical processing stages to control the microbial growth.

MATERIALS AND METHODS

Meat analog manufacture

The product was prepared in a vegetable protein pilot plant (Superbom Food Industry, SP, BR), following a procedure illustrated in the flow diagram shown in Fig. 1. The raw materials (Table 1), in the proportion of 12% textured soy protein (53% protein; 1% fat; 6% moisture; 12.7 mm particle size) and 15% wheat gluten (77% protein; 0.7% fat; 1% ash; 7.5% moisture) were mixed in amounts necessary to prepare one batch of the final vegetable protein product. A production lot of approximately 200 kg corresponded to 250 metallic cans each containing 850 g of the final product. The textured soy protein was always acquired from the same supplier (Bunge Alimentos, SC, BR), as was the wheat gluten (Alvol, Mendoza, Argentina). The chemical characteristics of the raw material were analyzed by a nationally certified laboratory (Adolfo Lutz Laboratory) following the AOAC (1996).

Soy preparation

The textured soy protein was placed in a stainless steel tank (Quiminox, SP, Br) and uniformly hydrated with water (1:3 wt/wt). The mixture was maintained at 70°C for 20 min using steam provided from an open coil (perforated underside) located at the bottom of the tank. Propellers installed in the center of the tank stirred the mixture constantly. The hydrated soy protein was transferred to a basket (400 nylon mesh) attached to a bowl centrifuge (1.0 m diameter; Westfalia Separator, SP, Br) and was washed by continuous jets of water (3.0 m^3/h, at room temperature 25°-30°C) introduced in the center of the product mass, while being centrifuged at 1,200 rpm for 15 min. After washing, the soy protein was centrifuged at 1,200 rpm for an additional 20 min, to attain a mass moisture of around 60% wt/wt. Washing and drying of the soy protein was needed to reduce the natural off-flavor of soy in the final product. Soy protein was minced into particles of an average size of 1.5 cm with a rotary disc meat cutter (IbrasmaK, SP, Br) with knives (25 cm in diameter spaced 3.5 cm in the center). The ground soy protein was held for no longer than two hours before mixing with other ingredients at 45°C for 15 min.

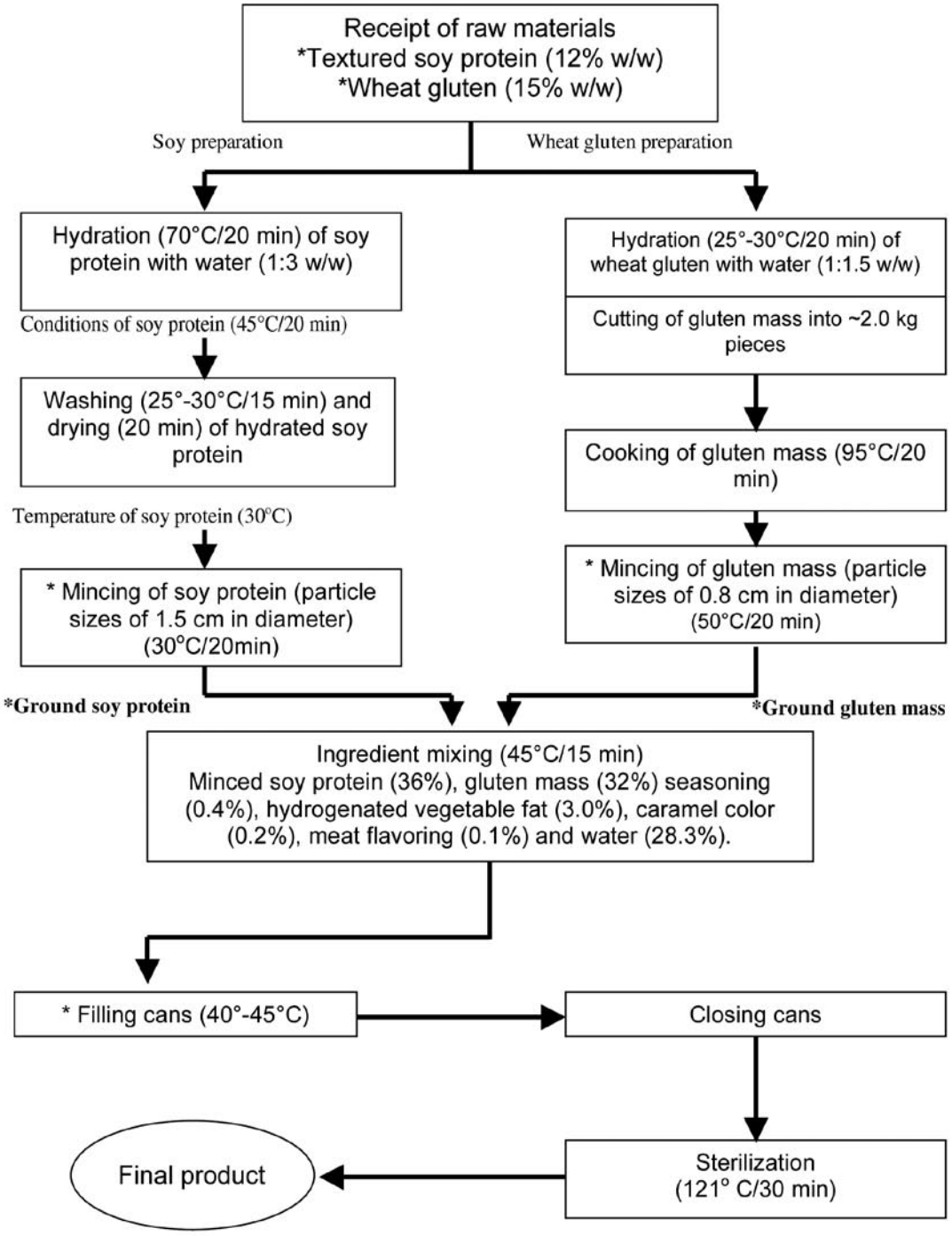


Fig. 1 - Flow diagram of vegetable protein production (*sampling points).

Table 1 - Chemical characteristics of the raw materials.

	Textured soy protein											
Year 2001	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Protein (N x 6.25) (dry basis)	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00
Fat (%)	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00
Carbohydrates (%)	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00
Fiber (%)	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00
Ash (%)	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00
Moisture (%)	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00
Year 2002												
Protein (N x 6.25) (dry basis)	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00	53.00± 0.00
Fat (%)	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00	1.00± 0.00
Carbohydrates (%)	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00	31.00± 0.00
Fiber (%)	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00	3.00± 0.00
Ash (%)	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00
Moisture (%)	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00
	Wheat gluten											
Year 2001	January	February	March	April	May	June	July	August	September	October	November	December
Protein (N x 5.7% (dry basis)	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00
Fat (%)	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00
Ash (%)	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
Moisture (%)	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00
Year 2002												
Protein (N x 5.7% (dry basis)	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00	77.00±0.00
Fat (%)	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00	0.70±0.00
Ash (%)	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
Moisture (%)	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00	7.50±0.00

CI = confidence interval (n = 12; p<0.05).

Gluten preparation

Wheat gluten was processed into a homogenous hydrated viscous mass with water (1:1.5 wt/wt) in a planetary blender (Usi Ram, SP, Br) at 80 rpm for 20 min, at a temperature of 25°-30°C. The gluten mass was then cut into pieces (about 2.0 kg/each) in a rotary disc meat cutter (IbrasmaK, SP, Br), and cooked in a water bath at 95°C for 20 min in the stainless steel tank (Quiminox, SP, Br), forming a gelatinous material stabilized by the denatured protein. The large gel pieces were cut into particles of 0.8 cm in diameter in the rotary disc cutter (IbrasmaK, SP, Br).

Mixture of ingredients and other steps

The minced soy protein (36%) and gluten mass (32%) were mixed with seasonings (0.4%), hydrogenated vegetable fat (3.0%), liquid caramel color (0.2%), meat flavors (0.1%) and water (28.3%) in a blender (Sigma-blade; 50 rpm; 250 L capacity; Usi Ram, SP, Br), at 45°C for 15 min to achieve the appearance of a moist dough with the light brown color characteristic of the meat analog product. Each cylindrical metal can (99x118 mm; with an inner facing of epoxiphenolic sanitary varnish and a lid for sealing) was filled with 850 g of the final product (kept at 40°-45°C) with an automatic filler (Welbba, SP, Br). The cans were placed in a mechanical vacuum-sealing machine, and the sealing process was completed in a vacuum of approximately 70 kPa. After passing through a warm (45°-50°C) water shower to remove any product adhering to the surface, the cans were distributed among three autoclave trucks (316 cans per truck) for sterilization (set at 121°C for 30 min, with total processing time of 88 min from initial ramping up to cool down).

Microbiological analyses

Microbiological analyses were performed on 288 lots covering two successive years of production. The number of viable microorganisms prior to sterilization was determined for each lot, and included analysis of the raw materials, the ground soy protein, the gluten mass, the ingredient mixture and the final canned meat analog product prior to retorting. Eight hundred and sixty-four samples were collected; sampling occurred during 4 production days per month with 9 samples being taken at different times throughout the production day. The samples (200-250 g) were aseptically collected in sterile bags in triplicate from every stage, as proposed by ANVISA (2001). Every effort was made to insure that the samples of the raw material were representative of all lots of the manufactured products.

Twenty-five grams from each sample was transferred into a sterile Stomacher bag (16.0x26.0 cm) and mixed with 225 mL of 0.1% (w/v) peptone water, homogenized in a Stomacher (Tekmar, Model 4001, Cincinnati, Ohio, USA) for 1 min. Serial decimal dilutions of 0.1% peptone water were prepared, and samples of appropriate dilutions were poured in duplicate on plate count agar ("PCA", Difco Laboratories, Sparks, MD, USA) plates for mesophilic (37°±2°C for 48h) and thermophilic (60°±2°C) counts. Dilutions were also plated on potato dextrose agar ("PDA", Difco) plates, acidified with sterile 10% tartaric acid (pH = 3.5) for yeast and mold (25°C for 72h) counts. The colony forming unit counts (CFU/g) were performed on plates of up to 200 colonies. Reinforced clostridial medium ("RCM", Difco) incubated at 35°±0.1°C for 72h in anaerobic jars (Gas-Pack System, Cockeysville, MD, USA) were used to measure anaerobic counts. For spore enumeration, samples were heated to 80°±0.2°C for 10 min prior to being poured. For total coliforms, each sam-

ple dilution was inoculated in triplicate into lauryl sulfate tryptose ("LST", Difco) tubes, which were incubated at 35°±1°C and the results are reported as the most probable number (MPN) of coliform bacteria per gram (APHA, 1992). The positive LST tubes were checked (i) for coliform bacteria by subculture and put into brilliant green bile ("BGB", Difco) broth tubes and incubated at 35°±0.5°C for 48±2 h; and (ii) for fecal coliform bacteria by subculture into *E. coli* ("EC", Difco) broth tubes, and incubated for 24±2 h at 45.5°±0.2°C in a controlled water bath.

Analysis of final retorted product

The final composition of the canned product was analyzed in accordance with AOAC (1996). The final retorted product was analyzed by the commercial sterility test by incubating 20 cans of each lot at 37°±2°C for 10 days. Cans were also examined for abnormal conditions such as leakage, swelling, and odor. The vacuum and pH were measured, and microscopic examinations were performed.

Microbiological tests of process water

The central industrial reservoir receives potable municipal water. The tank walls were sanitized with sodium hypochlorite (100 ppm total chlorine) biannually during mechanical cleaning. Water used in processing vegetable protein was treated with cartridge filters (1,000 L capacity, Acqua Domini, SP, Br) consisting of four layers (active charcoal with salts of colloidal silver, quartz, and dolomite). The water was tested according to Brazilian state and federal regulations and followed the methodology of APHA (1995). The free chlorine content in the processing water was monitored daily and maintained at 0.5-1.0 ppm. The analyses of standard aerobic mesophilic and coliform counts were conducted twice each week.

Microbiological testing of equipment, facilities and worker's hands

The analyses of samples from the equipment, facilities and worker's hands in the processing plant were performed according to Brazilian state and federal regulations and followed the methodology of APHA (1992).

Approximately 100 cm^2 per area was swabbed using a rayon-tipped applicator. Samples of surfaces of worker's hands were collected by the "swab contact method" and subjected to the same microbiological analyses as equipment swab samples. Before the first lot and at the end of the production day, the following analyses were performed after cleaning and sanitizing of the equipment: total count of mesophilic and coliform bacteria, *Staphylococcus aureus* (coagulase positive), yeasts and molds. Cleaning and sanitizing the equipment and facilities consisted of an initial rinse with potable water (45°C) to remove gross contamination, followed by washing with a 2% v/v alkaline (62% NaOH) non-ionic detergent solution (Quimistrol SU 186, Lever Industrial, SP, Br) at 45°C for 10 min and rinsing again with potable water. Next, the equipment and facilities were sanitized with a solution (1% v/v) of sodium hypochlorite (Divosan Sol L, pH 11.5; Lever Industrial SP, Br) at 5 ppm residual chorine for 5 min, followed by a water rinse.

Statistical analysis

All microbiological counts were converted to decimal logarithms for statistical analysis. Data were analyzed with the SGWIN program (Statgraphics Plus for Windows version 1.4 – Statistical graphics Corporation, 1995). The mean, standard deviation, and range were calculated for every n = 12 samples collected. Differences between means were detected by a one-way analysis of variance and are expressed as confidence inter-

vals (CI). Probabilities of ≤0.05 were considered significant.

RESULTS AND DISCUSSION

The canned meat analog (pH = 6.58±0.04) had a protein content greater than 18% which is the minimum required for vegetable protein products (SMITH and CIRCLE, 1972) in accordance with the following percentages of the product: protein (dry basis; Nx6.25%=20.69±0.80%), fat (1.92±0.37%), carbohydrates (2.90±0.34%), moisture (69.48±0.55%), fiber (1.47±0.18%), iron (1.65±0.12 mg/100 g) and phosphorous (63.30 mg/100 g), totaling 127.00±1.49 calories per 100 g of product. The high-protein and moisture content of the canned product make it a highly favorable medium for microorganisms before it is processed. It is essential to maintain sanitary conditions of the raw materials during the processing stages as this contributes to the final microbial bioburden of the product.

The microbiological characteristics for the water used in the different steps of preparation met the requirements of APHA (1995). The standard plate counts were less than 2 $\log_{10}$ CFU/mL of water analyzed and no coliforms were detected. Samples from the equipment and workers in the processing plant were negative for *S. aureus* and for yeasts and molds. In addition, coliforms and mesophilic groups were present at less than 2.0 $\log_{10}$ CFU/cm^2.

The samples of each raw material presented a range of standard counts in plates from a minimum of 0.50 $\log_{10}$ CFU/g to a maximum of 1.50 $\log_{10}$ CFU/g for wheat gluten; from a minimum of 4.61 $\log_{10}$ CFU/g to a maximum 4.96 $\log_{10}$ CFU/g for textured soy protein. Yeast and mold counts ranged from 0.42 to 1.75 $\log_{10}$ CFU/g for wheat gluten to an average of 2.0 $\log_{10}$ CFU /g for textured soy protein. The absence of coliforms and *S. aureus* in 1.0 g of analyzed sample, and of *Salmonella* spp. in 25 g of sample was certified in every raw material (Table 2). The high microbial populations present in the soy protein raw material shows a potential risk that must be controlled during processing.

KELLER *et al.* (2002) evaluated the efficacy of sanitation and cleaning methods in a small apple cider mill by surface swab methods as well as the microbiological characteristics of the incoming raw ingredients and the final product. They observed that, in general, the microbiological quality of the final product reflects the microbial load of the raw ingredients.

Soy preparation

After hydration, washing and grinding of the textured soy protein (Fig. 1), the average standard count increased by as much as 2 $\log_{10}$ cycles (Table 3) with respect to the microbial populations of the raw material. The highest total mesophilic aerobic count was observed in November 2002 (6.76±0.20), while the highest total mesophilic anaerobic count was observed in April 2001 (5.65±0.11 $\log_{10}$ CFU/g). The highest yeast and mold count was observed in September 2001 (3.64±0.16 $\log_{10}$ CFU/g). The total thermophilic anaerobic and aerobic counts were generally lower and ranged from 1 to 2 $\log_{10}$ CFU/g. Despite the apparent absence of coliforms within the soy protein raw material, an average population of greater than 3 $\log_{10}$ MPN/g for coliforms and fecal coliforms was observed in the ground soy protein. This increase may have resulted from the favorable temperature and time conditions present during the hydration and washing stages prior to retorting and /or the transfer of microorganisms from the equipment to the ground product.

At the end of hydration (70°C for 20 min) the temperature of the soy protein was approximately 45°C, an ideal

Table 2 - Microbiological characteristics of the raw materials. The counts are expressed in decimal logarithms.

	Textured soy protein(log CFU/g)											
Year 2001 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Standard plate count	4.61±0.20	4.92±0.23	4.92±0.23	4.94±0.28	4.92±0.23	4.90±0.21	4.94±0.28	4.96±0.25	4.92±0.23	4.69±0.27	4.90±0.21	4.92±0.23
*Yeasts and Molds (< 3.00)	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
*Coliforms (< 2.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Eschrichia coli* (< 1.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**S. aureus* (positive coagulase) (<2.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Salmonella* sp. (25 g sample)	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
Year 2002												
Standard plate count	4.61±0.20	4.92±0.23	4.92±0.23	4.94±0.28	4.92±0.23	4.90±0.21	4.94±0.28	4.96±0.25	4.92±0.23	4.69±0.27	4.90±0.21	4.92±0.23
*Yeasts and Molds (< 3.00)	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
*Coliforms (< 2.00 log NMP/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Escherichia coli* (< 1.00 log NMP/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**S. aureus* (positive coagulase) (<2.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Salmonella* sp. (25 g sample)	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
	Wheat gluten (log CFU/g)											
Year 2001	January	February	March	April	May	June	July	August	September	October	November	December
Standard plate count	0.58±0.38	0.67±0.44	0.67±0.28	1.00±0.42	1.00±0.42	1.50±0.30	1.25±0.35	0.58+ 0.29	1.42±0.29	0.58±0.29	0.58±0.29	0.75±0.26
*Yeasts and Molds (< 3.00)	1.67±0.00	1.25±0.00	1.25±0.00	1.75±0.00	1.75±0.00	1.50±0.00	1.50±0.00	1.58±0.00	1.50±0.00	0.83±0.00	1.00±0.00	1.50±0.00
*Coliforms (< 2.00 log NMP/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Escherichia coli* (< 1.00 log NMP/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**S. aureus* (positive coagulase) (<2.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Salmonella* sp. (25 g sample)	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent
Year 2002												
Standard plate count	0.50±0.30	0.92±0.45	0.67±0.28	0.92±0.38	1.00±0.00	1.50±0.30	1.00±0.00	0.50+ 0.00	0.75±0.26	1.25±0.00	0.92±0.45	1.00±0.42
*Yeasts and Molds (< 3.00)	0.42±0.00	0.83±0.00	0.58±0.00	0.58±0.00	1.17±0.00	1.25±0.00	0.25±0.00	0.75±0.00	1.50±0.00	1.08±0.00	1.58±0.00	1.17±0.00
*Coliforms (< 2.00 log NMP/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Escherichia coli* (< 1.00 log NMP/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**S. aureus* (positive coagulase) (<2.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
**Salmonella* sp. (25 g sample)	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent	absent

* Brasil, Resolution - ANVISA - Ministry of Health.
CI = confidence interval (n = 12; p<0.05).

temperature for coliform and fecal coliform development. Hydration of the soy protein is required for product quality and can promote microbial growth. The ground soy protein was stored for approximately 2 h prior to mixing the ingredients. This stage of production also contributed to the development of coliforms. Dried products (including the raw materials used here), in general, have been considered as one of the safest modes of food storage since the dry state does not promote microbial survival or growth. Low water activity (a_w < 0.85), among other factors (FUNG and GAILANI, 1986), prevents microbial development and may maintain populations at undetectable levels. Unfortunately, such hurdles are removed upon the addition of water for processing.

Although post-disinfection surface specifications for coliforms are not commonly available (because these values are likely to depend upon the environment, product and process) target values of <2.5 CFU/cm^2 have been suggested and are generally attainable for a range of surfaces (MOORE and GRIFFITH, 2002). It should be noted that the presence of very low concentrations (<2.0 $\log_{10}$ CFU/cm^2) of bacteria on surfaces may still be a significant risk factor for contamination thus emphasizing the importance of effective cleaning and disinfection procedures.

Wheat gluten preparation

In general, the ground gluten mass (Fig. 1) had lower viable counts (Table 4) compared to the soy protein (Table 3). The highest average counts were 3.19±0.18 (April 2002) and 1.83 $\log_{10}$ CFU/g (February and June 2001) for mesophilic aerobes and anaerobes, respectively. Less than or equal to 10 CFU/g were observed for all other counts, and fecal coliforms were never detected. It is likely that the cooking operation (95° for 20 min) required for the sliced hydrated gluten mass, helped to maintain its bioburden relatively unchanged from the raw material (Table 2).

Mixture of ingredients and filled canned product

The product had mean total mesophilic aerobes above 6.5 $\log_{10}$ CFU/g after mixing, with a high of 7.28 $\log_{10}$ CFU/g observed in December 2001 (Table 5). Total mesophilic anaerobic counts were above 5 $\log_{10}$ CFU/g, with a high of 6.21 $\log_{10}$ CFU/g observed in April of 2002. Similar results were observed in the canned product (Table 6), since the can filling process was automated and did not contribute to the bioburden of the final product. Mesophilic sporeforming aerobic and anaerobic plate counts were generally close to or higher than 4 $\log_{10}$ CFU/g in the mixed (Table 5) and filled (Table 6) product. Thermophilic aerobic and anaerobic counts increased by about 1 $\log_{10}$ CFU/g during mixing (Table 5) relative to the counts in the ground soy protein (Table 3), most likely due to the presence of thermophilic organisms in the non-protein ingredients. Other than this exception, counts of other organisms were generally similar to those obtained in the ground soy protein.

TESSI *et al.* (2002) evaluated the microbiological quality and safety of ready-to-eat cooked foods from a centralized school kitchen in Argentina. The detection of large aerobic and *Enterobacteriaceae* counts in ready-to-eat cooked foods showed that improvements in manufacturing, cooking and delivery were needed to enhance the microbiological safety of these foods. This study highlighted the importance of the control and maintenance of appropriate temperatures during various stages of the food preparation process.

Raw materials have been reported as the primary sources of microbial contamination during the processing of various types of food (FUNG and GAILANI,

Table 3 - Microbiological characteristics of the ground soy protein. The counts are expressed in decimal logarithms (log CFU/g).

Year 2001 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Total mesophilic aerobes	5.66±1.06	5.54±1.07	4.77+ 0.22	6.63±0.12	6.69±0.41	5.96±0.57	4.68±0.62	5.61±1.14	5.45±0.70	5.07±0.30	6.04±0.40	5.87±0.49
Total mesophilic anaerobes	5.08±0.44	5.23±0.74	4.68±0.07	5.65±0.11	5,59±0,18	5.39±0.80	5,18±0,01	5.18±0.74	5.42±0.13	4.94±0.36	5.41±0.66	5.36±0.44
Total thermophilic aerobes	1.50±0.40	1.92±0.00	1.50±0.40	1.58±0.40	1,17±0,00	1.83±0.00	1.50±0.40	1.67±0.00	1.67±0.00	1.25±0.00	1.58±0.00	1.50±0.40
Total thermophilic anaerobes	1.25±0.00	1.42±0.00	1.27±0.00	1.25±0.00	1,42±0,00	1.25±0.00	1.55±0.40	1.50±0.40	1.42±0.00	1.42±0.00	1.25±0.40	1.25±0.00
Yeast and Molds	2.74±0.40	3.02±0.26	3.36±0.11	3.50±0.10	2,96±0,22	2.87±0.11	3.12±0.48	3.01±0.26	3.64±0.16	3.47±0.06	3.31±0.08	2.74±0.40
Coliforms (log MPN/g)	3.15±0.19	3.32±0.11	3.07±0.49	3.53±0.16	3,50±0,02	3.50±0.02	3.30±0.04	3.32±0.11	3.53±0.16	3.43±0.08	3.54±0.14	3.13±0.19
Fecal Coliforms (log MPN/g)	3.76±0.08	3.72±0.14	3.73±0.07	3.16±0.48	3,43±0,12	3.25±0.22	3.06±0.29	3.72±0.14	3.22±0.26	3.30±0.00	3.48±0.03	3.76±0.08
Mesophilic sporeforming aerobes	3.81±0.17	3.95±008	4.21±0.31	4.33±0.05	3,82±0,02	3.84±0.29	3.64±0.07	3.95±0.08	4.24±0.18	4.20±0.12	3.87±0.37	3.83±0.13
Mesophilic sporeforming anaerobes	4.06±0.21	3.60±0.53	4.12±0.15	4.30±0.05	4,11±0,18	4.20±0.14	3.37±0.70	3.64±0.53	4.27±0.05	4.30±0.14	4.21±0.14	4.15±0.29
Thermophilic sporeforming aerobes	0.42±0.40	0.58±0.00	0.92±0.40	0.50±0.40	0,50±0,40	0.92±0.00	0.75±0.00	0.58±0.00	0.42±0.00	0.67±0.00	0.67±0,00	0.50±0.00
Thermophilic sporeforming anaerobes	0.58±0.40	0.50±0.00	0.67±0.00	0.67±0.00	0.58±0.00	0.83±0.40	0.67±0.00	0.50±0.00	0.67±0.40	0.58±0.00	0.83±0.00	0.58±0.00
Year 2002 **Groups of Microorganisms**	**January** **Mean±CI**	**February** **Mean±CI**	**March** **Mean±CI**	**April** **Mean±CI**	**May** **Mean±CI**	**June** **Mean±CI**	**July** **Mean±CI**	**August** **Mean±CI**	**September** **Mean±CI**	**October** **Mean±CI**	**November** **Mean±CI**	**December** **Mean±CI**
Total mesophilic aerobes	6.13±0.63	5.83±0.62	5.91±0.45	5.79±0.79	6.27±0.55	4.88±0.57	5.94±0.36	6.47±0,12	5.99±0.61	5.77±0.74	6.76±0.20	4.95±0.62
Total mesophilic anaerobes	5.49±0.30	5.37±0.32	5.16±0.27	5.27±0.54	5.14±0.64	4.75±0.20	5.38±0.66	5.22±0.17	5.12±0.17	5.09+ 0.68	5.14+ 0.64	4.54±0.06
Total thermophilic aerobes	1.58±0.40	1.58±0.00	1.67±0.40	1.67±0.40	1.58±0.00	1.58±0.40	1.58±0.00	1.83±0.40	1.58±0.40	1.67±0,00	1.58±0.00	1.42±0.40
Total thermophilic anaerobes	1.42±0.00	1.33±0.00	1.42±0.00	1.50±0.40	1.42±0.40	1.25±0.00	1.25±0.40	1.25±0.00	1.33±0.00	1.58±0,40	1.67±0.40	1.27±0.00
Yeast and Molds	3.20±0.26	3.38±0.07	3.32±0.16	3.04±0.26	3.02±0.26	3.42±0.10	3.47±0.08	3.55±0.10	2.89±0.10	3.09±0,26	3.50±0.26	3.39±0.00
Coliforms (log MPN/g)	3.45±0.15	3.07±0.50	3.54±0.14	3.37±0.11	3.34±0.10	3.11±0.40	3.52±0.13	3.53±0.15	3.46±0.02	3.34±0,10	3.54±0.29	3.14±0.49
Fecal Coliforms (log MPN/g)	3.45±0,12	3.82±0.29	3.58±0.03	3.73±0.14	3.72±0.14	3.73±0.07	3.48±0.03	3.16±0.48	3.25±0.21	3.72±0,14	3.72±0.14	3.78±0.28
Mesophilic sporeforming aerobes	4.00±0.10	4.36±0.31	3.87±0.37	3.97±0.04	3.95±0.08	4.20±0.17	3.93±0.28	4.34±0.04	3.80±0.16	3.95±0,08	3.98±0.00	4.31±0.19
Mesophilic sporeforming anaerobes	4.16±0.08	4.34±0.10	4.24±0.12	3.74±0.63	3.71±0.68	4.12±0.15	4.16±0.14	4.41±0.37	4.25±0.14	3.65±0.53	3.76±0.56	4.19±0.15
Thermophilic sporeforming aerobes	0.50±0.00	1.00±0.00	0.50±0.00	0.67±0.00	0. 58±0.00	0.83±0.00	0.83±0.00	0.58±0.00	0.83±0,00	0.42±0.00	0.50±0.00	0.83±0.00
Thermophilic sporeforming anaerobes	0.58±0.00	0.58±0.00	0.75±0.00	0.50±0.00	0.58±0.00	0.67±0.00	0.67±0.00	0.65±0.00	0.83±0,00	0.58±0.00	0.50±0.00	0.50±0.00

CI = confidence interval (n = 12; p<0,05).
MPN = most probable number.

Table 4 - Microbiological characteristics of the ground gluten mass. The counts are expressed in decimal logarithms (log CFU/g).

Year 2001 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Total mesophilic aerobes	2.71±0.23	2.93±0.22	3.15±0.00	3.17±0.00	2.96+ 0.44	3.08±0.40	2.86±0.01	2.74±0.04	3.04±0.16	3.12±0.24	3.18±0.02	3.14±0.18
Total mesophilic anaerobes	1.50±0.40	1.83±0.00	1.25±0.00	1.67±0.00	1.67±0.00	1.83±0.00	1.67±0.40	1.50+ 0.00	1.50±0.40	1.75±0.00	1.42±0.40	1.50±0.40
Total thermophilic aerobes	0.58±0.00	0.67±0.40	0.58±0.00	0.58±0.00	0.50±0.00	0.67±0.00	0.58±0.00	0.50±0.00	0.50±0.40	0.58±0.00	0.58±0.00	0.58±0.00
Total thermophilic anaerobes	0.58±0.40	0.75±0.00	0.58±0.40	0.58±0.40	0.58±0.40	0.58±0.40	0.67±0.00	0.58±0.40	0.58±0.40	0.58±0.40	0.58±0.40	0.50±0.40
Yeast and Molds	0.92±0.40	1.00±0.00	0.92±0.40	0.92±0.40	0.92±0.40	1.00±0.00	0.92±0.40	0.92±0.40	0.92±0.40	0.83±0.00	0.92±0.40	0.92±0.40
Coliforms (log MPN/g)	0.92±0.00	0.83±0.00	0.75±0.00	0.92±0.40	0.92±0.00	0.75±0.40	0.92±0.00	0.92±0.00	0.83±0.00	0.83±0.00	0.83±0.00	0.75±0.00
Fecal Coliforms (log MPN/g)	absence	absence	absence	absence	absence	absence	absence	absence	absence	absence	absence	absence
Mesophilic sporeforming aerobes	0.92±0.00	0.92±0.00	1.00±0.00	0.92±0.00	0.58±0.40	0.92±0.00	0.75±0.00	0.83±0.00	0.83±0.40	0.83±0.00	1.00±0.00	0.92±0.00
Mesophilic sporeforming anaerobes	0.92±0.00	0.92±0.00	0.92±0.00	0.92±0.00	0.92±0.00	0.92±0.00	0.92±0.00	0.83±0.00	0.83±0.00	0.58±0.00	0.92±0.00	0.83± 0.00
Thermophilic sporeforming aerobes	1.00±0.00	0.83±0.00	0.92±0.00	0.92±0.00	1.00±0.00	0.92±0.40	0.92±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	0.92±0.00
Thermophilic sporeforming anaerobes	0.83±0.00	0.33±0,40	0.75±0.00	0.75±0.00	0.58± 0.00	0.75±0.40	0.75±0.40	0.67±0.00	0.58±0.40	0.50±0.40	0.67±0.00	0.75±0.40
Year 2002 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Total mesophilic aerobes	3.17±0.32	2.58±0.00	2.91±0.20	3.19±0.18	3.09±0.00	2.71±0.23	3.18±0.10	3.17±0.39	3.07±0.08	3.14±0.05	2.86±0.40	2.88±0.40
Total mesophilic anaerobes	1.75±0.00	1.50±0.00	1.33±0.40	1.50±0.40	1.67±0.00	1.50±0.40	1.33±0.40	1.67±0.00	1.50±0.40	1.50±0.40	1.67±0.00	1.25±0.40
Total thermophilic aerobes	0.58±0.00	0.50±0.00	0.50±0.40	0.58±0.00	0.58±0.00	0.58±0.00	0.58±0.00	0.50±0.00	0.58±0.00	0.58±0.00	0.58±0.00	0.58±0.00
Total thermophilic anaerobes	0.50±0.40	0.58±0.40	0.67± 0.40	0.58±0.40	0.67±0.00	0.58±0.40	0.42±0.40	0.58±0.40	0.58±0.40	0.58±0.40	0.42±0.40	0.83±0.00
Yeast and Molds	0.92±0.40	0.92±0.40	0.92±0.40	0.92±0.40	0.92±0.40	0.92±0.40	0.92±0.40	0.92±0.00	0.83±0.40	0.92±0.40	0.83±0.40	0.83±0.00
Coliforms (log MPN/g)	0.83±0.00	0.92±0.00	0.92±0.00	0.75±0.00	0.92±0.00	0.92±0.00	0.75±0.40	0.83±0.00	0.75±0.00	0.83±000	0.67±0.40	0.83±0.00
Fecal Coliforms (log MPN/g)	absence	absence	absence	absence	absence	absence	absence	absence	absence	absence	absence	absence
Mesophilic sporeforming aerobes	0.92±0.00	0.83±0.00	0.67±0.40	0.83±0.40	0.75±0.00	0.92±0.00	0.92±0.00	0.83±0.00	1.00±0.00	1.00±0.00	0.83±0.00	0.67±0.40
Mesophilic sporeforming anaerobes	0.83±0.00	0.83±0.00	0.92±0.00	0.83±0.00	0.92±0.00	0.92±0.00	0.83±0.00	0.92±0.00	0.92±000	0.92±0.00	0.75±0.00	0.75±0.00
Thermophilic sporeforming aerobes	0.75±0.00	0.83±0.00	0.92±0.00	0.75±0.00	0.92±0.00	1.00±0.00	1.00±0.00	1.00±0.00	0.92±0.00	0.92±0.00	1.00±0.00	0.67±0.00
Thermophilic sporeforming anaerobes	0.67±0.00	0.75±0.40	0.67±0.00	0.58±0.40	0.83±0.00	0.83±0.00	0.58±0.40	0.67±0.40	0.67±0.00	0.67±0.40	0.58±0.00	0.67±0.00

CI = confidence interval (n = 12; p<0.05).
MPN = most probable number.

Table 5 - Microbiological characteristics of the mixture of ingredients. The counts are expressed in decimal logarithms (log CFU/g).

Year 2001 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Total mesophilic aerobes	6.85±0.42	6.89±0.32	6.52±0.28	7.03±0.54	6.78±0.07	7.15±0.12	7.09±0.20	7.06±0.06	7.00±0.02	7.11±0.08	6.86±0.33	7.28±0.12
Total mesophilic anaerobes	5.52±0.20	5.25±0.64	5.88±0.64	5.74±0.77	5.42±0.68	6.03±0.14	5.11±0.24	5.83+ 0.66	5.58±0.68	6.03±0.02	6.13±0.36	6.02±0.64
Total thermophilic aerobes	2.62±0.91	2.75±0.55	2.94±0.37	2.77±0.58	2.93±0.37	2.82±0.52	3.00±0.15	3.18±0.15	3.20±0.16	2.98±0.12	2.88±0.52	3.04±0.44
Total thermophilic anaerobes	2.93±0.40	3.13±0.09	2.93±0.52	3.19±0.09	3.20±0.18	3.07±0.16	3.12±0.06	3.26±0.03	2.93±0.54	3.12±0.12	3.23±0.02	3.19±0.09
Yeast and Molds	2.95±0.34	3.10±0.28	3.35±0.20	3.14±0.20	3.20±0.21	3.20±0.18	3.13±0.28	3.22±0.17	3.38±0.06	3.22±0.14	3.13±0.34	3.18±0.12
Coliforms (log MPN/g)	3.15±0.22	3.30±0.15	3.29±0.06	3.36 + 0.15	3.35±0.12	3.17±0.15	3.28±0.15	3.38±0.00	3.29±0.05	3.17±0.16	3.20±0.37	3.37±0.18
Fecal Coliforms (log MPN/g)	3.43±0.44	3.37±0.08	3.32±0.02	3.62 + 0.52	3.45±0.06	3.43±0.44	3.37±0.08	3.46±0.03	3.37±0.01	3.44±0.44	3.50±0.30	3.68±0.52
Mesophilic sporeforming aerobes	3.54±0.55	4.01±0.48	4.07±0.46	3.69±0.12	4.13±0.44	3.76±0.15	4.07±0.48	4.31±0.12	4.20±0.14	3.80±0.15	3.63±0.43	3.73±0.12
Mesophilic sporeforming anaerobes	3.79±0.16	3.95±0.31	3.94±0.07	4.03±0.18	4.05±0.08	3.82±0.05	3.98±0.28	4.08±0.08	3.98±0.05	3.87±0.05	3.79±0.16	3.99±0.18
Thermophilic sporeforming aerobes	0.75±0,00	0.75±0.40	1.00±0.00	0.58±0.40	1.00±0,00	0.58±0.40	0.59±0.00	0.50±0.00	0.75±0.00	0.67±0.40	0.67±0.00	0.50±0.40
Thermophilic sporeforming anaerobes	0.58±0,00	0.92±0.00	0.92±0.00	0.50±0.00	0.92±0,00	0.58±0.00	0.92±0.00	0.92±0,00	0.95±0.00	0.75±0.00	0.58±0,00	0.50±0.00
Year 2002 **Groups of Microorganisms**	**January** **Mean±CI**	**February** **Mean±CI**	**March** **Mean±CI**	**April** **Mean±CI**	**May** **Mean±CI**	**June** **Mean±CI**	**July** **Mean±CI**	**August** **Mean±CI**	**September** **Mean±CI**	**October** **Mean±CI**	**November** **Mean±CI**	**December** **Mean±CI**
Total mesophilic aerobes	6.78±0.18	6.71±0.20	7.06±0.26	7.18±0.00	7.01±0.12	6.64±0.58	7.18±0.22	7.20±0.04	7.01±0.02	6.79±0,01	7.04±0.28	6.89±0.40
Total mesophilic anaerobes	5.03±0.24	5.89±0.78	5.45±0.72	6.21±0.14	6.18±0.14	5.20±0.20	6.07±0.58	6.15±0.74	6.08±0.80	5.42±0.68	5.55±0.78	5.65±0.40
Total thermophilic aerobes	2.92±0.52	3.08±0,00	3.00±0.40	2.85±0.54	3.10±0.34	2.74±0.38	3.01±0.54	2.92±0.58	2.96±0.32	2.96±0.37	3.20±0.10	3.09±0.06
Total thermophilic anaerobes	2.94±0.34	3.04±0.56	2.95±0.52	3.11±0.12	3.13±0.03	3.25±0.00	3.27±0.01	3.16±0.06	3.11±0.12	3.22±0.18	3.17±0.06	3.15±0.18
Yeast and Molds	3.19±0.18	3.56±0.19	3.13±0.38	3.31±0.08	3.28±0.22	3.26±0.15	3.22±0.10	3.27±0.15	3.38±0.08	3.20±0.21	3.28±0.00	3.46±0.15
Coliforms (log MPN/g)	3.34±0.15	3.37±0.03	3.27±0.00	3.29±0.02	3.31±0.02	3.40±0.15	3.41±0.18	3.37±0.18	3.25±0.20	3.37±0.12	3.43±0.05	3.29±0.18
Fecal Coliforms (log MPN/g)	3.44±0.00	3.28±0.08	3.30±0.35	3.44±0.19	3.71±0.16	3.62±0.52	3.68±0.52	3.68±0.51	3.44±0.18	3.45±0.06	3.69±0.38	3.50±0.23
Mesophilic sporeforming aerobes	4.15±0.28	4.10±0.52	4.09±0.38	3.86±0.03	3.56±0.26	3.71±0,04	3.74±0.12	3.73±0.12	3.93±0.02	4.15±0.44	3.82±0.14	3.84±0.03
Mesophilic sporeforming anaerobes	4.07±0.04	4.05±0.22	3.94±0.34	3.77±0,29	3.91±0.00	4.06±0.20	4.02±0.12	3.94±0.06	3.71±0.00	4.06±0.02	4.04±0.16	3.85±0.05
Thermophilic sporeforming aerobes	0.67±0.40	0.75±0.40	0.42±0.40	0.58±0.00	0.33±0.40	0.33±0.00	0.25±0.00	0.42±0.00	0.42±0.40	0.92±0.00	0.75±0.40	0.67±0.40
Thermophilic sporeforming anaerobes	0.67±0,00	0.58±0.00	0.67±0.00	0.58±0.00	0.58±0.00	0.83±0.00	0.58±0.40	0.50±0.00	0.92±0.00	0.92±0.00	0.50±0.00	0.58±0.00

CI = confidence interval (n = 12; p<0.05).
MPN = most probable number.

Table 6 - Microbial characteristics of the canned product. The counts are expressed in decimal logarithms (log CFU/G).

Year 2001 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Total mesophilic aerobes	6.24±0.58	6.83±0.40	6.87±0.56	6.96±0.16	6.80±0.42	6.61±0.40	7.00±0.00	6.77±0.30	6.74±0.02	6.95±0.38	7.01±0.02	6.67±0.40
Total mesophilic anaerobes	5.91±0.56	5.48±0.42	5.42±0.72	5.13±0.10	5.60±0.40	5.68±0.72	5.81±0.52	5.37±0.80	5.67±0.15	5.85±0.30	6.14±0.12	6.06±0.38
Total thermophilic aerobes	1.42±0.80	1.17±0.00	1.33±0.00	1.50±0.40	0.58±0.40	1.08±0.40	1.67±0.00	0.92±0.40	1.25±0.80	1.17±0.40	1.50±0.40	1.50±0.40
Total thermophilic anaerobes	1.58±0.00	1.08±0.40	1.58±0.00	1.00±0.80	1.08±0.40	1.25±0.40	1.33±0.00	0.92±0.80	1.25±0.80	1.42±0.00	1.33±0.40	1.50±0.00
Yeast and Molds	2.87±0.40	3.03±0.28	3.24±0.40	3.39±0.12	3.40±0.32	3.29±0.45	3.44±0.20	3.11±0.12	3.47±0.52	3.46±0.20	3.52±0.20	3.04±0.40
Coliforms (log MPN/g)	3.17±0.03	3.17±0.03	3.28±0.00	3.31±0.00	3.33±0.00	3.31±0.00	3.32±0.03	3.32±0.00	3.32±0.00	3.37±0.03	3.32±0.03	3.17±0.03
Fecal Coliforms (log MPN/g)	3.26±0.00	3.32±0.03	3.18±0.12	3.32±0.00	3.29±0.00	3.34±0.04	3.28±0.03	3.36±0.00	3.35±0.03	3.31±0.03	3.27±0.03	3.34±0.00
Mesophilic sporeforming aerobes	4.19±0.12	4.28±0.00	4.33±0.00	4.41±0.00	4.46±0.04	4.40±0.04	4.41±0.00	4.41±0.03	4.39±0.02	4.47±0.02	4.43±0.00	4.23±0.06
Mesophilic sporeforming anaerobes	4.20±0.12	4.19±0.04	4.25±0.04	4.28±0.02	4.28±0.02	4.24±0.40	4.29±0.08	4.22±0.10	4.27±0.00	4.30±0.02	4.30±0.08	4.22±0.06
Thermophilic sporeforming aerobes	0.83±0.00	0.67±0.40	0.67±0.00	0.67±0.00	0.67±0,40	0.75±0.00	0.58±0.00	0.75±0.40	0.50±0.40	0.92±0.00	0.42±0.00	0.17±0.00
Thermophilic sporeforming anaerobes	0.67±0.00	0.67±0.00	0.67±0.00	0.58±0.00	0.50±0.40	0.42±0.40	0.67±0.00	0.67±0,40	0.50±0.40	0.58±0.40	0.58±0,00	0.67±0.00
Year 2002 Groups of Microorganisms	January Mean±CI	February Mean±CI	March Mean±CI	April Mean±CI	May Mean±CI	June Mean±CI	July Mean±CI	August Mean±CI	September Mean±CI	October Mean±CI	November Mean±CI	December Mean±CI
Total mesophilic aerobes	6.91±0.18	6.95±0.02	7.15±0.08	7.06±0.02	6.65±0.16	7.14±0.00	7.14±0.05	7.01±0.06	7.00±0.00	7.05±0,00	7.07±0.02	7.05±0.00
Total mesophilic anaerobes	5.50±0.76	5.90±0.46	5.88±0.32	5.99±0.35	6.10±0.38	5.46±0.30	5.53±0.76	5.90±0.62	6.02±0.32	5.70±0.10	5.96±0.52	5.67±0.70
Total thermophilic aerobes	1.42±0.80	1.83±0,00	1.17±0.40	1.58+ 0.40	1.58±0.40	1.50±0.40	1.33±0.80	1.58±0.00	1.83±0.40	1.50±0.00	1.67±0.00	1.67±0.00
Total thermophilic anaerobes	1.42±0.80	1.58±0.40	1.42±0.40	1.42±0.00	1.42±0.40	1.42±0.80	1.33±0.80	1.67±0.40	1.67±0.40	1.33±0.80	1.50±0.40	1.42±0.40
Yeast and Molds	3.53±0.32	3.49±0.08	3.49±0.20	3.50±0.04	3.09±0.18	3.35±0.00	3.57±0.32	3.48±0.04	3.52±0.08	3.34±0.03	3.56±0.10	3.24±0.52
Coliforms (log MPN/g)	3.33±0.00	3.31±0.03	3.36±0.00	3.33±0.00	3.17±0.03	3.35±0.00	3.36±0.00	3.35±0.00	3.36±0.00	3.32±0.00	3.34±0.03	3.33±0.03
Fecal Coliforms (log MPN/g)	3.36±0.03	3.29±0.03	3.31±0.00	3.29±0.03	3.38±0.00	3.32±0.00	3.37±0.03	3.32±0.03	3.30±0.00	3.31±0.00	3.32±0.00	3.31±0.03
Mesophilic sporeforming aerobes	4.43±0.04	4.41±0.00	4.45±0.02	4.44 + 0.00	4.25±0.06	4.45±0,06	4.40±0.04	4.45±0.40	4.43±0.02	4.43±0.06	4.42±0.02	4.42±0.00
Mesophilic sporeforming anaerobes	4.29±0.00	4.28±0.08	4.32±0.00	4.31±0,08	4.22±0.06	4.29±0.00	4.30±0.06	4.33±0.14	4.29±0.08	4.29±0.02	4.34±0.14	4.33±0.14
Thermophilic sporeforming aerobes	0.83±0.40	0.50±0.40	0.75±0.00	0.58±0.40	0.50±0.40	0.50±0.00	0.58±0.00	0.58±0.40	0.67±0.00	0.58±0.00	0.67±0.40	0.50±0.40
Thermophilic sporeforming anaerobes	0.67±0,40	0.67±0.00	0.42±0.40	0.67±0.00	0.67± 0.00	0.50±0.00	0,67±0,40	0.67±0.00	0.67±0.00	0.67±0.00	0,67±0.00	0.67±0.00

CI = confidence interval (n = 12; p<0.05).
MPN = most probable number.

1986; KELLER *et al.*, 2002). Microorganisms from the raw materials accumulate on the equipment and work surfaces, and are therefore distributed to all products passing through the system (MOORE and GRIFFITH, 2002).

Retorting canned meat analog involves stabilizing the heating and cooling temperatures of the product in pressurized cookers or autoclaves. The shelf-life of this canned product is established during hygienic processing and the final thermal treatment. In spite of the high mesophilic counts (including spore formers) in the canned product, the autoclaved cans, which were incubated for 10 days at 35°C (ANVISA, 2001), proved that the autoclaving was sufficient and the product attained commercial sterility, with no detection of any of the microorganisms analyzed. In Brazil, the directive for processed food in hermetic packing, which must be stable at room temperature, has not established a microbiological standard for the vegetable protein product (meat substitute) or at any stage of the process, including the canned products before the autoclaving operation.

The present study describes the importance of microbial control of the raw vegetable proteins and at all processing stages. Due to the high levels of microorganisms in the soy protein raw material, exposure of this protein to the optimal growth conditions during processing caused a considerable increase in the bioburden of the canned product, previous to moist heat treatment.

ACKNOWLEDGMENTS

The authors thank the Brazilian Committees for Scientific Technology Research (CNPQ and FAPESP) for financial support and Produtos Alimentícos Superbom. Thanks to Kristin M. Schaffner for carefully proofreading of the document for style and grammar.

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Revised paper received December 10, 2004 Accepted February 2, 2005

PAPER

DIETARY FIBRE COMPONENTS OF HIGH-FIBRE COMMERCIAL PRODUCTS. EFFECT OF COOKING PROCESS

EFFETTO DEL PROCESSO DI COTTURA SUI COMPONENTI DELLA FIBRA DIETETICA DI PASTE AD ALTO CONTENUTO IN FIBRA

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ABSTRACT

Six commercial high-fibre pastas were chosen to evaluate their composition in relation to the main dietary fibre components (Total Dietary Fibre, TDF; β-glucan; Resistant Starch, RS), which are directly beneficial to human health through several physiological and metabolic effects. The changes in fibre components due to cooking and overcooking processes were also assessed. The fibre component levels varied in the six pasta samples. Significant RS formation occurred during cooking and the

RIASSUNTO

Sei paste commerciali dichiarate, in etichetta, ad alto contenuto in fibra sono state analizzate per evidenziare eventuali differenze nella composizione relativamente ai principali componenti della fibra (fibra dietetica totale, β-glucano, amido resistente), che sono in grado di contribuire direttamente al miglioramento della salute attraverso diversi effetti fisiologici e metabolici. Sono presentati, inoltre, i risultati relativi alle variazioni nella composizione della fibra indotte dai processi di

- Key words: β-glucan, high-fibre pasta, resistant starch, Total Dietary Fibre -

amount of RS increased gradually with cooking time. Analytical results indicate that the cooking process may modify pasta starch directly by increasing the nondigestible fraction with important effects on the bioavailability and nutritional value of the pasta.

cottura e sovracottura. I risultati evidenziavano che i sei campioni di pasta presentavano differenti livelli di fibre e che il processo di cottura determinava una significativa formazione di amido resistente. I dati mostravano che il processo di cottura modificava direttamente la componente amilacea della pasta e, come conseguenza, la quota di carboidrati complessi non digeribile con importanti effetti sulla loro biodisponibilità e sul valore nutritivo del prodotto cotto.

INTRODUCTION

In recent years, the governments of the many developed countries have promoted and financed nutritional programmes that provide recommendations about eating to ensure good health and prevent chronic diseases. Dietary guidelines have been issued to encourage healthy lifestyles that minimise the risk of developing diet-related diseases. These guidelines emphasize the importance of a nutritionally balanced diet to promote and improve health and well-being and recommend including fruit, vegetables, legumes, whole grains and fish in the diet, while limiting the intake of animal products that are rich in saturated fats. By encouraging the consumption of plant-based food, the dietary guidelines recognize the beneficial physiological properties of fibre. The results of epidemiological studies have shown that dietary fibres play a protective role against obesity, cancer and cardiovascular disease, suggesting that their intake should be increased in the human diet (DAVIDSON, 1998; ONIANG'O, 1998; SCHNEEMAN, 1998; TUNGLAND and MEYER, 2002).

Recommendations for the developed countries (MILLER JONES, 2003) suggest a dietary fibre intake in the range of 20-35 g/day for a healthy adult (about twice what the majority of people currently consume).

Cereals, especially whole grains, are considered an important food (ADAMS and ENGSTROM, 2000; JACOBS *et al.*, 2000; SLAVIN *et al.*, 2000) that contains many protective factors including vitamins, minerals, antioxidants, phytoestrogens and fibre (soluble and insoluble). Cereals are normally processed to make products such as bread, pasta, cookies, etc. During processing the grain is refined, which lowers the dietary fibre content, because the highest fibre concentrations, found in the outer part of the kernel, are removed during milling (CAMIRE, 2004). To promote increased fibre consumption, there has been an increased availability of high-fibre products on the market over the last decade. These products, labelled as "rich source of fibre", include pasta products, that have traditionally played a leading role in the Mediterranean diet. They are produced by enriching durum wheat semolina with fibre or by directly processing durum wheat grain without removing the bran.

Dietary fibre is a mixture of two categories of fibre, that are both beneficial to health (ENGLYST and KINGMAN, 1990). Soluble fibres, that include pectins, gums and mucilage, play a ma-

jor role in reducing the risk of coronary heart disease (FULGONI III and MUELLER, 2001; WOOD, 2001). Insoluble fibres, including the structural portion of the plant cell (cellulose, hemicelluloses and lignin), have direct effects on the colon by preventing constipation and associated problems (DAVIDSON, 1998; LAIRON, 2001). In addition, the resistant starch fraction, affected by food processing (MUIR *et al.*, 1995) and classified (ENGLYST *et al.*, 1992) as the part of starch that resists digestion and therefore acts physiologically as dietary fibre, not only improves bowel function (intestinal flora effects), but also provides further potential health benefits including the control of blood sugar and cholesterol (RANHOTRA, 2001), weight control and colon cancer prevention. The processes involved in pasta production (milling, extrusion and heat treatments) may affect the dietary fibre content and its properties (POUTANEN, 2001). On the other hand, the physico-chemical properties of dietary fibre, that depend on the type and concentration of fibres, may affect the sensory characteristics, the potential nutritional quality as well as the technological aspects involved in producing high-fibre products (MARLETT *et al.*, 1996; ALLDRICK, 2001; MEUSER, 2001; TUDORICA *et al.*, 2002).

In the present study, six different commercial high-fibre pasta samples were examined in order to quantify the main dietary fibre compounds, Total Dietary Fibre (TDF), β-glucan and Resistant Starch (RS). In addition, considering that heat treatment could affect fibre content and composition (THEANDER *et al.*, 1993), changes due to cooking and overcooking were also considered. The results suggest that a new approach to nutritional food labelling should be adopted. The label should include a complete fibre characterisation in order to provide all the needed information about the type of dietary fibre contained in the product.

MATERIALS AND METHODS

Materials

Two packets of six commercial Italian dry high-fibre pastas, spaghetti or similar shapes, purchased from supermarkets, were analysed. The high-fibre pastas were produced by using various combinations of the outer layers of the grain using different processing techniques (Table 1). Samples 1 and 2 were produced from whole-durum wheat semolina (spaghettini and spaghetti, respectively); samples 3 and 4 were made with semolina of durum wheat enriched with fine bran and bran, respectively. Samples 5 (spaghetti) and 6 (bucatini) were made with milled, organic whole grain durum wheat.

All of the products claimed to be "high in fibre" i.e. containing at least 4g of dietary fibre per serving or 6% w/w (YUE and WARING, 1998; COUSSEMENT, 2001). A traditional pasta produced solely with durum wheat semolina served as control. All samples were stored under refrigerated conditions (T=4°C) until analysed. The samples were analysed uncooked, cooked and overcooked. The pasta products were ground with a Cyclotec mill (Cyclotec 1093, Tecator-FossItalia, PD, Italy) to pass through a 1 mm sieve.

Cooking procedure

All samples were prepared as follows: 100 g of pasta in 1L boiling tap water without kitchen salt for the recommended time according to package instructions, Table 1, (cooked) and also for five minutes more than the indicated time (overcooked). Immediately after cooking, the water was drained off through a colander. Following the suggestions of BJORK *et al.* (1987) for preserving heat-treated wheat starch and the indications of EGGUM *et al.* (1993), wet samples were immediately frozen at -20°C and then lyophilised (temperature of dry-

Table 1 - Type and characteristics of the six high-fibre Italian pastas studied.

Sample number	Information on the package label						
	Durum wheat ingredients	Pasta shape	Recommended cooking time (min)	Nutritional components % as is			
				TDF[1]	Carbohydrate	Total protein	Lipid	Ash
1	Whole semolina	Spaghettini	10	6.80	65.4	13.0[2]	2.40	not reported
2	Whole semolina	Spaghetti	9	9.48	61.0	13.2	2.96	not reported
3	Semolina enriched with fine bran	Linguine	6	7.00	64.3	13.0[2]	1.75	1.5
4	Semolina enriched with bran and wheat germ	Spaghetti	10	8.00	62.5	14.0	3.00	not reported
5	Whole grain	Spaghetti	8	9.40	65.2	11.7	0.70	1.6
6	Whole grain	Bucatini	8	9.40	65.2	11.7	0.70	1.6
	Average	-	8.5	8.35	63.9	12.8	1.91	-
	St. Dev.	-	-	1.25	1.80	1.14	1.05	-

[1] Total Dietary Fibre; [2] The label of these samples reported N x 6.25.

ing = 15°C). The resulting cooked pasta samples were kept in desiccators.

Chemical composition

Moisture was determined with a thermo balance (Sartorius MA40, Goettingen, Germany) at 120°C. The protein content was estimated through micro-Kjeldhal nitrogen analysis (N x 5.7). The ash content was determined according to the UNI-ISO 2171 method (1991). The total lipid content was determined with a semi-automatic system (Soxlet apparatus, FossItalia, Padua, Italy) using 2-propanol as solvent.

Fibre analysis

Total Dietary Fibre (TDF) content was determined using an enzymatic-gravimetric method (AOAC, 1995) and an automatic filtration of the hydrolysed products (Fibertec system, FossItalia, Padua, Italy). β-glucan and Resistant Starch (RS) contents were evaluated by enzymatic methods using Megazyme kits (Megazyme International, Victoria, Australia) (MC CLEARY and CODD, 1991; MC CLEARY and MONAGHAN, 2002; MC CLEARY *et al.*, 2002).

Statistical analyses

All the analyses and the cooking test were carried out in two replicate samplings. Anova analysis was performed using the MSTAT Programme, Model Number 9. Duncan's test and LSD values at p=0.05 were also calculated. The relationship between the parameters was determined by simple correlation analysis.

RESULTS

Some of the information given on the label of the six commercial high fibre pasta samples investigated is reported

in Table 1. Chemical composition of the uncooked pastas and the control are reported in Table 2 along with the significance of sample differences (Duncan's test and LSD values).

The ash content in the uncooked high-fibre samples varied from 1.46 to 2.21% d.m. These values fall within the limits set by Italian law for "whole grain pasta" or a "dietetic high fibre product" (DPR, 2001). The protein content in the high-fibre pastas varied between 10.9 and 14.4% d.m. Samples 1, 3 and 4 were higher than the average (12.85% d.m.). The values for samples 1-5 were in good agreement with those reported on the label and were also in compliance with the legal requirements for this type of product. Only sample 6 had a value that was notably less than that indicated on the package and required by law.

The lipid content ranged from 1.17 to 3.56% d.m. while the control value was 0.91% d.m. The non-nitrogen compounds (calculated by subtracting the other constituents) varied between 67.57 and 77.75, with a mean value of 74.42% d.m.

The results of ANOVA analysis reported in Table 3 show some significant differences for the fibre components in relation to the variability factors considered.

The Total Dietary Fibre (TDF) content of the uncooked samples and the respective variations due to cooking and overcooking are reported in Table 4. The TDF contents of uncooked high fibre samples ranged from 7.1 to 12.3% d.m. with a mean value of 9.1. The ANOVA analysis showed significant differences among samples. The analytical method chosen showed that the TDF contents differed with respect to what was reported on the nutrition information on the packages (Table 1). The most marked differences were noted for samples 3 and 4 (42 and 53% higher TDF contents, respectively, than the values reported).

In general, the cooking process caused a significant increase in TDF content; the mean values were 10.4 and 10.7% d.m., respectively, for cooked and overcooked samples with a mean increment of 14 and 18%, respectively, in comparison to uncooked ones. For the control, the TDF value (2.28% d.m.) for the uncooked sample increased 53% during cooking and remained at this level after overcooking. VASANTHAN *et al.* (2002) re-

Table 2 - Chemical composition (% d.m.) of the six high-fibre Italian uncooked pastas.

Sample number	Moisture %	Ash (A)	Total protein (B)	Lipid (C)	Non nitrogen compounds[(1)] (D)
1	10.1	1.46 f	12.91 c	1.39 c	76.68
2	10.5	1.73 c	12.67 d	2.62 b	74.16
3	10.3	1.91 b	13.73 b	1.45 c	72.97
4	9.6	2.21 a	14.40 a	3.56 a	67.57
5	10.0	1.50 e	12.51 de	1.17 c	77.75
6	9.5	1.69 d	10.90 e	1.28 c	77.39
Average	10.0	1.75	12.85	1.91	74.42
LSD value (p=0.05)	-	0.026	0.182	0.293	-
Control	12.4	0.66	17.73	0.91	78.42

[(1)] calculated by difference: D=100-A-B-C-TDF determined; Duncan's test: a-f different letters in the same column indicate the samples were significantly different.

Table 3 - ANOVA analysis of the six high-fibre pasta samples.

Parameters		TDF[a]	β-Glucan	RS[b]
Mean values (%d.m.)		9.72	0.42	0.72
Variability Factors	DF[(1)]			
A) Pasta samples	5	***	***	**
B) Uncooked vs cooked pastas	1	***	n.s.	***
A x B	5	*	**	***

*, **, ***: significant at p = 0.05, 0.01 and 0.001, respectively; n.s.: not significant. [(1)] DF: degrees of freedom;
[a] Total Dietary Fibre;
[b] Resistant Starch.

Table 4 - Total Dietary Fiber (TDF), β-glucan and Resistant Starch (RS) variations (% d.m.) in the six uncooked, cooked and overcooked high-fibre pastas studied.

Pasta	TDF			β-glucan			RS		
samples	Uncooked	Cooked	Overcooked	Uncooked	Cooked	Overcooked	Uncooked	Cooked	Overcooked
1	7.56	8.56	8.91	0.30	0.34	0.39	0.49	1.55	2.11
2	8.82	10.09	10.35	0.44	0.44	0.44	0.50	0.94	1.09
3	9.94	10.64	10.75	0.37	0.43	0.45	0.29	0.73	1.06
4	12.26	15.00	15.35	0.46	0.52	0.50	0.46	1.14	1.15
5	7.07	7.67	7.86	0.47	0.37	0.42	0.36	0.61	0.98
6	8.74	10.34	11.14	0.46	0.43	0.43	0.50	1.25	1.54
average	9.06	10.38	10.73	0.41	0.42	0.44	0.43	1.04	1.32
Control	2.28	3.49	3.44	0.15	0.15	0.15	0.35	1.55	1.73
SE (n=2)[a]	0.483	0.252	0.327	0.026	0.024	0.009	0.036	0.084	0.060

[a] p = 0.05.

ported increases of TDF for barley flours due to extrusion-cooking processes and attributed this to increases in SDF (soluble dietary fibre) as well as to the formation of RS_3 (enzyme retrograded resistant starch).

The content of one of the soluble components of TDF, β-glucan (Table 4), ranged from 0.30 to 0.47% d.m. for uncooked high-fibre samples, showing that fibre enrichment caused small but significant increases in comparison with the traditional product (0.15% d.m.). Samples 4, 5 and 6 had higher values than the mean value. The cooking and overcooking processes did not significantly alter the β-glucan content. The major soluble dietary fibre present in semolina (arabinoxylan) was not specifically measured.

Resistant Starch content (RS) was also determined in all the samples. In the uncooked high-fibre pastas, the RS content (Table 4) varied between 0.29 and 0.50% d.m. with a mean value of 0.43 with significant differences among samples. The RS content increased markedly and significantly during cooking and overcooking with mean values of 1.04 and 1.32% d.m., with mean increments of 136 and 200%, respectively; these values are lower than data reported for cooked spaghetti by BRIGHENTI *et al.* (1998), who used a different analytical procedure. During the recommended cooking time, the RS content increased in all the prod-

ucts varying between 69 and 216% of the initial value. The RS content values were typical of the analyzed pasta samples, which were made of different durum wheat ingredients with slight differences in product thickness. In the additional five minutes of overcooking RS increased in all the samples except in pasta integrated with bran (sample 4). The highest RS content (2.11% d.m.) was in sample 1 after overcooking. The control had the highest percentage of increase (343% for the cooked sample and 393% for the overcooked one) which was probably due to the presence of more starch. Due to its nature and physical form (BERRY *et al.*, 1988), it can be modified during the cooking treatment as reported by THARANATHAN and THARANATHAN (2001) for various other processes. No relationship between the level of lipids (Table 2) and resistant starch formation during cooking was found which is in agreement with the conclusions of BERRY (1986). COLONNA *et al.* (1990) reported that cooking losses occur in spaghetti in the first period of boiling without leading to significant differences in the amylose content of the starch fraction that remains in the pasta, and, as shown in a previous paper (D'EGIDIO *et al.*, 1983), contains both the starch components. On the basis of these indications, the cooking losses were not evaluated in this study.

Table 5 reports the β-glucan and RS contents as percent of TDF. On the average, β-glucan, accounted for 4.7% of TDF in uncooked high-fibre products with a slight decrease after cooking and overcooking. There was a large variability among uncooked samples; the two samples made with whole-grain durum wheat (samples 5 and 6) had the highest β-glucan percentages as well as the control that had the lowest TDF content (2.3% d.m.). The RS values in the uncooked high-fibre products varied between 2.9 and 6.5% of TDF; the traditional pasta (control) had a very high percentage (15.4% TDF). Cooking and overcooking caused marked variations in TDF composition; in fact, the RS increased to 50% of TDF. Most of samples had a different composition after cooking, which was probably due to slight losses of soluble components into the cooking water and significant increases in RS.

In Table 5 the Dietary Fibre (DF) residue, obtained by subtracting the β-glucan and RS components from TDF which cannot to be separately determined with the methodology used (AOAC, 1995), is

Table 5 - β-glucan and Resistant Starch (RS) as % of Total Dietary Fibre (TDF=100).

Pasta	β-glucan			RS			DF residue[1]		
samples	Uncooked	Cooked	Overcooked	Uncooked	Cooked	Overcooked	Uncooked	Cooked	Overcooked
1	3.9	3.9	4.3	6.5	18.1	23.7	89.6	78.0	72.0
2	4.9	4.3	4.3	5.7	9.3	10.5	89.4	86.4	85.2
3	3.7	4.0	4.2	2.9	6.9	9.9	93.4	89.1	86.0
4	3.7	3.5	3.3	3.8	7.6	7.5	92.5	88.9	89.3
5	6.6	4.8	5.4	5.1	8.0	12.5	88.3	87.3	82.2
6	5.3	4.2	3.9	5.7	12.1	13.8	89.0	83.7	82.3
Average	4.7	4.1	4.2	4.9	10.3	13.0	90.6	87.4	86.6
St.Dev.	1.14	0.42	0.68	1.34	4.23	5.68	2.07	4.20	5.91
Control	6.6	4.3	4.4	15.4	44.5	50.4	78.0	51.2	45.2

[1] Dietary Fibre residue calculated by subtracting β-glucan and RS percentages.

also reported. Cooking and overcooking caused marked changes in these values, as seen in the significant changes in TDF and RS (Table 4), particularly in samples 1, 3, 5, 6 and the control.

These results suggest that the effect of the cooking process on the TDF of these pastas was mostly due to modifications in the starch, that significantly increased the level of the resistant starch fraction. Similar results were reported by BERRY (1986) and EGGUM (1993). ENGLYST *et al.* (1992) reported that the main RS fractions present in spaghetti boiled for 10 min were RS_3, retrograded amylose, and RS_1, a partially physically inaccessible starch. The formation of this important fibre component, that is fermented in the large intestine by beneficial bacteria helping to improve colon health, was favoured. This was particularly evident in the traditional pasta which showed the highest increase in resistant starch during cooking and overcooking.

To confirm the effect of cooking time on RS formation, the RS trend in relation to cooking time was determined (Fig. 1). For the sample used, the recommended cooking time as indicated on package (10 min) was used as well as additional times that were lower and higher than the recommended time. A marked increase (>200%) was reported before the disappearance of the core, with a mean value of 1.63% d.m. compared to the uncooked sample (0.5% d.m.). The RS content increased gradually with cooking time. At 20 min, the RS was 2.25% d.m., a 45% increase with respect to the value obtained in the complete starch gelatinisation phase (ten min).

CONCLUSIONS

Numerous epidemiological studies have shown that several illnesses common in Western society are inversely correlated to fibre consumption. Other studies have demonstrated the positive effect of fibre on glucidic and lipid metabolism and on bowel health. Since fibre components seem to behave differently in the intestine and have varying effects on metabolic functions, it would be beneficial to know the composition of the fibre found in commercial foods that claim to be high in fibre content.

The results of this study show that there is a large variability in the composition of high-fibre pasta products, and that extended cooking time results in an increase in TDF, particularly resistant starch. The significant results of the pasta x cooking treatment interactions indicate that the effects of cooking

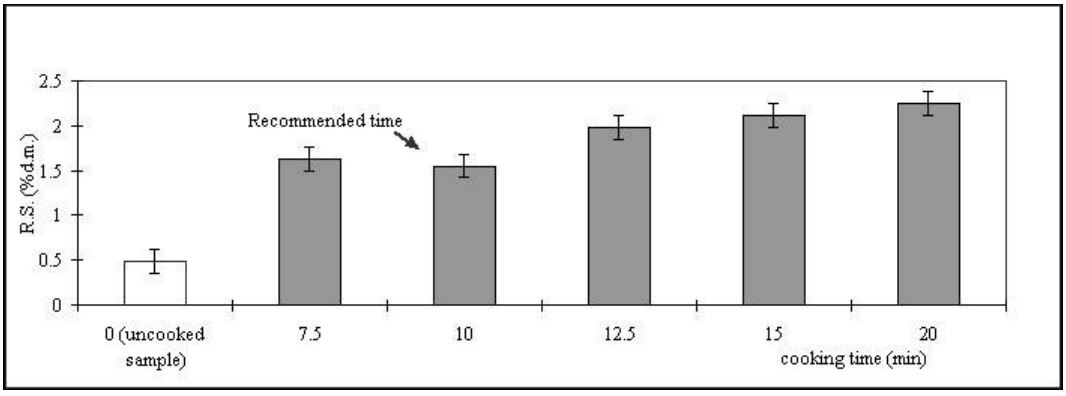


Fig. 1 - Resistant starch trend in relation to cooking process. The bars indicate ± SE (n=4).

are also related to the uncooked sample characteristics. The greatest RS formation was observed in pasta 1 (where the whole durum wheat grains were directly processed into whole semolina spaghettini, without removing the outer grain layers), in 6 (whole grain pasta in bucatini shape, with more surface area exposed to water) and in the control (spaghetti traditionally produced with semolina). During cooking the intrinsic characteristics of these pasta samples favoured the transformation of starch granules from the typical A-type pattern into the resistant form due to the reorganization of the amylose chain into a B-type, characteristic of the retrograded form. The positive health effect of the starch modification induced by processing is of interest because, as a natural consequence, the less available glycemic food starch remains in the cooked product.

Finally, the results of this study show the wide range of nutritional quality of high fibre pasta products and the effects of cooking on the main fibre components. This provides further insight into the mechanisms responsible for the health benefit of pasta products that both satisfy nutritional requirements and safeguard health.

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Revised paper received May 5, 2005 Accepted June 28, 2005

PAPER

EFFECT OF DIRECT ACIDIFICATION AND PASTEURIZATION OF BUFFALO MILK ON THE QUALITY OF MARAJOARA CHEESE

EFFETTI DELL'ACIDIFICAZIONE DIRETTA E DELLA PASTORIZZAZIONE DEL LATTE DI BUFALA SULLA QUALITÀ DEL FORMAGGIO MARAJOARA

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ABSTRACT

The quality of Marajoara cheese, a soft cheese manufactured from raw buffalo milk in Amazonian regions, was evaluated using four methods: natural acidification of raw milk (T_1), acidification with citric acid (T_2), pasteurization at 65°C for 30 min (T_3) and pasteurization at 71°C for 5 min (T_4). Milk from treatments T_3 and T_4 was acidified using starter cultures. Cheese from T_3 and T_4 treatments had lower mesophilic counts (P<0.05) on day 1 of storage at 4°C, however counts increased after

RIASSUNTO

L'obiettivo di questo studio è stato la valutazione della qualità del formaggio Marajoara, un formaggio a pasta morbida prodotto con latte crudo di bufala nella regione amazzonica, impiegando quattro diversi metodi: acidificazione naturale del latte crudo (T_1), acidificazione con acido citrico (T_2), pastorizzazione a 65°C per 30 min (T_3), e pastorizzazione a 71°C per 5 min (T_4). Il latte trattato secondo i metodi T_3 e T_4 è stato acidificato impiegando colture microbiche starter. I formaggi T_3 e T_4 hanno pre-

- Key words: analysis, buffalo milk, heat treatment, Marajoara cheese -

day 15. T_1 cheese had higher psychrotrophic counts (P<0.05). Texture parameters (firmness, gumminess and chewiness) were lower (P<0.05) in T_1 and T_2 cheese on days 1 and 15. Sensory evaluation showed higher final scores (P<0.05) for T_1 and T_2 cheeses, whereas T_3 had the lowest score. Results indicate that heat treatment of buffalo milk, as used in this trial, led to lower bacterial counts in cheese, but also decreased overall quality of Marajoara cheese.

sentato quantità minori di batteri mesofili (P<0,05) il primo giorno di conservazione a 4°C, mentre questa quantità è aumentata dopo il quindicesimo giorno di conservazione. Il formaggio T_1 ha presentato una carica di batteri psicrofili piú alta (P<0,05). I parametri strutturali (consistenza, gommosità e masticabilità) sono risultati minori (P<0,05) nei formaggi T_1 e T_2 nel 1° e 15° giorno. Le valutazioni sensoriali hanno mostrato punteggi finali più alti (P<0,05) per i formaggi T_1 e T_2, mentre il formaggio tipo T_3 ha presentato il punteggio più basso. I risultati indicano che il trattamento termico del latte di bufala, così come utilizzato in questo esperimento, ha determinato le minori cariche microbiche riscontrate nel formaggio, ma ha anche determinato uno scadimento della qualitá del formaggio stesso.

INTRODUCTION

Marajoara cheese is a soft cheese traditionally manufactured from raw buffalo milk in the Amazonian region of Brazil. As other buffalo dairy products, this cheese has high nutritional value and large market potential in other regions of the country, being an excellent alternative for the use of milk from this species (NEVES, 1985).

According to FINOTELO (1979), the typical composition of creamy-type Marajoara cheese is 50 to 52% moisture, 21 to 23% fat, and 24 to 27% protein. Because of its relatively high moisture content, wet yields of 11.8% are typically obtained when making Marajoara cheese from buffalo milk (FINOTELO, 1979; NEVES, 1985).

The artisan procedure of Marajoara cheese production, followed by most farm producers, is characterized by low production costs, and does not require sophisticated industrial equipment. This process is performed by means of natural acidification of the milk, leading to a decrease in pH due to the microbial fermentation of raw milk. However, this method leads to the production of cheese with great differences in characteristics, because the microbiota in the milk may be highly variable due to milking hygiene, conditions of transportation and preservation and time of the year (FINOTELO, 1979).

In Brazil and many other countries, thermal pasteurization of milk for use in cheese manufacture is mandatory, and may be performed at 62°-65°C for 30 min (IDF, 1995). This time-temperature relationship is normally applied to bovine milk, and for buffalo milk, other combinations of time and temperature may be used, due to differences in composition. Thermal treatment that eliminates pathogenic microorganisms also destroys lactic acid bacteria naturally

present in the milk, hence the addition of starter cultures is essential for products to be uniform (ISEPON and OLIVEIRA, 1995). BHATTACHARYA *et al.* (1971) recommend that buffalo milk destined for cheese production should be heated at 71°C for 5 min in order to prevent the occurrence of undesirable changes in the original properties of the milk.

Some organic acids, such as citric acid, have been widely used in dairy industries to increase the speed of pH reduction in milk used for the manufacture of cheese by acid coagulation (SPREER, 1991). Although artificial acidification has several advantages, the addition of acid to milk should be controlled in order to prevent problems in the manufacture of the products, such as the flocculation of protein in milk, or premature / late cut of the curd, which may lead to sensory changes or decrease in yield (DEMOTT, 1983).

LOURENÇO *et al.* (2002) reported a short shelf-life of Marajoara cheese, around 15 days, based on microbiological analysis of the product. However, there are few reports on the evaluation of Marajoara cheese quality, and there are no studies on the use of thermal treatment of milk destined for the manufacture of this kind of cheese. The objective of the present trial was to evaluate physical, chemical, microbiological and sensorial parameters and the yield of Marajoara cheese produced from naturally and artificially acidified buffalo milk, and from pasteurized buffalo milk followed by the addition of starter cultures.

MATERIALS AND METHODS

Milk collection and experimental design

Milk used in the trial came from only one herd and was obtained by hand milking, in adequate hygienic conditions. After withdrawal, milk was immediately cooled to 5°C and transferred to previously cleaned and disinfected polypropylene containers. It was then transported to the dairy factory at the University of São Paulo campus in Pirassununga, Brazil, where processing occurred. Milk was accepted when mesophilic counts were below $1.0x10^5$ colony forming units (CFU)/mL.

The total volume (200 L) was heated to 40°C and partially skimmed (up to 0.5% fat). Cream was immediately cooled and stored at 5°C, to be used later. Skimmed milk was divided into four equal parts of 40 L and immediately used in the manufacture of Marajoara cheese.

Seven replicates of the experiment were performed, approximately one per month. The four 40-L volumes of milk were treated as follows: natural coagulation of raw milk at room temperature for 24 hours (T_1); direct acidification of raw milk to pH 5.0 using citric acid (T_2); low temperature long time pasteurization at 65°C for 30 min (CHAPMAN and SHARPE, 1987), followed by the addition of starter culture for acidification (T_3); pasteurization at 71°C for 5 min (BHATTACHARYA *et al.*, 1971), followed by the addition of starter culture for acidification (T_4).

Manufacture of Marajoara cheese

Cheese was produced according to FINOTELO (1979) in double-walled processing tanks. Direct acidification (T_2) was performed by adding a 10% (w/w) citric acid solution to milk at 20°C at a rate of 2 mL/L/min, while simultaneously warming the milk to 36°C at a rate of 2°C/min. The milk was held at 36°C for nearly 15 min, until it had been acidified to pH 5.0. Milk from treatments T_3 and T_4 was inoculated (3% inoculum) with starter culture (*Strepococcus thermophilus* and *Lactobacillus delbruekii* ssp. *bulgaricus*, Wisbyvac, Germany) and incubated at 42°C for approximately 3 hours, until reaching pH 5.0.

After milk coagulation according the

treatments above, the curd was cut and gently stirred, and the whey was partially drained off. Curd was placed in 1 kg forms for manual draining of the whey. After that, curd was again placed in the double-walled tank and washed with 4 L of water (10% of the initial volume of milk) at 65°C, stirring slowly in order to separate the maximum volume of whey still inside the curd. After this procedure, 4 L of fresh buffalo milk were added (10% of the initial volume), and the curd was heated under constant stirring at 80°C for 10 min. Separation of excess whey was performed after transferring of the curd to 1 kg forms, which were placed in a 0.5 kg-mechanical press for 30 min. Curd was then shredded in a helicoidal grinder until fragments of nearly 5 mm were obtained. Ground curd was salted (2%) and transferred to another tank where final thermal treatment was applied (95°C for 5 min). Then, 1.2 L of cream previously obtained in the skimming procedure (3% of the initial milk volume) was added, and the mixture was submitted to constant stirring until an elastic and homogenous product was obtained. After cooling at room temperature, the cheese was placed in 500 g forms, manually packed in polyethylene bags and cooled at 4°C.

Cheese yield was calculated using R (%) = (Wf/Wi) x 100, where R is the yield, Wf is the final weight of the cheese and Wi is the initial total weight (milk and ingredients) according to YUNES and BENEDET (2000).

Physical, chemical and microbiological analyses

Cheese samples were analysed after 1 and 15 days of storage at 4°C for titratable acidity, pH, dry matter, fat and total protein, according to AOAC (1990). Microbiological tests included standard plate count for mesophilic and psychrotrophic bacteria and coliform count (APHA, 1992).

Texture of the cheese was analyzed at 12°C by means of Texture Profile Analysis (TPA), using a texturometer TA.XT2i (Stable Micro Systems, Godalming, UK), using a cylindrical sample of 20x20 mm, with a 3 mm probe and speed of 0.8 mm/s, controlled by the Texture Expert v.1.15 (SMS) software. The following parameters were measured: TPA firmness (g), defined as the maximum force measured during the compression cycle; TPA springiness (dimensionless), which is the recovered height of the sample after the 1st compression and before the 2nd compression; TPA gumminess (dimensionless), energy required to disintegrate a semi-solid food in a way that it may be swallowed; and TPA chewiness (g), calculated as the product of the other three properties.

Sensory analyses

Samples of cheese were submitted to a non-trained panel of 85 individuals (IFT, 1981) after 1 and 15 days of storage at 4°C. Panelists were recruited among students and staff of the University that have never tasted Marajoara cheese previously. Samples were cut into portions of approximately 30 g and submitted to the panel without addition of any other ingredients, at approximately 10°C. Panelists were instructed to follow a hedonic scale of 9 points and attribute scores to each of the following cheese characteristics: appearance, consistency, odor and taste. The descriptions used for the hedonic scale were: 9=excellent, 8=very good, 7=good, 6=fairly good, 5=indifferent, 4=fairly poor, 3=poor, 2=very poor, 1=extremely poor.

Statistical analyses

Analytical results of duplicate samples of the seven lots and sensory data were submitted to one-way ANOVA (GACULA and SINGH, 1984). Treatment means were compared by the Tukey test, using

the SAS® General Linear Model (SAS INSTITUTE, 1992). Statistical significance was accepted at $P<0.05$. Microbiological results (CFU) were transformed into $\log_{10}$.

RESULTS AND DISCUSSION

Average yields of Marajoara cheese obtained in the different treatments varied from 10.6 to 12.2% (mean of 11.3%), and no differences were observed between the treatment means ($P>0.05$). These values were lower than those described in the literature for other cheese produced with buffalo milk, such as Mozzarella obtained by means of artificial acidification (18%) and enzymatic coagulation (17.3%) (NEVES, 1985), but were similar to values reported by FINOTELO (1979) for this kind of cheese.

Results of physical-chemical and microbiological analyses of Marajoara cheese are presented in Table 1. On day 1 after manufacture, acidity was greater ($P<0.05$) in T_2 cheese when compared with the other treatments, due to the addition of citric acid. Acidity in treatments T_2 and T_3, on the 15th day of storage, was greater than that of treatment T_4 ($P<0.05$). Accordingly, pH decreased with storage. On day 1, pH values were lower ($P<0.05$) in cheese produced by means of direct acidification (T_2), as expected. However, The pH of Marajoara cheese in all treatments was above the values described by FINOTELO (1979) for this kind of product (less than 5.2). FINOTELO (1979) worked in the Brazilian Amazonian region (state of Pará) using natural acidification of the milk, thus the difference in pH values in both studies may be explained by the regional variations in the original microbial flora of the milk.

Fat, protein and dry matter content ranged from 22.4 to 24.8%, 26.5 to 28.1% and 50.3 to 53.0%, respectively, which is in agreement with FINOTELO (1979) for these parameters (fat: 21 to 23%; protein: 24 to 27%; dry matter: 48 to 50%). No significant differences ($P>0.05$) were observed between treatments for these parameters, and no large variations were observed on days 1 and

Table 1 - Average values of chemical and microbiological characteristics of Marajoara cheese manufactured from buffalo milk[1].

Treatment	Titratable acidity (% lactic acid/g)	pH	Fat (%)	Total protein (%)	Dry matter solids (%)	Mesophilic (Log CFU/g)	Psychrotrophic (Log CFU/g)
Cheese on storage day one							
T_1	2.27±0.74^{a}*	5.73±0.41^{a}	23.01±2.25^{a}	26.71±2.32^{a}	50.45±4.22^{a}	3.43±3.74^{a}	3.11±3.61^{a}
T_2	3.10±0.86^{b}	5.32±0.12^{b}	23.38±1.85^{a}	27.45±2.03^{a}	52.07±5.04^{a}	4.71±4.98^{b}	1.14±1.54^{b}
T_3	2.44±0.62^{a}	5.63±0.21^{a}	23.22±1.89^{a}	27.12±2.33^{a}	51.22±5.23^{a}	2.66±2.87^{c}	2.31±2.67^{c}
T_4	2.13±0.50^{a}	5.76±0.18^{a}	22.42±1.96^{a}	26.51±1.67^{a}	50.35±4.86^{a}	2.81±2.97^{ac}	1.85±2.28^{bc}
Cheese on storage day 15							
T_1	2.66±0.54^{ab}	5.65±0.23^{a}	23.33±3.45^{a}	27.24±2.98^{a}	51.87±5.20^{a}	2.80±2.72^{a}	3.87±4.24^{a}
T_2	3.41±1.06^{a}	5.25±0.16^{b}	24.83±2.17^{a}	28.13±1.87^{a}	52.97±2.78^{a}	4.17±4.62^{b}	1.89±2.00^{b}
T_3	3.46±0.46^{a}	5.31±0.17^{ab}	23.66±1.37^{a}	27.35±3.03^{a}	50.30±5.58^{a}	3.81±4.01^{b}	2.64±2.86^{b}
T_4	2.23±0.61^{b}	5.52±0.38^{ab}	22.88±2.46^{a}	27.56±1.89^{a}	51.54±5.07^{a}	4.59±4.67^{b}	4.05±4.46^{a}

* Within a column, means followed by the same letter are not significantly different ($P>0.05$).
[1] Mean±standard deviation of duplicate analyses of seven batches examined.
T_1: Natural acidification; T_2: Acidification with citric acid; T_3: Pasteurization at 65°C, 30 min; T_4: Pasteurization at 71°C, 10 min.

15 after production. The thermal treatment of the milk used in cheeses T_3 and T_4 did not influence the composition of the product, unlike what was observed by PATEL *et al.* (1998) in the manufacture of Mozzarella cheese using buffalo milk. They observed a loss in fat content due to previous pasteurization of the milk.

Mesophilic and psychrotrophic counts (Table 1) were relatively low in all treatments (mesophiles < 4.71 Log CFU/g; psychrotrophs: < 4.05 Log CUF/g), which may be related to the elimination of microbial load during final heat treatment of the curd, as part of the manufacturing procedure, as stated by LOURENÇO *et al.* (2002). However, treatment using citric acid (T_2) had higher (P<0.05) mesophilic counts on day 1 of storage, which were still high on day 15. This fact indicates that artificial acidification was not able to reduce the original microbial load of the raw milk. Cheese T_1, which was not submitted to thermal processing or acidification, had higher counts than the other treatments on day 1, but decreased on day 15. The other cheese in this treatment also had higher psychrotrophic counts (P<0.05). Cheeses T_3 and T_4 had the lowest mesophilic counts (P<0.05) on day 1, as compared with the other treatments, possibly due to the previous pasteurization of the milk, which presented similar efficiency in the elimination of the original microbial load of the milk. Cheese in these treatments also had higher mesophilic counts on day 15 of storage, which may be attributed to the starter culture that survived the heat treatment given to the cheese, and to other bacteria, mainly those naturally present in cream incorporated in the curd at the end of the process. No total or fecal coliforms were detected in the samples analyzed.

The results of texture profile analysis are reported in Table 2. TPA firmness was greater (P<0.05) in cheeses T_3 and T_4 on day 1 after manufacturing, when compared with the other treatments, and was constant until the 15th day. The lowest values for this parameter were obtained for cheese T_1 (P<0.05). Firmness of T_1 decreased even more with storage, unlike what was observed in cheese T_2, which was constant during the 15 days of storage. TPA springiness values did not present any differences (P>0.05) among the different treatments, and no significant variations were observed during storage. As for TPA chewiness and gumminess, cheese T_1 presented the lowest values, whereas the results obtained for cheeses T_3 and T_4 were greater on days 1 and 15, when compared with the other treatments.

The texture of cheese may be greatly influenced by the composition of the initial milk. Water content, fat and protein distribution, as well as pH changes, are the main factors responsible for changes in texture characteristics of cheese during storage (Marshal, 1990). As these parameters did not differ between treatments or have significant changes during the 15-day storage time, it may be concluded that differences observed in the texture of cheeses T_3 and T_4 were related to changes that occurred in the milk due to pasteurization, such as precipitation of whey proteins, since the standard pasteurization (T_3) would cause only a small amount of whey protein denaturation (Lewis, 1993). Also, the starter culture used in the manufacture of cheeses T_3 and T_4 may have contributed to different proteolysis profiles, when compared to cheese T_1, which contained only the original microbial load, thus leading to differences in texture parameters. Finally, the difference between T_3 and T_1 is more likely a result of the different amount of proteolysis that occurred during acidification, with more occurring in T_1 than in T_3.

Direct acidification has the effect of solubilizing more calcium phosphate so that it is lost from the curd during whey drainage (WALSTRA and JENNESS, 1987),

Table 2 - Average values of texture parameters of Marajoara cheese manufactured from buffalo milk[1].

Treatment	Firmness (g)	Springiness	Gumminess	Chewiness (g)
Cheese on storage day one				
T_1	345.6±109.7[a] *	0.9868±0.0045[a]	192.6±59.8[a]	189.6±58.8[a]
T_2	496.0±78.2[a]	0.9814±0.0141[a]	285.0±58.4[b]	281.3±58.1[ab]
T_3	610.9±276.1[b]	0.9871±0.0099[a]	359.7±182.3[c]	356.7±181.7[b]
T_4	671.4±299.5[b]	0.9673±0.0358[a]	357.2±166.2[c]	349.3±172.4[b]
Cheese on storage day 15				
T_1	243.7±55.7[a]	0.9847±0.0079[a]	164.9±36.4[a]	162.5±35.6[a]
T_2	478.8±81.6[b]	0.9825±0.0117[a]	270.3±63.5[ab]	233.0±66.2[ac]
T_3	648.3±122.2[c]	0.9887±0.0085[a]	401.1±108.9[c]	397.8±108.4[b]
T_4	617.4±111.7[c]	0.9736±0.0312[a]	338.9±116.6[bc]	332.6±120.7[bc]

* Within a column, means followed by the same letter are not significantly different (P>0.05).
[1] Mean±standard deviation of duplicate analyses of seven batches examined.
T_1: Natural acidification; T_2: Acidification with citric acid; T_3: Pasteurization at 65°C, 30 min; T_4: Pasteurization at 71°C, 10 min.

which might have contributed to lower values of firmness for T_2 cheese. On the other hand, the presence of proteolytic enzyme activity in the milk can result in a softer cheese, as also observed in T_1 cheese, because in the raw milk there could be many proteolytic bacteria present that can hydrolyze the casein during acidification. Cheeses from treatment T_2 would also have had a low level of proteolysis, as it had a shorter acidification time, so it would be expected to have higher firmness than T_1, but, because of its lower calcium content, it would be expected to have less firmness than T_3.

Results of the sensory analysis of cheese on days 1 and 15 after manufacture are reported in Table 3. The average scores for the attributes among treat-

Table 3 - Average values of sensory attributes of Marajoara cheese manufactured from buffalo milk[1].

Treatment	Appearance	Consistency	Odour	Taste
Cheese on storage day one				
T_1	7.42±1.56[a] *	6.60±1.99[a]	6.81±1.79[ab]	6.81±1.82[a]
T_2	7.32±1.42[a]	7.33±1.29[b]	6.95±1.28[a]	7.20±1.36[a]
T_3	5.51±2.48[b]	5.15±1.82[c]	6.31±1.54[b]	5.99±1.74[b]
T_4	5.27±2.47[b]	6.68±1.67[ab]	6.56±1.42[ab]	6.87±1.56[a]
Cheese on storage day 15				
T_1	6.97±1.74[a]	7.67±1.12[a]	7.16±1.34[a]	7.09±1.73[a]
T_2	6.60±1.47[ab]	7.35±1.09[ac]	6.80±1.30[a]	7.19±1.41[a]
T_3	6.28±1.79[b]	6.63±1.53[b]	6.93±1.40[a]	6.25±1.81[b]
T_4	6.72±1.45[ab]	6.94±1.28[bc]	6.94±1.58[a]	6.72±1.52[ab]

* Within a column, means followed by the same letter are not significantly different (P>0.05).
[1] Mean±standard deviation of individual scores obtained from 85 panellists.
T_1: Natural acidification; T_2: Acidification with citric acid; T_3: Pasteurization at 65°C, 30 min; T_4: Pasteurization at 71°C, 10 min.

ments were in the range of 6 to 8, which corresponded to the descriptions of "fairly good" "good" and "very good" on the hedonic scale, except for appearance, consistency and taste of cheese on storage day 1 from treatment T_3, and appearance of T_4, which had average scores between 5 ("indifferent") and 6 ("fairly good"). It is important to note that panelists were not familiar with the Marajoara cheese attributes, and had not tasted it previously. On day 1, higher scores were attributed to the appearance of cheese in treatments T_1 and T_2 ($P<0.05$). As for consistency on day 1, treatments T_2 and T_3 presented the highest and the lowest scores, respectively. Considering that T_2 cheese had lower ($P<0.05$) firmness and similar ($P>0.05$) chewiness than T_3 cheese, it is concluded that firmness has probably a bigger impact on the acceptability of Marajoara cheese. Treatment T_3 also presented the lowest scores for odor and taste ($P<0.05$). These results indicate that pasteurization used in treatments T_3 and T_4 produced sensorial changes in the cheese that were more intense when the time-temperature relationship is 65°C for 30 min (T_3). These changes are probably related to the effects of heat treatment of the milk, such as inactivation of original enzymes in the raw milk, formation of sulphydryl groups from denaturation of whey proteins (IDF, 1995) and increase in viscosity of milk (Walstra and Jenness, 1987).

On day 15, cheese in treatment T_3 and T_4 had higher scores than those obtained on day 1 for all attributes, leading to a decrease in the differences in relation to the other treatments. This fact is possibly related to the development of the surviving starter culture used, as stated by Isepon and Oliveira (1995), and to other bacteria incorporated during cheese making, mainly from the cream. Thus, the cheese obtained in treatment T_3 presented lower scores ($P<0.05$) for appearance, consistency and taste. The comparison between the results of sensory analysis on days 1 and 15 shows that Marajoara cheese undergoes a ripening process during storage under refrigeration, and these modifications are favorable to the sensory characteristics of the product. The only exception was cheese T_2, whose scores were stable throughout the study. For cheese produced with pasteurized milk (T_3 and T_4), ripening is essential for the development of their final characteristics.

CONCLUSIONS

The use of citric acid in the manufacture of Marajoara cheese showed promising results, as the product presented satisfactory physical, chemical and sensory characteristics. Pasteurization of buffalo milk, as used in the present trial, produced an additional effect on the elimination of mesophilic microorganisms in the cheese, but increased texture parameters (firmness, gumminess and chewiness) and decreased the sensory quality. The starter culture used in the study enabled an improvement of the sensory quality of the cheese produced with pasteurized milk after 15 days of storage at 4°C. However, further studies should be performed in order to select adequate microorganisms for improvement of sensory characteristics of the product.

ACKNOWLEDGEMENTS

The authors would like to thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Brazil; grant no. 2001/03817-0) for the financial support.

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Paper received December 30, 2004 Accepted April 12, 2005

PAPER

EVALUATION OF TOTAL CAROTENOIDS AND ASCORBIC ACID IN OSMOTIC PRETREATED GUAVAS DURING CONVECTIVE DRYING

VALUTAZIONE DEI CAROTENOIDI TOTALI E DELL'ACIDO ASCORBICO DURANTE L'ESSICCAZIONE CONVETTIVA IN FRUTTI DI GUAVA PRETRATTATI OSMOTICAMENTE

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ABSTRACT

Degradation of ascorbic acid and carotenoid content in osmotic pretreated and convective dried guavas was investigated. The osmotic dehydration was performed in a semi-continuous flow osmotic contactor at 40°C for two hours with sucrose solution at 60% (w/w). Pretreated guava sticks were dried at 60°C to 20 and 11% moisture (w.b.) content. The results showed that the carotenoid retention varied from 66 to 70% and that of ascorbic acid was

RIASSUNTO

È stato esaminato il processo di degradazione dell'acido ascorbico e del contenuto in carotenoidi in frutti di guava pretrattati osmoticamente ed essiccati con processo convettivo. La disidratazione osmotica è stata ottenuta grazie all'utilizzo di un separatore osmotico a flusso semicontinuo a 40°C per due ore, con una soluzione di saccarosio al 60% (p/p). Le striscie di frutto di guava pretrattate sono state poi essiccate a 60°C dal 20% fino all'11% di umi-

- Key words: ascorbic acid, carotenoid, drying, guava, osmotic dehydration -

around 20 to 35% of the original values in osmotic pretreated and dried guavas.

dità (peso fresco). I risultati dell'esperimento hanno mostrato che, nei frutti di Psidio protrattati osmoticamente ed essiccati, la conservazione dei carotenoidi è passata dal 66 al 70% mentre la concentrazione di acido ascorbico si è attestata tra il 20 ed il 35% dei valori iniziali.

INTRODUCTION

In recent years there has been great interest shown in the nutritional value of foods in order to understand the contribution made by individual food products to the daily nutritional needs and how food processing and preservation technologies affect nutritive composition. It is known that fruit and vegetables play an important role in the human diet and longevity. The protective effect of fruit and vegetables is generally attributed to the presence of natural substances such as antioxidants, fibres and other bioactive components. However, recent studies have demonstrated that the consumption of the individual substance as a food supplement does not have the same effect as the corresponding food source. This suggests that the mechanisms of the protective properties of fruit and vegetables is more complex than was previously thought. It is possible that other natural components are involved in the interaction of these properties, which influence the biological activity (LERICI *et al.*, 2000). Thus, studies are needed to understand how to preserve these properties in fruit and vegetables during processing.

Vitamin C is considered one of the most important antioxidants because of its activity against free radicals, and the important role it plays against carcinogenesis and cardiovascular diseases, as well as in stimulating the immune system. The vitamin C content varies greatly among the numerous species and varieties of vegetables and fruit and can be affected by size, ripening stage, cultivation practices, storage conditions and packaging techniques (GALGANO *et al.*, 2002). Carotenoids are another very important quality attribute for the fruit industry not only because of the colour they provide but also because of their health benefits. Carotenoid intake reduces the risk of certain types of cancer, arteriosclerosis, and cataract formation, and it is well known that they play a role in disease prevention (ARIAS *et al.*, 2000). Vitamin C and carotenoids are, however, very sensitive to degradation when fruit is subjected to long-term storage, high temperatures, low relative humidity, physical damage, and chilling injury (LEE and KADER, 2000; PÉREZ-GÁLVEZ *et al.*, 2004). In general, if ascorbic acid is well retained, the other nutrients are also well preserved. Hence, ascorbic acid is usually considered as an index of nutrient quality during food processing and storage (FAVELL, 1998; GALGANO *et al.*, 2002; UDDIN *et al.*, 2002).

Guava, a native fruit of the tropical and subtropical regions of America, is rich in vitamin C (88-300 mg/100 g). In fact, guavas have 3-6 times more Vitamin C than oranges (YAMASHITA *et al.*, 2000; UDDIN *et al.*, 2002). Many studies on guava have been carried out to determine how to prevent oxidative damage, particularly in terms of ascorbic acid

degradation during processing and storage (UDDIN *et al.*, 2002; PADULA and RODRIGUES-AMAYA, 1987).

Since dried guavas are processed at high temperatures and in the presence of oxygen, they are subject to oxidative damage that results in undesirable changes in colour and appearance. Oxidative damage can be avoided by optimising the processing conditions. ERLE and SCHUBERT (2001) proposed a combination of osmotic pre-treatment and microwave-vacuum dehydration to obtain apples and strawberries with high quality in terms of colour, vitamin C content, structure and volume. SHARMA *et al.* (1998) suggested that osmotic and vacuum drying could be used to obtain dried apples with higher ascorbic acid retention and less browning. SHI and LE MAGUER (2000) indicated that sugar osmotic solutions that remain in the surface layer of the fruit prevent oxygen from penetrating and oxidizing carotenoids. Osmotic pre-treatment prior to air-drying was advantageous for product quality with lycopene losses being less than with air-drying. The final product had properties similar to those of typical fruits. TORREGGIANI and BERTOLO (2001) showed that solute uptake, combined with the effect of dehydration, modified the fruit composition which improved colour, vitamin and aroma retention during air dehydration and/or frozen storage.

PÉREZ-GÁLVEZ *et al.* (2004) and MÍNGUEZ-MOSQUERA *et al.* (2000) observed that the effect of drying on the carotenoid content in peppers depends on the variety of fruit used. They demonstrated that the metabolic complexity of carotenoid synthesis in the pepper fruit involves two biosynthetic pathways, with one or the other being dominant depending on the degree of ripeness. In some cases, the concentrations of certain pigments increased during drying because the biosynthesis of carotenoids probably continued in the fruit being dried; if the fruit is not completely ripe, the metabolic pathways of pigment formation continue to function. For varieties that were harvested and dried when completely ripe, the carotenoid content was less after drying.

Studies on the effects of processing conditions on qualitative and quantitative changes of some nutrients in fruit are needed to determine the best conditions for processing guava in order to preserve carotenoid content and ascorbic acid retention. The objective of this study was to evaluate total carotenoid loss and ascorbic acid reduction of osmotically pre-treated and dried guavas.

MATERIALS AND METHODS

Materials

Ripe guavas of the cultivar *Paluma* (*Psidium guajava* L.) were obtained from producers in São Paulo State, Brazil. Fruit of the same size and ripening stage was selected, in order to have homogeneous samples. Samples were washed, peeled, cut into halves and the seeds were removed. The fruit was then soaked in a sucrose solution (60°Brix) at 40°C for two hours with a 1:5 fruit-to-syrup mass ratio in a semi-continuous contactor. The osmotic solution was reused for 20 batches. From time to time, the concentration of osmotic solution had to be adjusted. After the process, the samples were washed with distilled water and placed on absorbent paper to remove surface moisture. The guavas processed in each of the 20 batches were air-dried before the analyses were carried out.

Air-Drying

Osmotically pre-treated guava halves were cut into 5 to 7 mm thick sticks and dried in a tray dryer at 60°±2°C for 3 and 4 hours. Air velocity was 1.0 m/s. Samples were weighed periodically in order to

check the drying process. Two final moisture contents were established, 20 and 11% (w.b), because the sensorial quality is different at these two moisture levels (results not shown).

Physico-chemical properties of fresh, osmodehydrated and osmodehydrated-dried guavas

Moisture, titratable acidity and reducing sugar content were determined according to AOAC (1996) methods 22.013, 22.008 and 31.036, respectively. Soluble solids (°Brix) were measured with a Zeiss West refractometer (Zeiss West, Berlin, Germany). Water activity was determined with a Decagon CX-2T hygrometer (Aqualab - Decagon Devices Inc., Pullman, WA, USA) and the pH was measured with a potentiometer Analyser model 300 (São Paulo, Brazil). All determinations were made in triplicate for each sample. For each cycle, two samples were taken for analysis and their average value was considered in the evaluation.

Determination of total carotenoid content

Total carotenoids were determined on an aliquot of petroleum ether using a spectrophotometric method (SÁ, 2001). Guava samples (10 g) were homogenised in 50 mL of acetone with 4.0 g of Celite. Samples were successively extracted with acetone until the residue was colourless and then filtered under vacuum through Whatman paper N. 1 on a Buchner funnel. The filtrate was transferred to a separatory funnel containing 100 mL petroleum ether. The combined filtrate was washed with water several times to remove all the acetone and the lower layer was discarded. The upper phase, containing carotenoid pigments, was recovered and transferred to a volumetric flask. Sodium sulphate was added to absorb the residual water. The volume of recovered petroleum ether was then adjusted to 200 mL with petroleum ether and absorbance was measured in a spectrophotometer (Beckman model DU-7-B340, Germany) at 468 nm. Necessary precautions were taken to prevent degradation or alteration of the pigments during analysis (e.g. protection from light and high temperature, short analysis time). Total carotenoid contents were calculated as mg per gram of fruit. All analyses were carried out in quadruplicate. The values reported are the means with their standard deviations.

Determination of ascorbic acid

The vitamin C content in fresh, osmotically-treated and osmodehydrated-dried guavas was measured by a chemical method involving titration with 2,6-dichloro-indophenol as described by the Official Methods of Analysis (AOAC, 1996). A 2% solution of oxalic acid was used in the homogeniser to inhibit further vitamin C degradation. After homogenisation, the slurry was filtered, and the chemical procedure was applied to the filtrate.

Statistical analysis

All statistical analyses were performed using Statistica V. 5.0 software. The significance level was $p<0.05$ for the analysis of variance (ANOVA), regression analysis, and Tukey test.

RESULTS AND DISCUSSION

Analytical results

Table 1 shows the mean values and standard deviations of the physico-chemical characteristics of the fresh and osmotically pre-treated guavas. For samples pre-treated by osmotic dehydration, the total sugar con-

Table 1 - Physico-chemical characteristics of fresh and osmotically pre-treated guavas obtained with an osmotic solution at 60±2.3°Brix, 40°C for 2 hours and reused to process 20 batches.

Analysis	Fresh	1st batch	5th batch	10th batch	20th batch
Titratable acidity (g/10g)*	0.62±0.02	0.39±0.01	0.37±0.02	0.41±0.01	0.42±0.04
Total sugar (%)	9.34±0.93	16.50±0.01	13.96±0.01	16.43±0.01	15.72±0.01
Water activity (a_w)	0.989±0.003	0.978±0.003	0.976±0.003	0.977±0.003	0.979±0.003
pH	3.90±0.20	4.83±0.30	4.88±0.20	4.50±0.10	4.44±0.20
Moisture (%)	85.38±0.10	73.15±1.78	70.80±0.24	71.35±0.67	72.45±0.93
Water loss OD (%)	-	28.36±2.40	30.00±1.00	28.86±1.60	28.04±1.40

OD, osmotic dehydrated guavas;
*g citric acid/10 g guava.

tent increased in comparison with the fresh samples. This increase can be explained by the sugar uptake, incorporated as sucrose or its hydrolysed form. This incorporation is part of the osmotic dehydration process that is due to a mass transfer, caused by the concentration differences between the fruit and the solution (RASTOGI and RAGHAVARAO, 1997). In relation to the reused cycles, the increase in sugar levels was nearly constant for all twenty batches. Another consequence of the osmotic dehydration was that the water activity and the moisture content were less for the pre-treated samples. The titratable total acidity of osmotically-treated samples was significantly less than that of the fresh fruit, although it was constant in the re-used solutions. This result can be explained by the diffusion of the fruit acids into the dehydration solution as well as by chemical deterioration (VIAL *et al.*, 1991). As a consequence, the pH values increased. Some of the differences observed among the samples can be attributed to the use of raw material that was not completely uniform, due to different stages of maturity of the fruit (LEE and KADER, 2000). Osmotic dehydrated samples with the characteristics presented in Table 1 were then subjected to a convective drying step.

Effect of re-using the dehydration solution on the product obtained after drying

Fig. 1 shows the weight loss in fresh and pre-dehydrated guava samples in reused sucrose solution after 4 hours of drying. The rates were constant for all the batches analysed. The weight losses for the dehydrated fruit and the untreated ones were very similar. The similarity of the results were unexpected since the amount of water in the osmotic dehydrated fruit was significantly less (about 30%) at the beginning of the drying processes. SANJINEZ-ARGANDOÑA (1999) reported that it took half the time to dry osmotically dehydrated guava halves to a certain water activity level compared to fresh fruit.

Table 2 shows the data for water activity and moisture content of osmotically-treated and untreated samples. Although pre-treated guavas had a lower moisture content at the beginning of the drying process, there was no significant difference ($p<0.05$) in the drying time required to obtain an intermediate moisture content (a_w around 0.65 to 0.75) for the two products. The drying curves for guavas with and without osmotic pre-treatment and dried at 60°C are shown in Fig. 2.

This result could be attributed to the

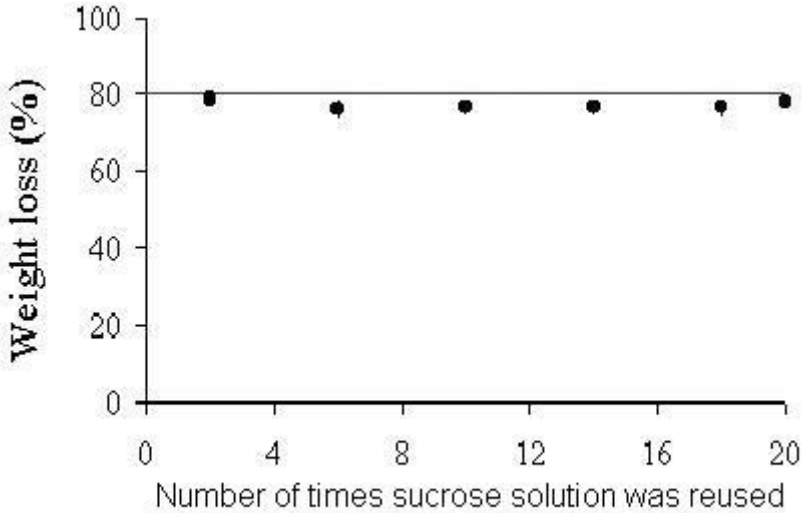


Fig. 1 - Weight loss after drying while reusing sucrose solution (●) and the fresh fruit subjected to drying (solid line).

Table 2 - Mean values of moisture, water activity, total sugar and titratable total acidity of guava sticks osmotically pretreated and dried for four hours and guava dried without pretreatment.

Sample	Moisture (%)	a_w	TS (%)	TTA (%)
Dried guava	13.84±2.0	0.743±0.020	16.09±1.3	5.7±0.2
OPDG 1st recycle	12.16±2.9	0.742±0.020	34.77±1.7	3.12±1.9
OPDG 5th recycle	10.93±1.2	0.658±0.040	40.91±0.7	3.61±0.8
OPDG 10th recycle	11.18±1.5	0.659±0.006	39.68±0.9	3.34±1.1
OPDG 15th recycle	11.84±2.68	0.679±0.007	36.95±1.9	3.22±0.7
OPDG 20th recycle	11.09±2.13	0.666±0.002	39.07±1.78	3.24±0.5
Standard deviation[1]	0.53	0.04	2.43	0.19

a_w: water activity, TS: total sugar and TTA: titratable total acidity.
OPDG: osmotically pre-treated and dried guavas.
[1]: Standard deviation among the values for the five recycles of osmodehydrated-dried guavas.

deposition of solids (sugar) on the surface of the treated samples which act as a barrier to water diffusion, causing a decrease in the drying rates of these samples, when compared to untreated samples. Another possible explanation could be associated with the fruit geometry used in this study (sticks). The hot air had a large contact area in the sticks of both the untreated and pre-treated guavas during drying which probably reduced the positive effect that osmotic treatment generally has on the drying rate of pre-treated foodstuffs.

The sugar content was higher for pretreated guavas (Table 2). The solid uptake observed in fruit during osmotic dehydration is inherent to the process and is important for maintaining product quality during the drying process.

The results for the samples that were osmotically pre-treated and then dried

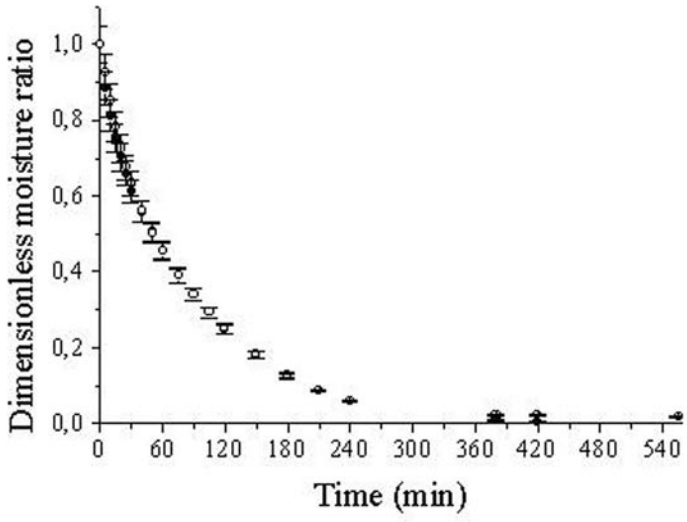


Fig. 2 - Dimensionless moisture content during drying of guava sticks with (●) and without (○) osmotic dehydration (OD).

for 3 h were similar to those found in the samples dried for 4 h; the moisture was around 20% (±2%); the water activity was 0.798 and the weight loss was 74%. Consequently, the evaluation of the ascorbic acid and total carotenoid contents was carried out on the 4 h-dried samples only.

Effect of drying time on ascorbic acid content of osmotically pretreated and dried guavas

The vitamin C content of osmotically dehydrated guavas, quantified as ascorbic acid, and as a function of the cycles of reused osmotic solution, was not significantly different; the retention value was 83.27% (±8.80%) of the initial amount.

The evolution of the residual ascorbic acid content during drying of osmotically pre-treated guavas at different times (from 30 to 360 min) is shown in Fig. 3. The ascorbic acid content in the raw guava was high, ranging from 88 to 300 mg/100 g, which is in accord with other literature data (PEREIRA and JUNIOR, 1986; YAMASHITA *et al.*, 2000; UDDIN *et al.*, 2002). Such variation in vitamin C content is influenced by many factors: degree of ripeness, climatic conditions during the growing season and post-harvest storage temperature (LEE and KADER, 2000). Conversely, no vitamin C was detected in dried guava without osmotic treatment. For osmotically pretreated guavas, dried at 60°C, the vitamin C content decreased as drying time increased. Experimental data showed that after 120 min of drying, the ascorbic acid retention was around 30-35%. A first order equation (Eq. 1) provides the best fit to describe the loss of vitamin C as a function of drying time; this is consistent with literature results (UDDIN *et al.*, 2002; DEWANTO *et al.*, 2002; ASSIRY *et al.*, 2003).

$$C_{At} = C_{Ao} + a.e^{-(t-t_o)/b} \qquad (1)$$

where, C_{Ao} (mg/100 g); is the concentration (mg/100 g) of ascorbic acid at initial

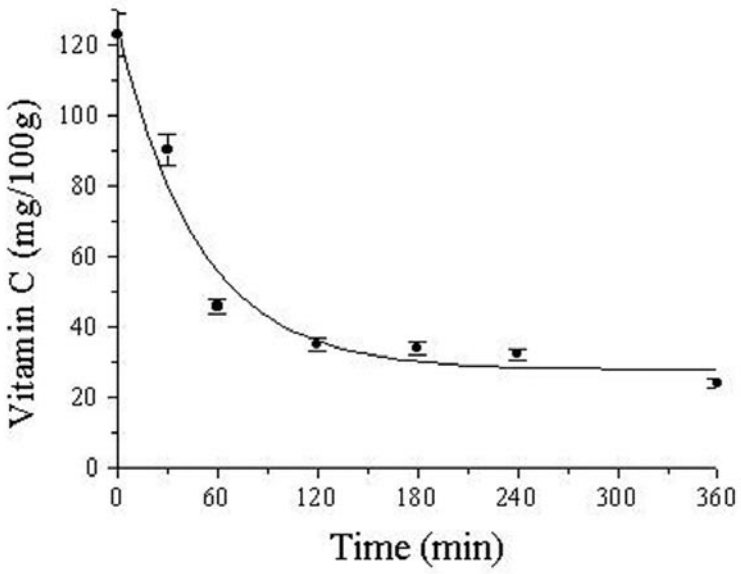


Fig. 3 - Evolution of ascorbic acid content as a function of drying time at 60°C in osmotically pretreated and dried guava sticks (●), sample data fitted to first order model (solid line).

time; C_{At} is the concentration of ascorbic acid at time t; t is time (min); t_o is the initial time (min) and *a* and *b* are equation constants.

Loss of vitamin C occurs primarily through oxidation of ascorbic acid to dehydroascorbic acid and other nutritionally inactive by-products. Ascorbic acid is easily oxidized, especially in aqueous solutions, and is greatly influenced by the presence of oxygen and high temperatures (LEE and KADER, 2000; GIOVANELLI *et al.*, 2002; ASSIRY *et al.*, 2003). A considerable loss of ascorbic acid has also been observed in tomato halves and tomato pulp after air-drying (ZANONI *et al.*, 1999). DEWANTO *et al.* (2002) showed that the vitamin C content decreased with increased heating time at 88°C in tomatoes. After 2, 15 and 30 min of heating at 88°C, the vitamin content decreased to 10.53, 15.79 and 28.95%, respectively. In spite of the significant loss of ascorbic acid, thermal processing enhanced the nutritional value by increasing the free flavonoid content which has a notable antioxidant activity. This effect could also occur in guavas. Moreover, the results of vitamin C retention of dried fruit without pre-treatment and the trend of the ascorbic acid loss to reach a pseudo-steady state, indicate that the impregnation of the samples with sucrose solution reduced vitamin degradation.

Effect of drying time on total carotenoid retention of osmotically pretreated and dried guavas

The total carotenoid retention for dried guavas, with and without osmotic treatment, is shown in Fig. 4. Total carotenoids slightly decreased from 6.01 to 5.34 mg/100 g (w.b.) during osmotic dehydration. Osmotically-treated and dried guavas showed greater total carotenoid retention (68% after 4 h of drying) than the untreated ones (25% after 4 h of drying). SHI *et al.* (1999) reported a similar behaviour for tomatoes. A possible explanation is that the sugar solution used for osmotic dehydration limited the contact between fruit and oxygen, reducing carotenoid oxidation, as previously observed by SHI and LE MAGUER (2000). Dried samples without osmotic pre-treatment showed

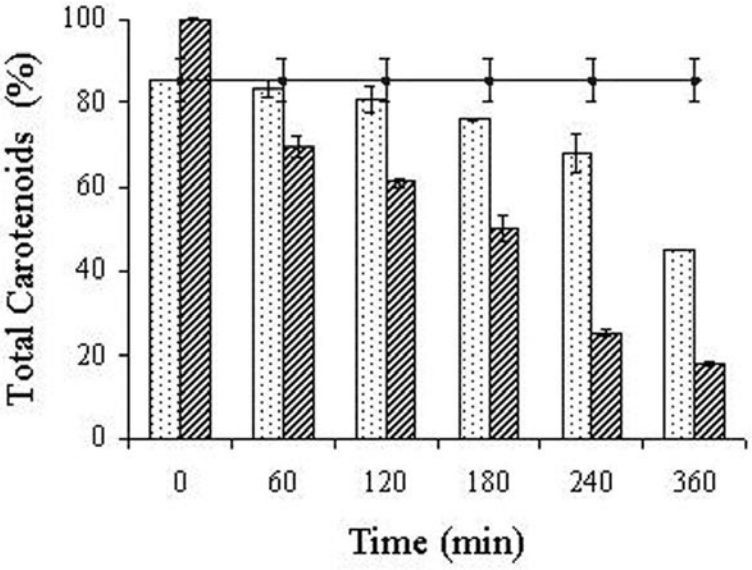


Fig. 4 - Evolution of total carotenoid content as a function of drying time (T= 60°C) in osmotically pre-treated ▨ and dried ⁘ guavas sticks.

a more pronounced carotenoid degradation, probably due to cellular tissue damage and increased exposure to oxygen, which resulted in pigment destruction (SHI *et al.*, 1999; SHI and LE MAGUER, 2000; NGUYEN *et al.*, 2001). TORREGGIANI and BERTOLO (2001) also showed that the fruit sugar uptake promoted by osmosis combined with drying can modify the fruit composition, favouring the retention of pigments, vitamins and flavour substances. However, the loss of carotenoids during osmotic dehydration can also be partially associated with pigment diffusion from the fruit to the solution (VALDEZ-FRAGOSO *et al.*, 1999).

For tomatoes, DEWANTO *et al.* (2002) found that thermal processing increased total antioxidant activity and enhanced available lycopene. On the other hand, the total amount of ascorbic acid present in the osmotic pre-treated guavas seems to be an important factor that influences carotenoid stability. CHOI *et al.* (2002) evaluated the interaction of ascorbic acid with anthocyanin and carotenoid pigments on the quality of orange juice, and found that the loss of carotenoids was limited compared to that of anthocyanin pigments under the same storage conditions, probably due to the stabilizing effect of ascorbic acid on carotenoids.

CONCLUSIONS

Osmotically pre-treated guavas with reused solutions and then dried did not show significant differences in weight loss, water activity, titratable acidity, total sugar and moisture between the different cycles. Convective drying of osmotically pre-treated guavas combined the benefits of both unit operations. Osmotic treatment influenced the properties of the final product giving better quality products in terms of vitamin C content and total carotenoid retention due to the sugar barrier layer formed at the surface of the osmotic pre-treated guavas. Vitamin C retention varied from 20 to 35% and the retention of the total carotenoids ranged from 66 to 70% for osmodehydrated guavas.

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Paper received October 19, 2004 Accepted April 16, 2005

PAPER

ANTIOXIDANT PROPERTIES OF THYME (*THYMUS VULGARIS* L.) AND WILD THYME (*THYMUS SERPYLLUM* L.) ESSENTIAL OILS

PROPRIETÀ ANTIOSSIDANTI DEGLI OLI ESSENZIALI DEL TIMO (*THYMUS VULGARIS* L.) E DEL TIMO SELVATICO (*THYMUS SERPYLLUM* L.)

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ABSTRACT

In this study, the composition of the volatile compounds and the antioxidant activity of thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) essential oils were investigated using three different methods: the DPPH radical scavenging method, the thiobarbituric acid reactive species (TBARS) assay and the β-carotene bleaching method. The results obtained for the total essential oils in both plants and their two fractions (hydrocarbon- and oxygen- containing fractions) were com-

RIASSUNTO

In questo studio sono state esaminate la composizione della frazione volatile e l'attività antiossidante degli oli essenziali ottenuti dal timo (*Thymus vulgaris* L.) e dal timo selvatico (*Thymus serpyllum* L.) utilizzando tre differenti metodologie: il metodo di cattura dei radicali liberi del 2,2′-diphenyl-1-picrylhydrazyl (DPPH), il saggio delle specie reattive dell'acido tiobarbiturico (TBARS) e il metodo della decolorazione del β-carotene. I risultati ottenuti per tutti gli oli essenziali in entrambe le piante e nel-

- Key words: antioxidant activity, β-carotene bleaching, DPPH radical scavenging, essential oil, TBARS, thyme, wild thyme -

pared with those of α-tocopherol, ascorbic acid and the synthetic antioxidants – butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Thyme and wild thyme essential oils exhibit significant *in vitro* antioxidant activity, while fractions of the essential oils that contain hydrocarbon compounds (CH fractions) only show antioxidant activity in the TBARS assay.

le loro due frazioni (CH e CHO, frazioni contenente idrocarbonili e carbossili, rispettivamente) sono stati confrontati con quelli dell'α-tocoferolo, dell'acido ascorbico e degli antiossidanti sintetici – butil idrossitoluene (BHT) e butil idrossianisolo (BHA). Gli oli essenziali di timo e di timo selvatico mostrano una significante attività antiossidante *in vitro,* mentre le frazioni di questi oli essenziali che contengono composti idrocarbonilici (frazioni CH) mostrano attività antiossidante soltanto nel saggio con metodo TBARS.

INTRODUCTION

Interest in natural antioxidants for the food industry has increased due to speculation about the possible toxic effects of synthetic antioxidants (BRANEN, 1975; NAMIKI, 1990; AESCHBACH *et al.*, 1990; POKORNY, 1991). Aromatic plants have been studied extensively because a rich source of natural antioxidants is available in their essential oils or diverse extracts. SHAHIDI *et al.* (1992) reported that the antioxidant effect of aromatic plants is closely related to the presence of hydroxyl groups in phenolic compounds.

Plants belonging to the *Lamiaceae* family are known to be rich in compounds possessing strong antioxidant activity (YOUDIM *et al.*, 1999; LEE and SHIBAMOTO, 2002; MIURA *et al.*, 2002). Thyme and wild thyme are also regarded as medicinal herbs with antispasmodic, expectorant and flatulence-reducing action (GUENTHER, 1949). The essential oils of the genus *Thymus* are mainly monoterpenic (C_{10}), and often contain large amounts of thymol and carvacrol (HEGNAUER, 1962). AESCHBACH *et al.* (1990) reported that thymol and carvacrol inhibit the peroxidation of liposome phospholipids in a concentration-dependent manner. Previous studies (KULISIC *et al.*, 2004) have shown that oregano essential oil that contains a high content of thymol and carvacrol has a strong antioxidant activity.

The objective of this research was to evaluate the antioxidant activity of thyme and wild thyme essential oils using a multiple-method approach in relation to their chemical composition. Three different methods were used: the β-carotene bleaching method, the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and the thiobarbituric acid reactive species (TBARS) assay (PRATT, 1980; MALLET *et al.*, 1994; CUENDET *et al.*, 1997; YEN and DUH, 1994; RUBERTO and BARATTA, 2000). The results were compared with the effect of α-tocopherol, ascorbic acid and the synthetic antioxidants, BHT and BHA.

MATERIAL AND METHODS

Materials

Thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) were collected in central Dalmatia and Bosnia and Herzegovina during the summer,

2002. After air-drying, the essential oils were extracted from the plant materials (flowered tops and stalks). A hundred grams of dried material from each plant were hydrodistillated for 3 h using a Clevenger-type apparatus. The distillated essential oils were dried over anhydrous sodium sulphate and stored under nitrogen in a sealed vial at -20°C until analysis. The voucher specimens of thyme and wild thyme plant material, as well as their essential oils, are stored in the Department of Biochemistry and Food Chemistry, Faculty of Chemical Technology, Split, Croatia.

The essential oils of thyme and wild thyme (0.5 g) were fractionated using a silica gel (30-60 µm, Mallinckrodt Baker B.V., Deventer, The Netherlands) column (length 20 cm; i.d. 2 cm). Pentane (50 mL) was used to obtain the fraction, which contained only non-polar hydrocarbons (CH fraction), while diethyl ether (50 mL) was used for the fraction containing polar (oxygen-containing, CHO fraction) compounds. These fraction solutions were concentrated to 0.5 mL and subjected to thin layer chromatography (TLC) on silica gel plates in order to check the results of the column chromatographic separation. Different solvents were used as mobile phase: *n*-hexane for the CH fraction and *n*-hexane:ethyl acetate 85:15 (v/v) for the CHO fraction. Two percent vanillin-sulphuric acid was used as a detection reagent. The fractions obtained by column chromatography were also subjected to GC/MS analysis.

GC-MS analysis

Volatile compounds were analysed on a Hewlett-Packard GC-MS system (GC 5890 series II; MSD 5971A, Hewlett Packard, Vienna, Austria). The fused-silica HP-20 M polyethylene glycol column (50 m x 0.2 mm, 0.2 µm thickness, Hewlett-Packard, Vienna, Austria) was directly coupled to the mass spectrometer. The carrier gas was helium (1 mL/min). The program used was 4 min isothermal at 70°C, then 4°C/min to 180°C and 10 min isothermal. The injection port temperature was 250°C and the detector temperature was 280°C. Ionisation of the sample was performed in the EI mode (70 eV).

The linear retention indices for all the compounds were determined by co-injection of the sample with a solution containing the homologous series of C_8-C_{22} *n*-alkanes (VAN DEN DOOL and KRATZ, 1963). The individual constituents were identified by their retention indices with reference to the compounds known from literature data (ADAMS, 1995), and also by comparing their mass spectra with spectra of either, known compounds or with those stored in the Wiley mass spectral database (Hewlett-Packard, Vienna, Austria).

Determination of antioxidant using 2,2´-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging

The antioxidant activity of the thyme and wild thyme volatile compounds was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical, DPPH (2,2´-diphenyl-1-picrylhydrazyl) according to the modified method of CUENDET *et al.* (1997). The colour of DPPH changes from purple to yellow as a consequence of radical scavenging by antioxidants through donation of a hydrogen atom to form the stable DPPH-H (VON GADOW *et al.*, 1997). The remaining DPPH, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant (BRAND-WILLIAMS *et al.*, 1995).

The ethanolic stock solution (50 µL) of two different concentrations (0.2 and 2 g/L) of antioxidants was placed in a cuvette with 1 mL of 0.004% ethanolic solution of DPPH. Absorbance measurements started immediately. The decrease in absorbance at 517 nm was determined with a Perkin-Elmer spectro-

photometer after 1 h. Ethanol served as a blank. The absorbance of the DPPH radical without antioxidant, i.e. the control, was measured daily and special care was taken to minimize the loss of free radical activity of the DPPH stock solution (storage under a nitrogen atmosphere; the glassware of the storage system was covered with aluminium foil according to BLOIS, 1956). All determinations were performed in triplicate. The amount of sample necessary to decrease the absorbance of DPPH (IC_{50}) by 50% was calculated graphically (% of inhibition was plotted against the sample concentration in reaction system). The percentage inhibition of the DPPH radical was calculated according to the following formula (YEN and DUH, 1994):

$$\% \text{ inhibition} = (A_{C(0)} - A_{A(t)} / A_{C(0)}) \times 100$$

where $A_{C(0)}$ is the absorbance of the control at t=0 min and $A_{A(t)}$ is the absorbance of the antioxidant at t=1 h.

Determination of antioxidant activity using thiobarbituric acid reactive species (TBARS)

A modified TBARS assay (RUBERTO and BARATTA, 2000) was used to measure the potential antioxidant capacity using egg yolk homogenate as lipid-rich media. Yolk homogenate, (0.5 mL of 10% water solution w/v) and 0.1 mL of different concentrations of ethanolic stock solutions of the samples (4.0, 20.0 and 40.0 g/L), prepared immediately before use, were added to a test tube and made up to 1.0 mL with distilled water. Subsequently, 0.05 mL of 2,2′-azobis (2-amidinopropane) dihydrochloride solution (0.07 M) in water was added to induce lipid peroxidation and 1.5 mL of 20% acetic acid and 1.5 mL 0.8% (w/v) thiobarbituric acid (TBA) in 1.1% (w/v) sodium-dodecyl sulphate solution were then added. The resulting mixture was vortexed and heated at 95°C for 60 min. After cooling, 5.0 mL of butan-1-ol were added to each tube, vortexed and centrifuged at 1,200 g for 10 min. The absorbance of the organic upper layer was measured using a spectrophotometer (Perkin-Elmer Lambda EZ 201, Roma, Italia), at 532 nm. All determinations were performed in triplicate and the values were calculated as the percentage antioxidant index (AI%) (RUBERTO and BARATTA, 2000):

$$AI\% = (1 - A_T/A_C) \times 100$$

where A_C is the absorbance value of the fully oxidized control and A_T is the absorbance of the test sample.

Determination of antioxidant activity using β-carotene bleaching (BCB)

Antioxidant activity of the thyme and wild thyme volatile compounds was determined according to a slightly modified version of the β-carotene bleaching method (PRATT, 1980). β-carotene (0.1 mg) was added to a boiling flask together with linoleic acid (20 mg) and Tween 40 (100 mg), all dissolved in chloroform. After evaporation to dryness, under vacuum at 50°C with a rotary evaporator, distilled water under oxygen gas flow (50 mL) was added and the mixture was emulsified for 1 min in an ultrasonic bath to form emulsion A. Two different concentrations (0.04 and 0.2 g/L) of ethanolic stock solution of the samples were prepared, of which, 200 µL were mixed with 5 mL of emulsion A in open-capped cuvettes. A control, without antioxidant, consisted of 200 µL of ethanol and 5 mL of emulsion A. Emulsion B consisted of 20 mg of linoleic acid, 100 mg of Tween 40 and 50 mL of distilled water under an oxygen gas flow. The mixture of 200 µL of ethanol and 5 mL of emulsion B served as a blank. Readings of all samples were taken immediately (t=0) and at 15 min intervals for 120 min on a Perkin-Elmer Lambda EZ 201

spectrophotometer at 470 nm. The cuvettes were incubated at 50°C between measurements. All determinations were performed in duplicate. The antioxidant activity coefficient (AAC) was calculated from the data with the following formula (MALLET *et al.*, 1994):

$$AAC = [(A_{A(120)} - A_{C(120)}) / (A_{C(0)} - A_{C(120)})] \times 1000$$

where $A_{A(120)}$ is the absorbance of the antioxidant at t=120 min, $A_{C(120)}$ is the absorbance of the control at t=120 min, and $A_{C(0)}$ is the absorbance of the control at t=0 min.

Statistical analysis

The results obtained from all the methods used were analyzed by ANOVA, using the computer software Mathematica 5.0.

RESULTS AND CONCLUSION

Chemical composition of thyme and wild thyme essential oils

GC-MS analyses of thyme and wild thyme essential oils indicate that they are qualitatively similar. The compounds identified in the total essential oils of thyme and wild thyme and the compounds identified after fractionation of the essential oils are reported in Table 1. Four compounds were identified in thyme essential oil without fractionation. The predominant compounds were: γ-terpinene (5.5%), *p*-cymene (11.6%) and phenolic monoterpenes – thymol (80.4%) and carvacrol (2.1%). In wild thyme essential oil eight compounds were identified without fractionation; *p*-cymene (5.2%), γ-terpinene (5.3%), thymol (30.0%) and carvacrol (49.4%) being the main compounds. After fractionation, the oils were subjected to a more comprehensive GC-MS analysis and eleven compounds were identified in the thyme hydrocarbon (CH) fraction; γ-terpinene (41.1%), *p*-cymene (26.5%) and *trans*-caryophyllene (23.9%) were the main components. On the other hand, eight compounds were identified in the wild thyme hydrocarbon (CH) fraction; α-terpinene (2.3%), γ-terpinene (29.5%), *p*-cymene (26.1%) and *trans*- caryophyllene (29.3%) were the main components. The oxygen-containing compounds (CHO) in the fractions in both cases were two phenolic compounds; namely thymol (91.8% in thyme, 35.4% in wild thyme) and carvacrol (5.8% in thyme, 62.7% in wild thyme).

Antioxidant activity of thyme and wild thyme essential oils

DPPH radical scavenging method

Table 2 shows the radical scavenging effect of thyme and wild thyme essential oils in comparison with those of the synthetic antioxidants BHT and BHA, α-tocopherol and ascorbic acid. The radical scavenging effect of thyme and wild thyme essential oils and oil fractions was much lower than those of the reference compounds. The concentrations needed for 50% inhibition of the DPPH radical were several times lower than those of thyme and wild thyme essential oils and their fractions.

Thyme essential oil was a slightly better scavenger of the DPPH radical than wild thyme essential oil. The CHO fraction of thyme essential oil was better than the wild thyme CHO fraction. The CH fraction of both essential oils did not show any radical scavenging effect. This is logical because it is known that the radical scavenging effect of the DPPH method is determined by the number and substitution pattern of the -OH groups (BRAND-WILLIAMS *et al.*, 1995; KOLEVA *et al.*, 2002).

Solvent effects are also important for the capability of the phenols to donate a hydrogen-atom (VALGIMIGLI *et al.*, 1995).

Table 1 - The composition of thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) essential oils.

N.	Compound	RI[1]	Area (%)			
			Thyme		Wild thyme	
			In total oil	In fraction	In total oil	In fraction
Hydrocarbon CH fraction (CH)						
1.	α-Pinene	1023	-	-	0.6	1.2
2.	α-Thujene	1031	-	0.7	-	-
3.	Δ-Carene	1131	-	0.3	-	-
4.	β-Myrcene	1148	-	-	0.4	1.0
5.	α-Terpinene	1231	-	0.8	0.7	2.3
6.	γ-Terpinene	1231	5.5	41.1	5.3	29.5
7.	*p*-Cymene	1247	11.6	26.5	5.2	26.1
8.	Terpinolene	1260	-	0.1	-	-
9.	Caryophyllene	1585	-	23.9	3.5	29.3
10.	α-Humulene	1683	-	0.5	-	0.6
11.	β-Cubebene	1694	-	0.7	-	-
12.	D-Germacrene	?[2]	-	1.5	-	-
13.	Δ-Cadinene	1716	-	2.8	-	-
14.	unknown compound		-	-	-	6.1
				Total 98.8		Total 96.3
Oxygen-containing compound fraction (CHO)						
15.	Linalool	1507	-	1.3	-	-
16.	Benzyl alcohol	1919	-	-	-	0.2
17.	Thymol	2115	80.4	91.8	30.0	35.4
18.	Carvacrol	2140	2.1	5.8	49.4	62.7
				Total 98.9		Total 98.3

[1]Retention indices relative to C_8-C_{22} alkanes on polar HP-20M column.
[2]Tentatively identified on the basis of mass spectra (MS) only.

Table 2 - Scavenging activity of thyme and wild thyme essential oils, their fractions, BHT, BHA, α-tocopherol and ascorbic acid on the DPPH radical (mean values ± standard deviation and ANOVA analysis are given).

Antioxidant	IC_{50}[1]	% inhibition of DPPH concentration in reacting system	
		0.2 g/L	2 g/L
Thyme essential oil	0.30±0.06	38.80±0.10	91.30±0.30
Wild thyme essential oil	0.40±0.05	30.50±0.04	82.00±0.07
Thyme CH fraction[2]	-	5.70±0.10	11.70±0.05
Wild thyme CH fraction[2]	-	5.30±0.08	12.60±0.50
Thyme CHO fraction	0.40±0.05	37.20±0.06	93.20±0.80
Wild thyme CHO fraction	0.70±0.06	28.60±1.00	82.20±0.70
BHT[3]	$1.80\times10^{-2}\pm0.20\times10^{-2}$		
BHA[3]	$5.40\times10^{-2}\pm0.20\times10^{-2}$		
α-Tocopherol[3]	$8.60\times10^{-3}\pm0.30\times10^{-4}$		
Ascorbic acid[3]	$4.40\times10^{-3}\pm0.30\times10^{-4}$		

[1]Concentration (g/L) for 50% inhibition. [2]CH fractions could not inhibit 50% of DPPH under test conditions. [3]At these concentrations (0.2 and 2 g/L), kinetic behaviour of BHT, BHA, α-tocopherol and ascorbic acid is so fast that they immediately achieve 100% DPPH inhibition. [4]ANOVA analysis gave 116.15 for F-ratio and 1.64×10^{-12} for null-hypothesis P-value.

"Water-like" solvents induce a dramatic decrease in the hydrogen-atom donating capability in comparison to chlorobenzene, methanol, ethanol (used in this method) or other alcohols.

TBARS

The thiobarbituric acid assay (TBA) involves the spectrophotometric detection of thiobarbituric acid reactive substances (TBARS), namely malonaldehyde (MDA), one of the secondary lipid peroxidation products, the quantity of which indicates the extent of lipid degradation (RUBERTO and BARATTA, 2000). Table 3 shows the antioxidant activity of thyme and wild thyme essential oils before and after fractionation, in comparison with those of α-tocopherol, ascorbic acid and the synthetic antioxidants, BHT and BHA. α-Tocopherol and BHA showed the highest antioxidant activities. α-Tocopherol had a high antioxidant activity even at the lowest concentration (AI of 72.6% at 100 ppm). Ascorbic acid did not show any antioxidant activity. The antioxidant activity of thyme essential oil was higher than that of wild thyme essential oil. In contrast, the CHO fraction of wild thyme essential oil was slightly better than that of the thyme essential oil. Non-polar CH fractions exhibited good antioxidant activities. RUBERTO and BARATTA (2000) reported similar results for non-polar compounds from essential oils. The authors tested about 100 pure components of essential oils and demonstrated that the monoterpene hydrocarbons γ-terpinene, *p*-cymene and α-terpinene had significant antioxidant activities. These compounds were representative of the CH fractions of thyme and wild thyme essential oils.

Egg yolk water emulsion was used in this method. Many antioxidants in emulsions will partition into both the lipid and water phase of emulsions. This portioning behaviour is critical in the activity of the antioxidant (DECKER *et al.*, 2005).

It has already been observed that compared to lipid-soluble antioxidants, water-soluble antioxidants like ascorbate yield better protection to strongly lipophilic food systems like pure oils. In contrast, antioxidants soluble in li-

Table 3 - Antioxidant activity of thyme and wild thyme essential oils, their fractions, BHT, BHA, α-tocopherol and ascorbic acid measured by the thiobarbituric acid method (mean values ± standard deviation and ANOVA analysis are given).

Antioxidants	AI (%)[1]		
	100 ppm[2]	500 ppm	1,000 ppm
Thyme essential oil	27.3±4.5	57.0±3.3	75.3±2.7
Wild thyme essential oil	27.4±3.1	36.6±1.7	55.3±2.4
Thyme CH fraction	10.0±1.7	42.3±3.6	60.7±4.3
Wild thyme CH fraction	45.2±3.7	66.2±7.5	77.0±3.5
Thyme CHO fraction	38.2±6.2	50.6±2.4	66.0±5.0
Wild thyme CHO fraction	51.8±2.9	65.3±4.2	73.5±3.7
BHT	37.5±1.9	64.0±2.5	68.6±1.1
BHA	38.5±3.2	78.9±5.1	88.7±2.9
α-Tocopherol	72.6±3.6	79.5±2.3	90.0±1.8
Ascorbic acid	12.5±1.2	5.3±2.4	2.7±1.6

[1]Antioxidant effectiveness expressed as antioxidant index (as described in Materials and methods). Values are the average of triplicates ± standard deviation. [2]Antioxidant concentration in reacting system. [3]ANOVA analysis gave 427.87 for F-ratio and 0.00 for null-hypothesis P-value.

pids like the tocopherols yield better protection to oil-in-water emulsions when compared to water-soluble antioxidants (PORTER, 1993). Polar antioxidants are more effective in bulk oils because they can accumulate at the air-oil interface, the location where lipid oxidation reactions would be the greatest due to high concentrations of oxygen and prooxidants. In contrast, non-polar antioxidants are more effective in emulsions because they are retained in the oil droplets and/or may accumulate at the oil-water interface. In emulsions polar antioxidants would tend to partition into the aqueous phase and they would not be able to protect the lipid (DECKER *et al.*, 2005). This could explain the good antioxidant activity of the non-polar CH fractions and the lack of the antioxidant activity of ascorbic acid in this method.

Table 4 - The antioxidant activity of thyme and wild thyme essential oils, BHT, BHA, α-tocopherol and ascorbic acid assessed with the β-carotene bleaching method (mean values ± standard deviation and ANOVA analysis are given).

Antioxidant	AAC[1] concentration in reacting system	
	0.04 g/L	0.2 g/L
Thyme essential oil	376±160	657±52
Wild thyme essential oil	400±24	545±181
Thyme CH fraction	82±3.6	314±53
Wild thyme CH fraction	5.1±3.1	168±28
Thyme CHO fraction	483±35	669±38
Wild thyme CHO fraction	299±12	555±34
BHT	693±59	852±37
BHA	980±18	992±14
α-Tocopherol	613±23	813±65
Ascorbic acid[2]	-	-

[1]AAC – the antioxidant activity coefficient (calculated as described in Materials and methods). [2]Using this method ascorbic acid did not show any antioxidant activity. [3]ANOVA analysis gave 25.50 for F-ratio and 1.10×10^{-7} for null-hypothesis P-value.

β-Carotene bleaching method

The antioxidant activites of compounds from thyme and wild thyme essential oils, BHT, BHA, α-tocopherol and ascorbic acid assessed with the β-carotene bleaching method, expressed as the antioxidant activity coefficient, are presented in Table 4. The synthetic antioxidants, BHA and BHT, and α-tocopherol were the strongest antioxidants. Ascorbic acid did not show any antioxidant effect with this method. This could be due to the previously described polar paradox. The polar antioxidants, such as ascorbic acid that remain in the aqueous phase of the emulsion are more diluted in the lipid phase and are thus less effective in protecting the linoleic acid (KOLEVA *et al.*, 2002; PORTER, 1993). Using this method, the thyme and wild thyme essential oils generally showed significant antioxidant activities. The antioxidant activity of thyme essential oil was slightly higher than that of wild thyme essential oil. The effect of the CHO fractions was almost the same as that of the total oils, probably because of the elimination of hydrocarbon fractions. The CH fractions of the thyme and wild thyme essential oils had low antioxidant activities.

The behaviour of different antioxidants in emulsion is not well understood (SCHWARZ *et al.*, 2000). Partitioning effects in emulsions may be more important than the antioxidative structure, the chemical properties of the emulsion components are also important and finally the unusual antioxidative properties of β-carotene is an additional important factor in the presentation of the results obtained with this method (KOLEVA *et al.*, 2002). Therefore, this method can only be used in combination with other methods to evaluate the antioxidant capacity of different compounds, especially in the case of complex samples such as plant extracts or essential oils.

CONCLUSION

The evaluation of the antioxidant activity of thyme and wild thyme essential oils based on the results of three different methods shows that these oils have an antioxidant activity (thyme is slightly better than wild thyme). However, the term antioxidant is limited by the specificity of the method, the chemical structure of the putative antioxidant, its polarity and concentration in the reaction medium and the possible interactions in complex systems like essential oils.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science, Education and Sport of the Republic of Croatia, Projects 0011-003 and HITRA TP-011701.

The authors thank Dr. Ante Bilusic (Faculty of Natural Sciences, Mathematics and Education, Split, Croatia) for the results of the ANOVA analysis.

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Revised paper received April 15, 2005 Accepted July 30, 2005

SHORT COMMUNICATION

SQUID (*SEPIA OFFICINALIS*) STORED IN ACTIVE PACKAGING: SOME CHEMICAL AND MICROBIOLOGICAL CHANGES

SEPPIE CONSERVATE IN IMBALLAGGI ATTIVI: VARIAZIONI CHIMICHE MICROBIOLOGICHE

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ABSTRACT

The effect of modified atmosphere packaging (MAP) with and without a moisture adsorbent, on the quality of squid (*Sepia officinalis*) during chilled storage, was evaluated. Chemical and microbiological tests were carried out to measure the amount of spoilage and the resulting loss of fish freshness during 12 days of storage at 3°C. After 11 days, trimethylamine nitrogen (TMA-N) increased from 0.4 mg/100 g in fresh

RIASSUNTO

In questo lavoro si è valutata l'efficacia di un imballaggio attivo sulla qualità di seppie (*Sepia officinalis*) conservate a 3±0,5°C. Sui diversi campioni di seppie è stato valutato il grado di deterioramento e la perdita di freschezza attraverso il monitoraggio dei principali parametri chimici e microbiologici. Il contenuto in trimetilammina (TMA-N), aumentava, a 11 giorni di conservazione, da 0,4 mg/100 g di pesce fresco, a

- Key words: active MAP, chill storage, psychrotrophs, squid, TMA-N, TVB-N -

samples to 24 mg/100 g in MAP with adsorbent and 35 mg/100 g in the other samples. Total volatile basic nitrogen (TVB-N) increased from 18 mg/100 g in fresh samples to 112 mg/100 g in MAP with adsorbent and 158 mg/100 g in control samples. Counts of aerobic psychrotrophic microorganisms and presumptive Pseudomonads were more than 10 times lower in both MAP treatments compared to the control, in which counts exceeded 10^6 cfu/g on day 9, and 10^7 cfu/g on day 12.

24 mg/100 g sui campioni conservati in MAP con adsorbente, e a 35 mg/100 g negli altri campioni. Il contenuto in azoto volatile basico totale (TVB-N), incrementava da 18 mg/100 g a tempo 0, a 112 mg/100 g nei campioni conservati in MAP con adsorbente, e a 158 mg/100 g nei campioni controllo a fine conservazione. La carica psicrotrofica aerobica e le presunte *Pseudomonas* ritrovate nei campioni conservati in MAP dopo 12 giorni di conservazione era 10 volte più basse rispetto al testimone, i cui valori superavano 10^6 e 10^7 cfu/g, rispettivamente a 9 e 12 giorni di conservazione.

INTRODUCTION

Seafood such as squid has a short shelf-life because fish muscle is rich in non-protein nitrogenous (NPN) compounds, such as trimethylamine oxide (TMAO), nucleotides, and free amino acids (RUIZ-CAPILLAS *et al.*, 2002). These substances serve as a substrate for the growth of contaminating bacteria, which are the principal cause of fish muscle spoilage.

The spoilage flora of seawater fish consist of Gram-negative, psychrotrophic, aerobic or facultative anaerobic bacteria (DEBEVERE and BOSKOU, 1996). A variety of psychrotrophic bacteria may be present in freshly caught products, but spoilage is caused by specific microorganisms, like *Shewanella putrefaciens* and *Pseudomonas* spp. in iced fresh fish, and *Photobacterium phosphoreum* in iced products from temperate waters preserved with modified atmosphere packaging (DALGAARD, 1995; GRAM and HUSS, 1996). During chilled storage, there is a shift in bacterial types. The bacterial levels at which the product is visibly deteriorated vary, and can be as low as 10^6-10^7 cfu/g, especially when species with large cell sizes and high TMAO reducing activity are involved (DEBEVERE and BOSKOU,1996; TAOUKIS *et al.*, 1999, PAARUP *et al.*, 2002 a, b).

The spoilage of fish and fish products is manifested via the formation of off-flavours, which are due to the transformation of NPN compounds into ammonia, trimethylamine (TMA-N) and other basic nitrogenous compounds, which together make up total volatile basic nitrogen (TVB-N) (GRAM and HUSS,1996). Thus, TMA-N and TVB-N content are directly related to bacterial spoilage in fresh or packed fish, and because of their high olfactory impact, they are commonly used as a quality index of stored fishery products (DEBEVERE and BOSKOU, 1996; RUIZ-CAPILLAS *et al.*, 2002). For instance, in the case of tattler (*Illex illecebrosus*), rejection thresholds of 10 mg TMA-N/100 g muscle and 45 mg TVB-N/100 g muscle have been reported (KE *et al.*, 1984).

Microbiology of seafood has been extensively studied and although few data are available on cephalopods the decrease of sensorial quality could be attributed to autolytic phenomena (LEB-

LANC and GILL, 1984). Many studies have used total aerobic counts as the only indicator of microbial spoilage (OHASHI *et al.*, 1991) and only recently has the composition of the specific spoilage flora of squid been studied (PAARUP *et al.*, 2002a, b). In a study on the shelf-life of chilled squid, PAARUP *et al.* (2002a) found that, when the sensorial properties of the product started to decline, Gram-negative, psychrophilic microorganisms of the *Pseudoalteromonas* genus constituted the main microflora of whole squid. In contrast, *Shewanella putrefaciens*, *Pseudomonas* and *Pseudoalteromonas* prevailed on gutted squid. The intestinal microflora of these samples was dominated by the psychrophile *Photobacterium phosphoreum*, probably implicated in the production of agmatine, a biogenic amine which is an important freshness indicator.

Modified atmosphere packaging (MAP) increases the shelf-life of fish products as CO_2 inhibits the growth of aerobic Gram-negative bacteria (REDDY *et al.*, 1992; PASTORIZA *et al.*, 1998). However, CO_2 tolerant species like *P. phosphoreum* may replace CO_2 sensitive *Pseudomonas* and *Shewanella*. Although MAP has been studied by many authors to predict the shelf-life of gadoid fish species (REDDY *et al.*, 1992; DALGAARD *et al.*, 1993; PASTORIZA *et al.*, 1996 a, b; SIVERTSVIK *et al.*, 2002), to date there have been no specific studies that provide information on the effect of MAP on the shelf-life of species like cephalopods.

The aim of the present study was to investigate the effect of MAP (20% CO_2 and 80% N_2) on the storage of gutted squid (*Sepia officinalis*) at 3°C. Furthermore since the CO_2-rich atmosphere gives rise to exudation from fish muscle (PASTORIZA *et al.*, 1998), which can be minimized by the use of adsorbent pads in the packaging (CHURCH, 1998), a new moisture adsorbent material for use in packs was used.

MATERIALS AND METHODS

Sample preparation

Squid (*Sepia officinalis*) samples were caught off the Salerno coast (Italy) and stored at 0°C for about 12 hours before processing. The squid were gutted, rinsed with water, portioned in 300-350 g samples (equal to the weight of two squid) and placed in polystyrene trays. There were three treatments:

(a) MAP: samples stored in 20% CO_2/80% N_2 and sealed with a high barrier ethylene vinyl alcohol film (EVOH, O_2 permeability (20°C dry) [cc/m^2 24h; 1 atm]<1.5; CO_2 permeability (20°C dry) [20°C cc/m^2 24h; 1 atm]<4.5);

(b) MAP+Ads: samples stored in 20% CO_2/80% N_2 with an adsorbent material (Italian patent Sa2003A – 000021) having high liquid retention (90.0% weight water/weight adsorbent) and sealed with EVOH;

(c) control: samples covered with a polyethylene film.

A total of 36 packaged squid samples from the same catch, (3 treatments x 3 replicates x 4 testing times: days 3, 5, 7, and 11 were prepared for physico-chemical analysis, and 45 packaged squid samples (3 treatments x 3 replicates x 5 testing times: days 2, 5, 7, 9 and 12 were prepared for microbiological analysis. On day 0, analyses were carried out on 3 samples (6 squid) with a total weight of about 1,000 g.

For the samples stored in MAP, a volume of 900 mL of gas mixture (20% CO_2; 80% N_2) per 300-350 g of squid (gutted and rinsed) was used.

The trays were designed with a false bottom, where the adsorbent material was inserted; the adsorbent constituted circa 6% of the total pack weight (weight adsorbent/weight gutted squid). The samples were stored at 3±0.5°C, and physico-chemical, and microbiological analyses were carried out on the days reported above.

Determination of pH

The pH was determined with a pH-meter (Basic 20, Crison, Spain) on fish homogenates using a penetration electrode.

Determination of Total Volatile Basic Nitrogen (TVB-N)

TVB-N determination was based on the method of ANTONACOPOULOS and VYNCKE (1989), modified as follows: thrichloroacetic acid (TCA) (7.5% w:w) was added to squid muscle homogenate (to precipitate muscle proteins) followed by centrifugation (4,000xg for 8 min) and distillation of the supernatant. The distillate was collected in H_2SO_4 (0.25N) and titrated with NaOH (0.2N).

Determination of thrimethylamine-nitrogen (TMA-N) and dimethylamine-nitrogen (DMA-N)

Sample preparation

Muscle extracts were obtained by blending 20 g of the minced arm and mantle with 100 mL of TCA (7.5% w:w) in an Ultra-Turrax homogenizer for 2 min. The mixture was stirred for 10 min and filtered. The filtered extract (50 mL) was introduced into a distillation tube (steam distillation BÜCHI 323) with 2 drops of phenolphthalein. NaOH solution (40% w:w) was added and distillation was carried out until 85 mL of distillate was obtained. This was collected in a volumetric flask (100 mL) containing 5 mL of HCl solution (0.1%).

Volatile amines were separated using an ion exchange chromatograph [(Dionex Corp., CA, U.S.A.), consisting of the following: GP 50 gradient pump; LC 50 chromatographic enclosure; ED 50 electrochemical detector; Ionpac CS 12 A column (250x4) mm; Ionpac CG 12 A Guard (50x4) mm]. Chemical suppression was achieved by a cation micromembrane Suppressor [CSRS-Ultra self-regeneration 4 mm, with 10-15 mL solution regenerant NaOH (0.2N)]. Acquisition and integration of chromatograms was performed with Peaknet G4G1T0 Dionex Corp. Software. The mobile phase used was double-distilled water (E_1) and methane sulfonic acid 0.1N (E_2) for a total running time of 16 min using the gradient: 84% E1-16% E2 for 5 min; to 60% E1-40% E2 in 3 min; 60% E1-40% E2 for 4 min; to 84% E1-16% E2 in 4 min.

Enumeration of psychrotrophic microorganisms and presumptive *Pseudomonas* spp.

Microbiological analyses were performed by homogenising the edible part of the each sample.

The mass (20-30 g) was mixed with an appropriate amount of Ringer's solution (ratio 1:10) in a Stomacher Lab-Blender 400. Serial dilutions were prepared and the microbial status was evaluated by plate counts. Psychrotrophic aerobic and anaerobic microorganisms were enumerated by spread plating on Plate Count Agar (OXOID) with 0.5% (w/v) NaCl and incubating at 10°C for 5 days. Anaerobiosis was obtained by incubation in Generbox jars with Generbag Anaer (bioMérieux, Mercy l'Etoile, France). Pseudomonas counts were obtained after 24-48 hours of incubation at 25°C on Pseudomonas Agar Base (OXOID) with Pseudomonas C-F-C Supplement. The green-blue, brown or fluorescent colonies were identified as presumptive *Pseudomonas* spp. but no further identification was carried out.

The adsorbent material was analysed in the same way as the samples.

Sensory evaluation

The sensorial assessment (odour and visual characteristics) of the squid was carried out over the storage time by a 5 member untrained panel. The odour of

raw squid, at the time of the opening of the package, was based on the following criteria: sea-fresh, neutral, fishy and off-odours. Owing to the informal test conducted, the results are not reported, but are summarized in the results section.

Statistical analysis of data

The data were analysed using one-way analyses of variance. Differences ($P<0.05$) between means were quantified with the Student-Newman-Keuls test.

RESULTS AND DISCUSSION

The pH of the control samples increased from 6.32 to 7.65 ($P<0.05$) by the 11th day of storage at 3°C (Table 1). The pH of squid samples stored in both types of MAP also increased ($P<0.05$), but by day 11 the values were lower with respect to the control. The increase in pH during the first 5 days was less in the samples stored in MAP only, but by the end of storage, the MAP+Ads packages had a significantly lower pH ($P<0.05$) than the control and MAP only samples.

Table 1 - Changes in pH during cold storage. Control: gutted squid packed in polyethylene bags under normal atmosphere; MAP: gutted squid packed in 20% CO_2, 80% N_2; MAP+Ads: gutted squid packed in 20% CO_2, 80% N_2 combined with adsorbent material.

Days	Control	MAP	MAP + Ads
0	6.32±0.08 a/x	6.32±0.08 a/x	6.32±0.08 a/x
3	6.52±0.02 b/x	6.49±0.11 a/x	6.58±0.13 b/x
5	6.82±0.01 c/x	6.55±0.08 a/y	6.68±0.01 b/z
7	6.82±0.04 c/x	6.91±0.13 b/x	6.88±0.06 c/x
11	7.65±0.01 d/x	7.06±0.12 b/y	6.85±0.11 c/z

Data are the average of three replicates ± standard deviation.
Different letters (a, b, c, etc.) in the same column indicate significant differences ($p<0.05$).
Different letters (x, y, z) in the same row indicate significant differences ($p<0.05$).

The growth of psychrotrophic microorganisms and of *Pseudomonas* are shown in Figs. 1a and 1b, respectively. The samples had low initial counts and the increase in aerobic psychrotrophic microflora (Fig. 1a) was slow in the early stages, i.e. the number of aerobic psychrotrophic bacteria was below 10^5 for all treatments in the first 7 days. In the control, the psychrotrophic bacteria approached and then exceeded 10^7 cfu/g at 9 and 11 days, respectively, when their count was significantly ($P<0.01$) higher than both MAP and MAP+Ads which were not significantly different from each other. A slower and more constant increase was observed in the case of psychrotrophic anaerobic bacteria which, after 12 days storage were still below 10^5 cfu/g. Again, the control sample counts were significantly higher than both the MAP and MAP+Ads samples.

GRAM and HUSS (1996) found that in the presence of CO_2 *Pseudomonas* spp. and *Shewanella putrefaciens* rarely exceeded log 5-6 cfu/g. This is confirmed for *Pseudomonas*, which (only in the case of the control) showed a rapid and constant growth, exceeding the threshold of 10^7 cfu/g on day 12. Enumeration of *P. phosphoreum* and identification of the microorganisms was not carried out in this work and no further inferences on the dominating spoilage flora can be made.

The presence of TMA-N is of particular interest because it is a spoilage indicator in fresh fish. It is formed by the bacterial reduction of osmoregulatory substances, such as trimethylamine oxide (TMAO) of seawater fish muscle (MALLE *et al.*, 1986), which gives rise to the typical odour of spoiled fish. An increase in TMA-N values during storage was observed in all samples (Fig 2a). However, the increase in samples sealed in MAP+Ads was less than in the control and in the MAP samples, and there were no significant differences between the control and MAP samples up to 3 days

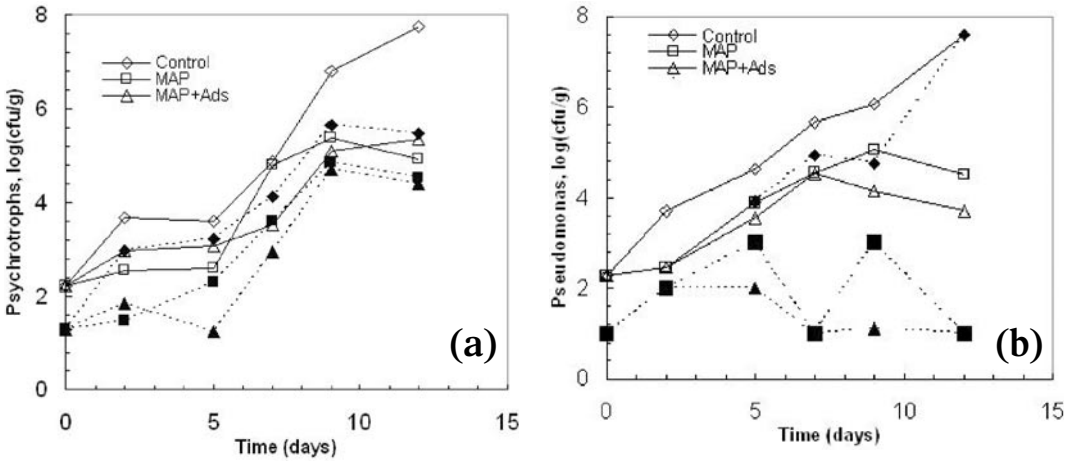


Fig. 1 - Growth of psychrotrophic microorganisms (a; empty symbols in aerobiosis; closed symbols in anaerobiosis) and of *Pseudomonas* (b; empty symbols total *Pseudomonas*; closed symbols presumptive *Pseudomonas aeruginosa*) during chilled storage (data are the average of three replicates). Control: gutted squid packed in polyethylene bags under normal atmosphere; MAP: gutted squid packed in 20% CO_2, 80% N_2; MAP+Ads: gutted squid packed in 20% CO_2, 80% N_2 combined with adsorbent material. Results are average of two replicates.

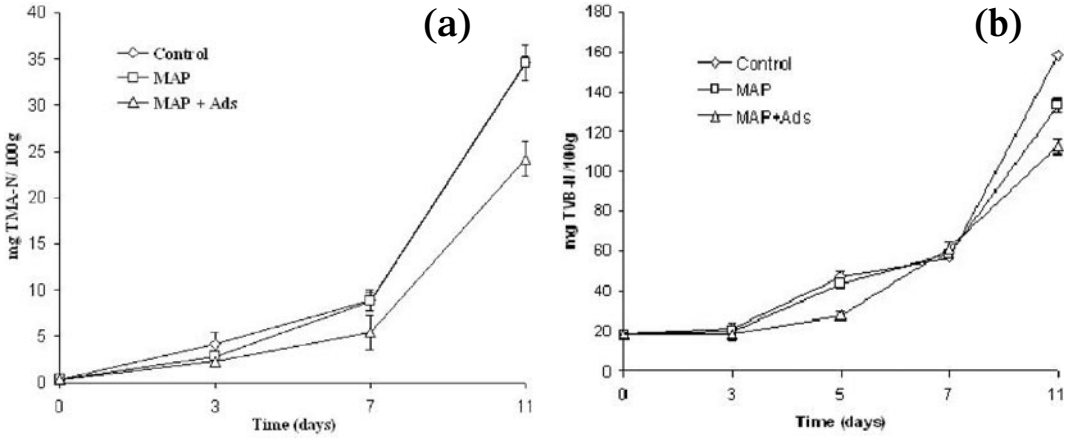


Fig. 2 - Development of trimethylamine nitrogen (TMA-N) (a) and of total volatile basic nitrogen (TVB-N) (b) during chilled storage (data are the average of three replicates). Control: gutted squid packed in polyethylene bags under normal atmosphere; MAP: gutted squid packed in 20% CO_2, 80% N_2; MAP+Ads: gutted squid packed in 20% CO_2, 80% N_2 combined with adsorbent material.

storage. Values for TMA-N/100 g exceeded 8 mg and 34 mg, respectively, on days 7 and 11 in control and MAP samples, with no differences between the two. In contrast, the concentration determined in squid muscle sealed in MAP+Ads was 5.4 mg after 7 storage days, and 24.2 mg after 11 days, significantly lower than in the control and MAP samples.

In our trials, the dimethylamine-nitrogen (DMA-N) concentration of the squid samples analysed during storage was always below the detection limit (0.1 mM) of the method utilised. This

is in agreement with the results of other authors (SOTELO *et al.*, 1995; MONSER and GREENWAY, 1996), who detected significant values of DMA-N in frozen but not in fresh fish.

The pattern of total volatile basic nitrogen (TVB-N) data was similar to that of TMA-N production, as described above. No statistically significant differences between the control and MAP samples were found during the first 7 storage days, although samples stored in MAP with adsorbent showed lower amounts of TVB-N ($P<0.05$) on days 5 and 11 (Fig. 2b). Thus, there was a high correlation between TMA-N and TVB-N during the storage time for control ($R^2=0.9897$), MAP ($R^2=0.9802$) and MAP+Ads samples ($R^2=0.9102$).

After 11 days of storage, during informal sensorial tests, off odours were perceived for both control and MAP samples, while only "fishy" odour was perceived for the MAP+Ads samples.

These results show that it is difficult (with classical packaging) to extend the storage of squid samples beyond 5-7 days. MAP slowed down the production of TMA-N and TVB-N, but only to a limited extent, confirming results reported by DEBEVERE *et al.* (1996).

The use of CO_2 in fish packaging extends the lag phase of Gram-negative aerobic bacteria, but favours fish meat exudation (decrease in water-binding capacity), thus causing acidifying of the fish muscle and reduction of the capacity of squid muscle protein to retain water (PASTORIZA *et al.*, 1998). In our trials, the exudate began to form after 5 days of storage, and did so only in MAP samples; at the end of storage it was about 2.5% of the total weight. The exudate was not visible in the MAP+Ads samples, where the adsorbent increased in weight after 12 days of storage. This endogenous liquid, which was in contact with the squid, is rich in nitrogenous compounds and is a good substrate for bacterial growth. Thus, spoilage could be related to the amount of exudate present in the packages, which in turn depended on the presence of the adsorbent.

CONCLUSIONS

Throughout the storage period, the bacterial counts were significantly higher in control samples than in MAP and MAP+Ads samples. Moreover, TMA-N and TVB-N increased in all squid samples, but to a lesser extent in MAP+Ads samples with respect to the control samples and to the samples stored in MAP only. The use of CO_2 in MAP caused an increase of exudate in the packages while the addition of adsorbent material reduced this phenomenon as a result of its capacity to absorb water. Thus, spoilage, confirmed by the production of TMA-N and TVB-N, could partially be related to the amount of exudate present in the packages, which in turn depended on the presence of the adsorbent.

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Revised paper received January 25, 2005 Accepted May 31, 2005

SHORT COMMUNICATION

PRELIMINARY NUTRITIONAL AND ORGANOLEPTIC ASSESSMENT OF XOCONOSTLE FRUIT (*OPUNTIA* SPP.) AS A CONDIMENT OR APPETIZER

EVALUTAZIONE PRELIMINARE NUTRITIZIONALE E SENSORIALE DEL FRUTTO DELLA XOCONOSTLE (*OPUNTIA* SPP.) COME CONDIMENTO OD APERITIVO

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ABSTRACT

The aim of this work was to analyze the nutritional value of the powdered fruit skin of six xoconostle fruit (*Opuntia* spp.) variants used to prepare a condiment or seasoning by mixing it with salt and chili peppers; an organoleptic evaluation was also carried out. Xoconostle pears are an acidic fruit (pH 3.3), rich in neutral-detergent fiber (14.2% dry matter) and potassium (2,248.3 mg/100 g dry matter)

RIASSUNTO

L'obiettivo di questa ricerca è stato quello di analizzare dal punto di vista nutrizionale la buccia polverizzata di sei varietà di xoconostle (*Opuntia* spp.), ed elaborare e valutare sensorialmente un condimento preparato con questa materia prima e addizionata di sale e di peperoncino. Il frutto della xoconostle è acido (pH 3,3), ricco di fibre (14,2% di peso secco) e potassio (2.248,3 mg/100 g ps), e povero di proteine grezze, gras-

- Key words: cactus pear, condiment, xoconostle pear, *Opuntia* -

and poor in crude protein, lipids, carbohydrates and sodium. For the six variants, peers were able to differentiate between the various xoconostle pear-chili-salt combinations (60:25:15, 70:15:15 and 100:00:00 xoconostle pear:chili: salt), but in general they rated samples as tasty.

si, carboidrati e sodio. Gli assaggiatori hanno apprezzato in modo differente le combinazioni del frutto della xoconostle con peperoncino e sale (60:25:15, 70:15:15 e 100:00:00 xoconostle pear: peperoncino:sale) delle sei varietà, e hanno qualificato i campioni generalmente come gradevoli al gusto.

INTRODUCTION

The most commonly known fruit of *Opuntia* are cactus pears, which are a thin-skinned fruit with a juicy, sweet pulp (BARBERA *et. al.*, 1992). In some regions of Mexico, there are other *Opuntia* fruit, locally called xoconostle pear, which have a thick, succulent, acidic skin with little pulp, and edible skin (BRAVO, 1978; COLUNGA *et al.*, 1986; REYES *et al.*, 2005a). Their common name is derived from the native Náhuatl words: xococ (acid) and nochtli (cactus pear) (SANTAMARÍA, 1992). There are 8 species of xoconostle (*O. duranguensis* Britton & Rose, *O. heliabravoana* Scheinvar, *O. imbricata* DC., *O. joconostle* A. Web., *O. leucotricha* DC., *O. matudae* Scheinvar, *O. velutina* F.A.C. Weber and *O. zamudioi* Scheinvar) and 7 species with variants that have the xoconostle fruit type (*O. chavena* Griffiths, *O. lasiacantha* Pfeiff., *O. megacantha* Salm-Dyck, *O. oligacantha* Hort. Vindob. ex Pfeiff., *O. robusta* Wendl., *O. spinulifera* Salm-Dyck, *O. streptacantha* Lem.) that grow in the wild nopaleras in semi-arid regions of Mexico, but some of these species are also cultivated in home gardens and commercial plantations (JANZEN, 1986; SCHEINVAR, 1999; VIGUERAS and PORTILLO, 2001; CASAS and BARBERA, 2002; REYES *et al.*, 2005b). According with HERNÁNDEZ and FLORES (2004), there are currently 1,300 hectares of xoconostle pear cultivated in six municipalities of Mexico State, at the center of the country. There is no information about the extension of wild nopaleras with xoconostle pear. Regarding the market a 20 kg box of fruit, has a wholesale price of 5 dollars (HERNÁNDEZ and FLORES, 2004).

The xoconostle pear skin has been used as food in the northcentral area of Mexico since pre-Columbian times (SAHAGÚN, 1989; SÁNCHEZ and ORTEGA, 1996). In traditional medicine this fruit is used as a remedy for diabetes, hypertension, obesity and respiratory ailments (SÁNCHEZ, 1982; SCHEINVAR, 1999). Due to the tasty acidic flavor, succulent texture and long-lasting permanence on the plant, the fruit is available throughout most of the year. It is regarded as a valuable food, that is consumed as fresh fruit, as a vegetable in salads and sauces, as a main ingredient in desserts, dried in sheets as an appetizer, and in alcoholic beverages. It is most frequently used, adding it fresh to soups to improve flavor (SÁNCHEZ and ORTEGA, 1996; ALMANZA, 1999). It is also processed on a small-scale in the food industry as a jelly, as whole fruit in syrup or brined, to be used to prepare ready-to-serve sauces and liquors, among other food products (CORRALES and FLORES, 2000). The nutritional characteristics of the cactus pear are relatively well known (SAWAYA *et al.*, 1983; DELGADO, 1985; EL KOSSORI *et al.*, 1998; SEPÚLVEDA, *et al.*, 2000; STINTZING *et al.*, 2001), but information about xoconostle pear is still scarce (SÁNCHEZ and ORTEGA, 1996).

In many parts of the world, plants (or parts of plants) have traditionally been used as spices or seasoning ingredients to add a strong, pungent or tasty flavor to foods and beverages. These materials are normally dehydrated and powdered in order to preserve them, facilitate their use and improve their presentation (FARRELL, 1990).

The aim of producing a xoconostle pear seasoning is to confer a new added value to this fruit, hence broadening its consumption and demand. The objectives of the present work were to assess the powdered fruit skin of six xoconostle pear variants from a nutritional standpoint, and then to develop and assess the acceptability of an appetizer or condiment made from the best combination of powdered xoconostle pear skin, powdered dried chili and salt.

MATERIALS AND METHODS

Fruit sources

The fruit used corresponded to the xoconostle pear species *O. duranguensis* Britton & Rose, *O. leucotricha* DC., Colorado and Xoconostle variants of *O. joconostle* A. Web. and Blanco and Cuaresmeño variants of *O. matudae* Scheinvar. The species were identified based on BRITTON and ROSE (1919), BRAVO (1978), GONZÁLEZ *et al.* (2001) and PINKAVA (1997). The fruit was collected in August and November 2002 (when commercially ripe and with no evidence of damage by pests or diseases) from wild nopaleras, home gardens and commercial plantations located in the states of San Luis Potosí, Zacatecas and México. Table 1 summarizes the average fruit morphological features of the six variants studied. It can be noted that there is a greater proportion of the skin than pulp or seeds which is characteristic of the xoconostle pears. An average of 20 kg of fresh fruit of each variant were collected, and only the skins were used in this investigation.

Sample processing

Fruit from each variant was washed separately with chlorinated water (20 $\mu L\ L^{-1}$), sliced in half longitudinally, and then epidermis, pulp and seeds were separated. The skins were placed in stainless steel trays and dried in a convection oven (Felisa FE-294A, Jalisco, Mexico) at 48°C for 30 h. The dried skin was powdered in an industrial blender (International L1-12, Jalisco, Mexico).

Table 1 - Morphological features of xoconostle pear fruit.

Attribute	*O. duranguensis*	*O. joconostle* variant Colorado	*O. joconostle* variant Xoconostle	*O. leucotricha*	*O. matudae* cv. Blanco	*O. matudae* cv. Cuaresmeño
Shape	Globose	Ovoid	Globose	Globose	Globose	Globose
Diameter (cm)	4.7	3.3	4.3	3.2	4.0	3.8
Length (cm)	6.4	3.8	4.8	3.1	5.9	4.6
Total fresh weight (g)	71.9	24.2	49.2	18.0	53.7	42.0
Skin color (Munsell Code)	5.8R 5/7	2.5R 4/8	4.2R 7/6	2.5GY 5/7	2.5GY 8/6	5.0RP 7/6
Pulp color (Munsell Code)	5.0R 6/6	5.0R 3/8	2.5R 4/4	2.5GY 8/4	2.5R 4/6	5.0R 4/8
Relative portion of skin (%)	72.0	81.4	87.2	83.9	87.2	80.0
Relative portion of pulp (%)	24.9	14.9	9.3	11.7	9.8	16.0
Relative portion of seeds (%)	3.1	3.7	3.5	4.4	3.0	4.0

Nutritional analysis

The dried powdered skin of fruits of each variant, chosen at random, was analyzed for pH (Corning 45 potentiometer, USA), crude protein, lipids (determined according to the Soxhlet method), carbohydrates, sodium and potassium (flame spectrophotometry) and neutral-detergent fiber. All the data are reported in terms of 100% dry matter. The analyses were carried out according to AOAC (1990) standard methods with the exception of the percentage of carbohydrates, which was determined using the Somogyi method (MC DONALD and HENDERSON, 1964), and the percentage of neutral-detergent fiber, determined according to the GOERING and VAN SOEST (1970) method. These data were subjected to analysis of variance and multiple comparisons of means through the procedure for General Linear Models and Tukey's test, respectively, as well as correlation analysis (SAS, 1990).

Organoleptic analysis

Powdered fruit skin from each xoconostle pear variant was mixed with chili powder (*Capsicum annuum* L.) and conventional sodium chloride (common salt) in three different proportions; respectively, based on a preliminary test: (1) 60:25:15; (2) 70:15:15; y (3) 100:00:00. They were then subjected to organoleptic tests to assess consumer acceptability. The peers had to judge how they would prefer to consume the assessed samples, i.e. as a seasoning ingredient, an appetizer or in any other way. Powdered fruit skin from each xoconostle pear was tasted directly.

Samples were offered to peers in clean, dry containers coded with three random numbers. A hedonic unstructured-scale test (USSEGLIO-TOMASSET, 1998) was used by a group of 15 consumers (nine Mexican females and six Mexican males, 24 to 45 years old). None of them were familiar with the product or its characteristics, nor the test procedure. Evaluations were carried out during two sessions; three series of samples were presented per session, each consisting of one variant with the three combinations of ingredients. Peers were asked to taste each series of samples and assess each of them according to preference based on a tasting form (Fig. 1); the acceptability of each variant and combination of ingredients was determined in this way. A question was included on the tasting form to find out in what way the peers preferred to consume the sample combinations.

The organoleptic assessment results, were analyzed by measuring the length (in cm) from the lower end of the diagonal square to the mark made by the judge.

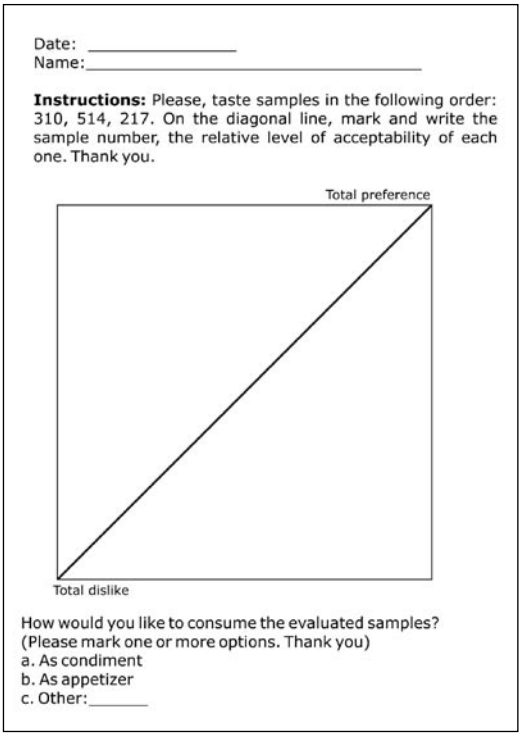


Fig. 1 - Record form for tasting combinations of powdered xoconostle pear with chili powder and salt (from USSEGLIO-TOMASSET, 1998).

This length constituted the numerical score (from 0.0 to 21.0 cm) of the acceptance level for each sample assessed (USSEGLIO-TOMASSET, 1998), where 0 represented total dislike and 21 was total preference. Answers to the question on the preferred way of consumption were evaluated in terms of frequency. The scores and frequency data obtained were then subjected to analysis of variance following a completely random experimental design and 6x3 (six variants by three combinations) and 6x4 (six variants by four ways of consumption) factorial arrangements, as well as by multiple comparisons of the means (SAS, 1990).

RESULTS AND DISCUSSION

Nutritional characteristics

The average nutritional attributes of the powdered fruit skin of the six variants studied are presented in Table 2. The xoconostle pear variants differed significantly in all the assessed attributes, indicating considerable diversity. The pH values were in agreement with that reported for the fresh skin of *O. joconostle* A. Web. (SÁNCHEZ and ORTEGA, 1996), but were lower than the values reported (5.5) for cactus pear skin of *Opuntia ficus-indica* (ODOUX and DOMINGUEZ-LOPEZ, 1996); the pH values ranged from 2.9 to 4.5 and confirm that all of these xoconostle pear variants are acidic fruit (BEDOLLA *et al.*, 2003). The overall average crude protein (3.0%) and potassium (2,248.3 mg/100 g) values recorded were lower than those reported by EL KOSSORI *et al.* (1998) for cactus pear skin of *Opuntia ficus-indica* (8.3% and 3,400.0 mg/100 g, respectively). The potassium content was higher than in some other powdered spices, including chili peppers (*Capsicum annuum* L.), onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) (1,916.0, 943.0 and 1,101.0 mg/100 g, respectively) (FARRELL, 1990). The per-

Table 2 - Nutritional characteristics of the dry matter from fruit skins of xoconostle pear (*Opuntia* spp.).

Attribute	*O. duranguensis*	*O. joconostle* variant Colorado	*O. joconostle* variant Xoconostle	*O. leucotricha*	*O. matudae* cv. Blanco	*O. matudae* cv. Cuaresmeño	Standard error	Average
pH	3.3 ^{b}	3.1 ^{bc}	3.2 ^{bc}	4.5 ^{a}	2.9 ^{c}	3.1 ^{bc}	0.0756	3.3
Crude protein (%)	2.9 ^{c}	3.1 ^{b}	3.2 ^{b}	3.2 ^{b}	3.4 ^{a}	2.2 ^{d}	0.0283	3.0
Lipids (%)	0.8 ^{cd}	0.9 ^{bc}	1.0 ^{b}	1.2 ^{a}	0.8 ^{cd}	0.8 ^{d}	0.0236	0.9
Carbohydrates (%)	12.0 ^{b}	9.1 ^{d}	10.7 ^{c}	15.0 ^{a}	9.3 ^{d}	14.9 ^{a}	0.2295	11.8
Neutral-detergent fiber (%)	14.4 ^{b}	11.6 ^{d}	16.7 ^{a}	13.5 ^{c}	14.4 ^{b}	14.4 ^{b}	0.1566	14.2
Na (mg/100 g)	14.4 ^{a}	5.9 ^{d}	12.1 ^{b}	10.9 ^{c}	4.8 ^{e}	10.5 ^{c}	0.1114	9.8
K (mg/100 g)	1,746.8 ^{c}	1,943.3 ^{b}	3,328.7 ^{a}	3,277.0 ^{a}	1,742.2 ^{c}	1,451.8 ^{d}	19.5275	2,248.3

^{a,b,c,d} Values with different letters in a row are statistically different ($p<0.05$).

centage of lipids (0.9%) was similar to that of powdered onion (1.1%), whereas carbohydrate and sodium (11.8% and 9.8 mg/100 g, respectively) were lower than those for seasoning ingredients in general (ranging from 44.0 to 80.7% and from 25.0 to 243.0 mg/100 g, respectively) (FARRELL, 1990). The neutral-detergent fiber content (14.2%) was lower than that reported for paprika (*C. annuum* L.) (20.9%), but higher than that of pepper (*Piper nigrum* L.), onion (*Allium cepa* L.) and powdered garlic (*Allium sativum* L.) (13.1, 10.5 and 1.9%, respectively) (FARRELL, 1990).

The fruits from the wild *O. leucotricha* DC. and from the home garden *O. joconostle* A. Web. variant Xoconostle had the highest lipid, carbohydrate, neutral-detergent fiber and potassium values, whereas the fruit from the commercial plantations of *O. matudae* Scheinvar cv. Cuaresmeño had the lowest crude protein, lipid and potassium values. This confirms the observations of HARLAN (1992) that wild variants are frequently richer in some nutrients than their cultivated relatives, because in the latter, size is favored, while nutritional quality may not necessarily increase proportionally. In the xoconostle pear variants studied, the *O. leucotricha* DC. fruit was the smallest (2.9 to 3.3 cm in length and weight 14.0 to 22.0 g) compared to the *O. matudae* Scheinvar fruit (3.8 to 7.4 cm in length and weight 23.2 to 75.0 g), grown in commercial plantations. Besides the high nutritional quality of *O. joconostle* A. Web., that is cultivated in home gardens (SÁNCHEZ and ORTEGA, 1996), its fruits are similar in size and weight to fruits of *O. matudae* Scheinvar. The correlation analysis showed that sodium and potassium increased with increasing pH ($p<0.05$), which could be due to the presence of organic acids which confer acidity to the fruit (citric, malic, ascorbic and succinic acids) (SÁNCHEZ and ORTEGA, 1996) and that may form salt compounds with them (MATHEWS and VAN HOLDE, 1998).

Organoleptic analysis

The xoconostle pear:chili:salt combinations with the highest acceptance level were 60:25:15 and 70:15:15, with average scores of 14.8 and 14.9, respectively. In contrast, the 100:0:0 combination received an average score of 7.9. The relatively high scores of the former two might be due to the fact that chili and salt are the preferred and most frequently used condiments in Mexican cuisine (ROZIN, 2002); hence, the absence of chili and salt in the 100:0:0 combination resulted in a decrease in the acceptance level. The average score for the six variants was 14.9. In general, the samples assessed were rated as highly tasty (10.5 to 21.0 points), meaning that the acceptance level by potential consumers might be high. Among the xoconostle pear variants, *O. duranguensis* Britton & Rose received the highest score, whereas *O. leucotricha* DC. received the lowest score. The differences observed among the scores obtained for all variants however, were not statistically significant.

The evaluation of the way in which the peers would prefer to consume the xoconostle pear:chili:salt combinations showed that, in general, the frequency of answers regarding consumption as a condiment or appetizer did not differ significantly ($p<0.05$). Among "another" way of use, the most frequent answer was "as a sweet", with a frequency that was again not significantly different from the former two (Table 3). This might be due to the fact that the acidity of xoconostle pear fruit gives it the strong, tasty flavor that is characteristic of plant products used as condiments and which has an appetite-stimulating effect. It is also likely that the characteristic xoconostle pear acidity probably resembles the highly acidic flavor of sweets that are fre-

Table 3 - Frequency of the preferred ways to consume the six variants of xoconostle pear (*Opuntia* spp.) combined with chili powder and salt.

Fruit	Way of consumption		
	Sweet	Condiment	Appetizer
O. duranguensis	8	9	6
O. joconostle variant Colorado	10	9	5
O. joconostle variant Xoconostle	10	8	6
O. leucotricha	7	10	5
O. matudae cv. Blanco	8	5	10
O. matudae cv. Cuaresmeño	9	5	9
Total	52 [a]	46 [a]	41 [a]

*Sum of the frequencies for each variant is greater than 15, the number of evaluators, because each evaluator had the option of selecting more than one answer.
Varying factor ($p>0.05$); forms of consumption factor ($p<0.001$); interaction ($p>0.05$).
[a]Values are statistically similar ($p<0.05$).

quently consumed by youngsters (ROZIN, 2002).

Despite their nutritional differences, all of the xoconostle pear variants assessed are acidic fruit with high neutral-detergent fiber and potassium contents and low crude protein, lipids, carbohydrates and sodium contents. Of the six variants, *O. leucotricha* DC. and *O. joconostle* A. Web. variant Xoconostle obtained the highest values for most of the attributes. The results of the organoleptic assessment revealed that powdered xoconostle pear in combination with chili powder and salt (60:25:15 and 70:15:15 xoconostle pear:chili:salt) could attain commercial success as a condiment, appetizer or sweet, regardless of the xoconostle pear variants used.

ACKNOWLEDGEMENTS

The authors wish to thank the Fondo de Apoyo a la Investigación of the Universidad Autónoma de San Luis Potosi (Agreement C02-FAI-10-20.60), and the Sistema Nacional de Inspección y Certificación de Semillas of the Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación from Mexico, for the partial funding provided to carry out this project; and to Biologist Adriana González Durán, who identified the species mentioned in the text; to Irina Pucaric, who translated the abstract to Italian; and to the referees, who with their critic, helped us to improve the quality of this paper.

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Revised paper received March 9, 2005 Accepted May 13, 2005

SHORT COMMUNICATION

VOLATILE PROFILES OF SICILIAN PRICKLY PEAR (*OPUNTIA FICUS INDICA*) BY SPME-GC/MS ANALYSIS

PROFILO DELLE SOSTANZE VOLATILI DI FICHI D'INDIA SICILIANI TRAMITE SPME-GC/MS IN SPAZIO DI TESTA

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ABSTRACT

The aroma compounds present in the headspace of homogenized slurries of fresh prickly pears (*Opuntia ficus indica*) from Sicilian cultivars were determined. Solid Phase Microextraction (SPME) followed by Gas-chromatography/Mass Spectrometry (GC/MS) analysis was used to characterize the volatile compounds. The volatile flavor profiles of the three cultivars investigated (red, white and yellow) showed significant differences. The aromatic profile of pealed prickly pears stored for one day was different from that of the fresh samples, mainly due to the occurrence of some oxidative and hydrolytic reactions.

RIASSUNTO

Sono stati determinati i componenti volatili d'aroma di fichi d'India della Sicilia occidentale, campionandoli in spazio di testa con la Micro Estrazione in Fase Solida (SPME) ed analizzandoli con Gascromatografia accoppiata alla Spettrometria di massa (GC/MS). Si evidenziano significative differenze tra le tre cultivar studiate (gialla, rossa e bianca). Inoltre il profilo aromatico dei frutti conservati per un giorno dopo sbucciatura risulta fortemente modificato per l'insorgere di reazioni di tipo ossidativo ed idrolitico.

- Key words: Gas-chromatography/Mass Spectrometry (GC/MS), *Opuntia ficus indica,* Prickly Pear, Solid Phase Microextraction (SPME), Volatile Flavor Profiles -

INTRODUCTION

Prickly pear (*Opuntia ficus indica* (L.) Mill. *Cactaceae*) is a bushy, tree-like cactus, native to Mexico and widespread throughout Sicily and the whole Mediterranean area, South Africa and Central and South America (BARBERA and INGLESE, 1993; MUÑOZ DE CHÀVEZ *et al.*, 1995). In Sicily the principal areas of cultivation are located in three zones: Etnea (eastern Sicily), the district of San Cono (east-central Sicily) and the western area of the Belice Valley (Santa Margherita-AG). The flowers and fruits form on the margin of the succulent leaf (cladodes). The fruit, usually eaten raw after peeling, is sweet and juicy, with the flesh enclosing many small edible seeds.

The different parts of the plant have a wide range of applications. The fruits and cladodes are used for human and animal consumption (BARBERA *et al.*, 1988), the cladodes are also used in traditional medicine to reduce serum cholesterol levels, regulate blood pressure and treat several pathologies such as ulcers, fatigue, dyspnea glaucoma, capillary fragility, liver conditions, rheumatic pain and wounds (MUÑOZ DE CHÀVEZ *et al.*, 1995; BARBERA *et al.*, 1988). Further, prickly pear may be used for the treatment of gastritis, hyperglycemia, arteriosclerosis, diabetes and prostate hypertrophy (HEGWOOD, 1990; FRATI *et al.*, 1990; PALEVITCH *et al.*, 1993). A flower infusion is used in traditional medicine in Sicily as a diuretic (BARBERA *et al.*, 1988). Other recent studies have shown antioxidant activities of Sicilian prickly pear (BUTERA *et al.*, 2002; TESORIERE *et al.*, 2003).

The pulp of the Sicilian cultivars is characterized by three different colors: yellow, purple red and pale green (white). The red and yellow pigments have been identified as betacyanins and betaxanthines, respectively (PIATTELLI and MINALE, 1964; PIATTELLI *et al.*, 1964; FORNI *et al.*, 1992). These water soluble pigments can be used as natural food colorants (BARBERA and INGLESE, 1993; BARBERA *et al.*, 1988). A comparison of the chemical and biochemical parameters (total acidity determination, sugar analysis and vitamin C contents, transition metal ions and pectin methylesterase activity) of different Sicilian cultivars was reported by GURRIERI *et al.* (2000).

In this study the volatile flavor profiles of different cultivars of Sicilian prickly pear from western Sicily were compared, particularly the yellow, so-called "Surfarina" (the color of sulphur), the white "Muscaredda" (sweet like muscatel) and red "Sanguigna" (bloody) cultivars. In addition, tests were conducted to detect if there was any variation in the aromatic profile when peeled fruit was stored before selling. The results were compared with those of other researchers on fruits from eastern Sicily (ARENA *et al.*, 2001).

Headspace Solid Phase Micro-Extraction (HS-SPME) was used followed by Gas-Chromatographic/Mass Spectrometry (GC/MS) analysis. These techniques offer several advantages with respect to other established methods. In fact, SPME GC/MS (VAS and VÉKEY, 2004) does not require the use of solvents, complex sample handing or complicated apparatus (FLATH and TAKAHASHI, 1978); it is fast and ensures excellent sensitivity and reproducibility. It has been used recently for the analysis of the volatile components responsible for the aroma in various fruit (AUGUSTO *et al.*, 2000; WAN *et al.*, 1999).

MATERIALS AND METHODS

All the fruit used in this study came from the Belice Valley. The plants were subjected to a forcing technique: summer flowers were removed, and fruits formed from a late blossoming were picked from September to December.

Five fruits of each cultivar were peeled

and gently squeezed. The fresh juice of each fruit (4 g) was placed in 15 mL vials with pierceable silicone rubber septa coated with PTFE film. The vials were stirred with a magnetic device in a thermostated water bath which gave temperature control within, at least 36°±0.5°C.

Sampling was carried out by using a SPME (Supelco, Bellafonte, PA) fiber coated with a 100 µm film of polydimethylsiloxane (PDMS) in the headspace; a 85 µm carboxen/PDMS fiber was also tested but the aromatic profile obtained was not as rich. After several tests at increasing times, it was determined that 15 min was suitable to obtain equilibrium and to reproduce the extraction procedure. Desorption time in the chromatograph injector at 250°C was fixed at 5 min in the splitless mode.

The analyses were run on a GC/MS Varian Saturn 3-Ion Trap System, equipped with a 30 m x 0.25 mm i.d.

Table 1 - Retention Index (RI) and Reconstructed Ion Current (RIC) % of compounds from fruits examined immediately after peeling.

RI	Compound	Red (RIC%)	Yellow (RIC%)	White (RIC%)
911	Butanoic acid, methyl ester		3.48	
1001	Butanoic acid, 2-methyl-, methyl ester	0.12	9.1	0.35
1083	Hexanal			0.13
1116	Benzene, 1,2-dimethyl			0.11
1125	β-Myrcene	0.51	0.83	0.59
1165	Hexanoic acid, methyl ester	0.78	2.96	0.20
1180	Eucalyptol	0.23	0.63	
1237	Unknown		1	3.5
1244	β-Ocimene	0.67	0.97	0.72
1275	3-Pentenoic acid, 3-methyl-, methyl ester		1.11	
1279	3-Hexenoic acid, methyl ester	1.19	3.84	0.36
1285	Acetic acid, hexyl ester	4.34	3.81	1.52
1301	Heptanoic acid, methyl ester	0.38	0.6	
1321	3-Hexenoic Acid, ethyl ester	0.29		
1331	4-Hexen-1-ol, acetate	1.1	0.75	0.50
1349	2-Hexen-1-ol, acetate	5.58	8.1	4.36
1368	1-Hexanol	13.7	6.6	14.37
1391	3-Hexen-1-ol	1.13	1.02	3.15
1401	Octanoic acid, methyl ester	3.63	5.53	
1420	Octanal	3.6		10.69
1428	2-Hexen-1-ol (E)	16.3	15.99	19.50
1459	Octanoic acid, ethyl ester	0.31	0.25	
1517	Nonanoic acid, methyl ester	0.33	0.58	
1567	Linalol	3.02	1.8	2.24
1576	1-Octanol	0.91		0.51
1612	Decanoic acid, methyl ester	2.27	2.29	2.14
1631	4-Decenoic acid, methyl ester	8.2	9.25	4.26
1666	1,9-Nonanediol	3.10	1.24	1.57
1687	(6Z)-Nonen-1-ol	1.09	0.63	1.23
1694	1-Dodecanol	2.15	0.73	1.48
1722	2-Nonen-1-ol (E)	21.93	13.98	16.95
1765	3,6-Nonadien-1-ol			0.94
1788	2,6-Nonadien-1-ol	3.14	2.93	8.63
	TOTAL	100	100	100

fused-silica capillary column Supelcowax – 10 (Supelco, Bellafonte, PA) coated with Polyethylene glycol with a film thickness of 0.25 µm. The carrier gas (helium) pressure was fixed at 12 psi at the head of the column. The transfer line and ion trap temperature was 180°C. The GC temperature program started at 35°C (5 min hold), first step 5°C/min to 130°C (10 min hold), second step 10°C/min to 220°C (5 min hold). The ion trap mass spectrometer parameters were: electron ionization mode (EI) 70 eV, mass range 40-400 Da and the scan frequency was 3 scans-second. The collected data were processed with the data system of the instrument and the chromatographic and spectrometric results showed excellent

Table 2 - Retention Index (RI) and Reconstructed Ion Current (RIC) % of compounds from fruits examined after being peeled and stored for 24 h.

RI	Compound	Red (RIC%)	Yellow (RIC%)	White (RIC%)
911	Butanoic acid, methyl ester	0.15		
1001	Butanoic acid, 2-methyl-, methyl ester	1.46		
1047	Unknown		2.5	
1083	Hexenal			0.19
1125	β-Myrcene	0.64	0.65	0.66
1160	Limonene	0.50	1.8	0.32
1165	Hexanoic acid, methyl ester	0.13	3.47	
1180	Eucalyptol	1	0.55	
1186	Cyclohexanol, 2-methyl-3-(1-methylethenyl)		5.7	
1218	Furan, 2-Pentyl-	0.43	0.36	1.13
1228	Heaxanoic acid, ethyl ester	0.83	4.14	0.13
1244	β-Ocimene	0.74	0.76	1.17
1279	3-Hexenoic acid, methyl ester		1.17	
1285	Acetic acid, hexyl ester	1.2	3.02	0.56
1301	Heptanoic acid, methyl ester	0.16	2.37	
1306	Unknown		7.1	
1317	Unknown	0.41	3.46	
1331	4-Hexen-1-ol, acetate	0.43	0.53	0.41
1345	Heptanoic acid, ethyl ester		2.77	
1349	2-Hexen-1-ol, acetate	0.54	4.63	0.29
1352	Unknown	1.9	2.65	
1368	1-Hexanol	0.59	1.9	1.23
1401	Octanoic acid, methyl ester	2.37	9	0.21
1428	2 Hexen 1 ol (E)	1.34	1.96	0.79
1459	Octanoic acid, ethyl ester	5.25	0.92	0.40
1517	Nonanoic acid, methyl ester	0.64	0.51	0.12
1564	2-Nonenal, (E)	11.22	11.48	21.04
1567	Linalol	3.14	1.53	0.8
1605	2,6 Nonadienal, (E,Z)	2.06	1.8	5.8
1612	Decanoic acid, methyl ester	1.2		0.65
1625	Cyclooctyl alcohol	1.3		0.80
1631	4 Decenoic acid, methyl ester	2.46		0.50
1687	(6Z)-Nonen-1-ol	1.84		3.16
1694	1-Dodecanol	2.9	2.36	4.12
1722	2-Nonen-1-ol (E)	41.57	14.42	41.89
1788	2,6-Nonadien-1ol	11.58	6.52	13.63
	TOTAL	100	100	100

reproducibility. Each determination was carried out three times. The variation in the peak area did not exceed 5%.

The standard compounds, 1-hexanol, 3-hexen-1-ol, and 2-hexen-1-ol, were purchased from Fluka (Buchs, Switzerland) and octanoic acid methyl ester and 1-octanol were obtained from Carlo Erba (Milano, Italy).

The volatile constituents of fresh and one-day stored prickly pears of the yellow, white and red cultivars were extracted by SPME in headspace and analyzed by GC/MS. The compounds were identified by linear retention indexes (RI) calculated using the Van Den Dool and Kratz's equation (VAN DEN DOOL and KRATZ, 1963) and by mass spectra through a critical and reasoned comparison with the NIST 2002 registry of mass spectral data; 1-hexanol, 3-hexen-1-ol, 2-hexen-1-ol, octanoic acid methyl ester, 1-octanol were also confirmed using standard compounds.

RESULTS AND DISCUSSION

The percentage of reconstructed total ion current (RIC) and the retention index

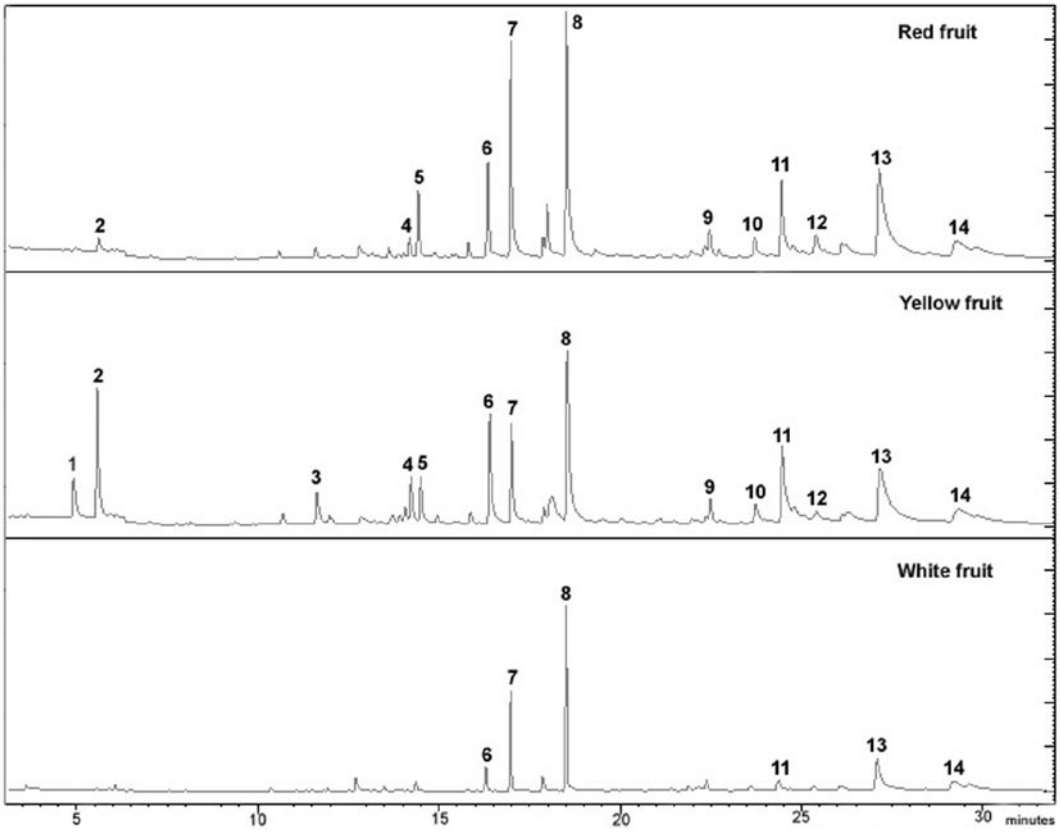


Fig. 1 - Gas-chromatograms of the fresh-peeled red, yellow and white fruit: 1=Butanoic acid, methyl ester; 2=Butanoic acid, 2-methyl-, methyl ester; 3=Hexanoic acid, methyl ester; 4=3-Hexenoic acid, methyl ester; 5=Acetic acid, hexyl ester; 6=2-Hexen-1-ol, acetate; 7=1-Hexanol; 8=2-Hexen-1-ol (E); 9=Linalol; 10=Decanoic acid, methyl ester; 11=4-Decenoic acid, methyl ester; 12=1,9-Nonanediol; 13=2-Nonen-1-ol (E); 14=2,6-Nonadien-1-ol.

of each compound for fresh peeled and one-day stored fruits are reported in Tables 1 and 2, respectively. The use of RIC % in a quantitative approach has severe limitations, as is evident in the peak areas that do not reflect the real amounts of the different compounds, but, such semi-quantitative data is very useful as a tool for comparison.

Gas chromatograms of the fresh peeled fruit are showed in Fig. 1; where the most abundant compounds are indicated. The most abundant compound in the yellow and white cultivars is 2-hexen-1-ol followed by 2-nonen-1-ol; this order is reversed in the red cultivar. In red and white cultivars 1-hexanol is quite abundant.

Similar qualitative results have been reported (ARENA *et al.*, 2001) for prickly pears cultivated in eastern Sicily (in a volcanic area), with the same three alcohols being the prevalent compounds. However there were remarkable differences in the quantitative ratio of these compounds compared with our results; this could be attributable to the differences in the soil composition, but also to the ripening and to the different extraction procedures used.

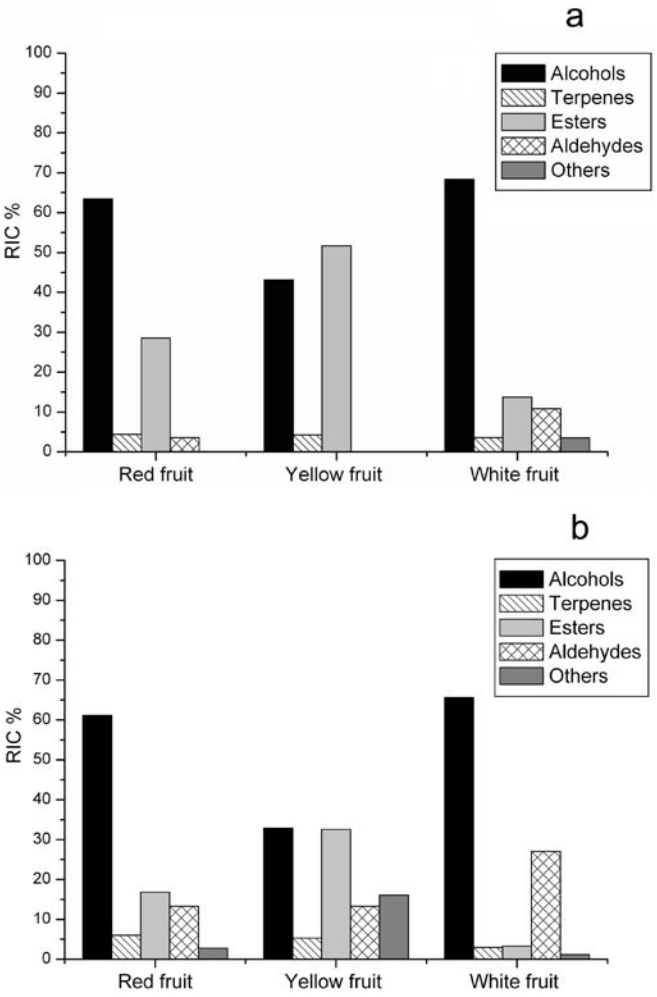


Fig. 2 - RIC % for each class of compound of red, yellow and white fruits examined (a) immediately after peeling and (b) after 24 h of storage.

Esters are also present with the most abundant one being 4-decenoic acid methyl ester, followed by 2-hexen-1-ol acetate and acetic acid hexyl ester; remarkable amounts of butanoic acid 2-methyl-methyl ester were found in the yellow cultivar only. Terpenes were always present in small amounts and traces of aldheydes were only found in the white fruit.

For an overall view of the aromatic profiles of just-peeled fruit, the histograms of the compounds arranged on the basis of chemical families (alcohols, esters, terpenes, etc.) are reported in Fig. 2a. The three cultivars show different aromatic profiles; the esters predominate in the yellow-type, whereas alcohols predominate in both the white and red fruit. This could account for the fact that the intensity of fruit aroma follows the same scale as the ester components, yellow > red > white.

Differences were determined between the aromas of fresh, just picked and peeled fruit (Table 1) and those of fruit stored for 24 h after peeling (Table 2). The histograms of the volatile components (alcohols, esters, terpenes, and aldhehydes) present 24 h after peeling are shown in Fig. 2b.

The most important variation in the aromatic profile in the fruits stored for 24 h after peeling (Table 2) was the increase of two alcohols (2-nonen-1-ol and 2,6 nonadien-1-ol) and the corresponding aldhehydic derivatives (Table 1). These results suggest that hydrolysis and some oxidation have occurred in the presence of atmospheric oxygen (DI CESARE *et al.*, 1993; GROSCH and SCHWARZ, 1971; HATANAKA *et al.*, 1975).

Moreover there was a marked decrease in the amount of esters, probably due to hydrolysis, and/or their high volatility, before the analysis was run. However, the appreciable, rapid change in the aromatic profile shows that the practice of some vendors and consumers of storing pealed prickly pears should be avoided. The data suggest that headspace SPME-GC/MS is a useful tool for determining the flavor profile of fruit, in general, and prickly pear in particular, as well as for detecting changes as a result of storing after peeling.

ACKNOWLEDGMENT

The authors thank the University of Palermo (Fondi di ricerca scientifica ex 60%) for financial support.

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Paper received February 21, 2005 Accepted May 25, 2005

SHORT COMMUNICATION

MICROBIAL POPULATIONS IN MEDICINAL AND AROMATIC PLANTS AND HERBAL TEAS FROM CROATIA

POPOLAZIONI MICROBICHE PRESENTI IN PIANTE OFFICINALI ED AROMATICHE ED INFUSI DI ERBE DELLA CROAZIA

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ABSTRACT

The type and level of microbial populations in common medicinal and aromatic plants and in their respective commercial herbal tea mixtures available in Croatia were examined. Aerobic mesophilic bacteria, spore-formers and moulds had the highest plate counts. Sulphite-reducing clostridia were also found. Yeast genera *Saccharomyces* and *Candida* were detected most often, as well as mould species of genera *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria*, *Mucor*, *Fusarium*, *Rhizopus*,

RIASSUNTO

Sono state esaminate le tipologie di popolazioni microbiche presenti nelle comuni piante aromatiche e officinali e nelle relative miscele commerciali per infusi disponibili in Croazia. Le popolazioni batteriche aerobiche mesofile, quelle produttrici di spore e le muffe sono risultate le più rappresentate nei campioni esaminati. Sono stati isolati anche batteri solfito-riducenti del genere Clostridium. Più frequentemente sono stati riscontrati lieviti dei generi *Saccharomyces* e *Candida*, così

- Key words: herbal teas, medicinal and aromatic plants, microbial population, processing -

Absidia, and *Trichoderma*. The processing of medicinal and aromatic plants seems to increase the microbial populations, and some herbal teas seem to be more prone to such contamination than others.

come specie di muffe dei generi *Penicillium, Aspergillus, Cladosporium, Alternaria, Mucor, Fusarium, Rhizopus, Absidia* e *Trichoderma*. Un aumento delle popolazioni microbiche delle piante officinali e aromatiche sembra collegata alla loro lavorazione, ed alcune miscele per infusi sembrano più suscettibili a subire alcune contaminazioni rispetto ad altre.

INTRODUCTION

Medicinal and aromatic plants are rich in phytochemicals which give them refreshing and/or health-enhancing properties. Research on phytochemicals supports their beneficial roles against cancer, coronary heart disease, diabetes, high blood pressure, inflammation, microbial, viral and parasitic infections, psychotic diseases, spasmodic conditions, ulcers, etc. (CRAIG, 1999; DILLARD and GERMAN, 2000). The plant material, however, is a favourable medium for microbial growth, and therefore it is essential to keep the counts of undesirable microorganisms in the final products as low as possible.

This work reports the results of a study on the type and level of microbial populations in samples of raw medicinal and aromatic plants, as well as in samples of commercial herbal teas. A preliminary examination of the effect of processing on microbial counts was also performed.

MATERIALS AND METHODS

The samples listed in Table 1, chosen on the basis of their market popularity, were obtained from a tea manufacturing factory and subjected to microbiological analyses in duplicate (raw plant material implies harvested, air dried crops, while herbal teas were produced from such material which was cut, sieved and blended before packaging).

The groups of microorganisms examined in the samples were: aerobic mesophilic bacteria (grown on medium for total bacteria), aerobic sporeforming bacteria (on medium for total bacteria), yeasts (on Saboraud maltose agar), moulds (on Czapek agar), coliforms (on violet red bile lactose agar) and sulphite-reducing clostridia (on sulphite agar). Microbiological investigations were performed using standard culture procedures, consistent with pertinent Croatian regulations on microbiological analyses of food (NARODNE NOVINE, 1980). Twenty grams of sample were suspended in 180 mL of sterile saline and serial dilutions were plated on appropriate agar media. After incubation (72 h at 30°C for aerobic mesophilic bacteria, 7 days at 28°C for moulds, 24-48 h at 37°C for coliforms, 72 h at 30°C for aerobic sporeformers, 2-5 days at 25°C for yeasts and anaerobic incubation for 48 h at 44°C for sulphite-reducing bacteria), the colonies were counted and results are expressed in log colony forming units per 1 g of sample (log CFU/g). Inoculum for aerobic sporeformers and sulphite-reducing bacteria was previously pasteurized at 80°C for 10 min. Identi-

Table 1 - Raw medicinal plant materials and final products.

Raw aromatic and medicinal plant material (n=35)		End products of aromatic or medicinal plants (n=8) *
Plant	Plant part	Herbal tea from single plants
Achillea millefolium L. (yarrow)	herb	camomile flower tea
Althaea officinalis L. (marshmallow)	leaf	marshmallow leaf tea
Arctostaphylos uva ursi L. (bearberry)	flower	dog rose fruit tea
Artemisia absinthium L. (wormwood)	herb	
Betula alba L. (birch)	leaf	*Herbal tea blends*
Calluna vulgaris L. (hull)	herb	herbal tea blend no. 1:
Coriandrum sativum L. (coriander)	fruit	bean seedpod, birch leaf, bilberry leaf,
Crataegus oxyacantha L. (hawthorn)	flower	black mulberry leaf, heartsease (*Viola*
Equisetum arvense L. (horsetail)	herb	*tricolor* L.) herb and flower, corn silk and
Erica multiflora L. (multiflower heather)	herb	nettle leaf
Erythraea centaurium L. (centaury)	herb	herbal tea blend no. 2:
Foeniculum vulgare L. (sweet fennel)	fruit	birch leaf, dog rose leaf, alder buckthorn
Juglans regia L. (walnut)	leaf	(*Rhamnus frangula* L.) bark, sweet fennel
Juniperus communis L. (juniper)	fruit	fruit, juniper fruit, lemon balm leaf, parsley
Linum usitatissimum L. (flax)	seed	fruit, elderberry flower and yarrow herb
Matricaria chamomilla L. (camomile)	flower	herbal tea blend no. 3:
Melissa officinalis L. (lemon balm)	leaf	walnut leaf, nettle root, nettle leaf,
Mentha piperita L. (peppermint)	leaf	camomile flower and birch leaf
Morus alba L. (white mulberry)	leaf	herbal tea blend no. 4
Ononis spinosa L. (spiny restharrow)	root	bearberry leaf, birch leaf, parsley fruit,
Petroselinum hortense L. (parsley)	fruit	flax seed, horsetail herb and corn silk
Phaseolus vulgaris L. (bean)	seedpod	herbal tea blend no. 5
Pyrola uniflora L. (single delight)	bark	multiflower heather herb, horsetail herb,
Rosa canina L. (dog rose)	fruit	spiny restharrow root, birch leaf, corn silk
Rubus idaeus L. (red raspberry)	leaf	and bearberry leaf
Salvia officinalis L. (sage)	leaf	
Sambucus nigra L. (elderberry)	leaf & flower	
Teucrium montana L. (mountain germander)	herb	
Thymus serpyllum L. (wild thyme)	herb	
Tilia platyphyllos L. (lime)	flower	
Tussilago farfara L. (coltsfoot)	leaf	
Urtica urens L. (nettle)	leaf	
Vaccinium myrtillis L. (bilberry)	leaf & fruit	
Viscum album L. (mistletoe)	leaf	
Zea mays L. (corn)	corn silk	

* - three samples of herbal teas from single plants and five samples of herbal tea blends.

fication of mould and yeast genera was carried out according to procedures described by GILMAN (1959) and FASSATIOVA (1986). The *Aspergillus* species were identified using the methods of RAPER and FENNEL (1965).

RESULTS AND DISCUSSION

The results of the microbial populations found in samples of raw medicinal or aromatic plant materials and herbal

teas are presented in Table 2. While the raw materials had rather high counts of aerobic mesophilic bacteria, all the samples analyzed had less than 7 log CFU/g, the value allowed by Croatian regulations (NARODNE NOVINE, 1994). The highest contamination (6.7 log CFU/g) was found in camomile flowers and similar high counts (> 6 log CFU/g) were found in samples of bean seedpod, elderberry leaf and fruit, lime flower, marshmallow leaf, spiny restharrow root, and heather, horsetail, wormwood and wild thyme herb. Most other samples had much lower counts, with the lowest values found in juniper berries.

The highest aerobic sporeforming bacteria loads were found in centaury herb, spiny restharrow root, elderberry leaf and fruit and fennel fruit. Aerobic spores were not detected in bearberry leaf samples.

Most of the raw material samples had low yeast populations. The highest yeast count (4.3 log CFU/g) was found in elderberry, while no yeasts were found in the samples of walnut, raspberry, mistletoe and nettle leaf, horsetail, multiflower heather and wormwood herb, hawthorn flower, sweet fennel fruit, and single delight bark. The genera *Saccharomyces* and *Candida* were prevalent in all samples.

The highest mould contamination (5.0 log CFU/g) was found in wormwood herb; four other raw plant material samples including nettle and coltsfoot leaf, lime flower, and bean seedpod, exceeded the upper limit for mould contamination (4 log CFU/g) (NARODNE NOVINE, 1994). The predominant mould genera were *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria*, *Mucor*, *Fusarium*, *Rhizopus*, *Absidia*, and *Trichoderma*.

Processed medicinal and aromatic plants, marketed as single plant herbal teas or herbal tea blends, generally had microbial population patterns similar to those of the respective raw materials. However, the aerobic plate counts of tea blends were not analogous to the level of contamination of their constituents. This was especially evident in herbal tea blend no. 5 (7.3 log CFU/g), which exceeded the accepted limits (NARODNE NOVINE, 1994). None of its medicinal plant constituents had counts over 6 log CFU/g. The mean herbal tea aerobic mesophilic bacteria contamination was also higher than the mean plate count of the raw plant materials. The mean total microbial loads determined for the other groups of microorganisms also increased.

The effect of processing was then examined in more detail by duplicate measurement of microbial populations in three samples of medicinal plants before and after processing (cutting, sieving, blending and packaging). The results are given in Fig. 1. Processing brought about the greatest increase in the aerobic plate count of marshmallow leaves, but all the other microbial populations increased as well. A decreased aerobic

Table 2 - Microbial populations of raw medicinal plant materials and final products.

	log CFU/g				Sulphite reducing clostridia[1] (%)	*A. flavus*[2] (%)
	Total mesophilic bacteria	Spore-formers	Yeasts	Moulds		
Raw materials (n=35)	5.9±6.1	4.2±4.5	3.3±3.7	3.9±4.3	60	18
Herbal teas (n=8)	6.7±6.8	4.4±4.3	3.6±3.7	4.1±4.1	64	9

[1] –sulphite-reducing clostridia positive; [2] –*Aspergillus flavus* positive.

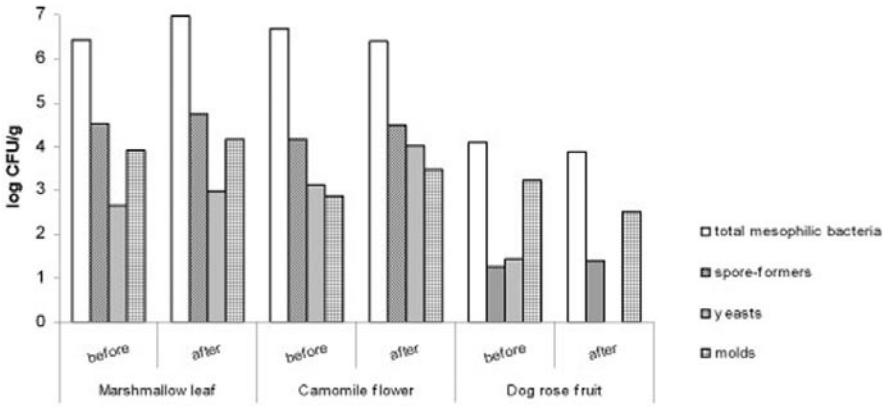


Fig. 1 - Microbial counts in selected medicinal plants before and after processing which included cutting, sieving, blending and packaging.

plate count was determined in camomile flowers after processing, while all the other microbial counts increased. The most pronounced effect was observed for the yeast load which increased by around 25% during processing of camomile. Processing seemed to have the most favourable effect on the microbial populations of dog rose fruit. The original lower microbial level, compared to the other two samples, decreased further, except for the aerobic sporeformer load which remained fixed. These preliminary results suggest that the plant part (leaf, flower, fruit, etc.) is an important factor influencing the effect of processing on microbial loads of end products. ZAGORY (1999) reported that separation of leaves and flowers from the rest of the plant, unlike the fruit, injures plant tissues and encourages microbial growth (ZAGORY 1999). In addition, the sanitary conditions during the processing of medicinal plants and herbs must be controlled in order to prevent further contamination (EUROPAM, 1998). It is also possible that sieving removes impurities more readily from fruit and seeds compared to leaves and flowers.

None of the investigated samples were contaminated with coliform bacteria; a number of samples tested positive for sulphite-reducing clostridia in both the raw materials and herbal teas (Table 2). As reported by other authors (AZIZ *et al.*, 1998; ROY *et al.*, 1988; ROY and CHOURASIA, 1990a,b), the aflatoxigenic mould *Aspergillus flavus* is the most frequently found species in medicinal plants and herbs and is indicative of the possible presence of mycotoxins.

The types and levels of microbial populations found in both the raw materials and final products are quite comparable to the literature data (BECKMANN *et al.*, 1996; CZECH *et al.*, 2001; MARTINS *et al.*, 2001; SCOLARI *et al.*, 2001; KNEIFEL *et al.*, 2002; PALIJAN and BERTIĆ, 1985). Total mesophilic bacteria, aerobic spores and moulds are the predominant microbial populations in medicinal and aromatic plants, although some of the authors listed above reported a much higher incidence of coliforms. According to the EUROPEAN PHARMACOPOEIA 5.0 (2005), the herbal medicinal and aromatic plants examined in this study can be assigned to the category 4 B (herbal medicinal products consisting of one or more herbal drugs, used whole, reduced or powdered, with treatment with boiling water). Total counts of mesophilic bacteria (< 7 log CFU/g) and fungi (< 5 log CFU/g), as well as the absence of *Escherichia coli* determined in both raw and processed material are in

complete agreement with the Pharmacopoeia criteria.

The microbial counts and quantities of toxins in herbal tea infusions are dependent on the levels of these microorganisms in the final herbal tea products (HALT, 1998; CZECH *et al.*, 2001; LUTOMSKI and KEDZIA, 1980; ROY and CHOURASIA, 1990a,b). Taking into account the possible plant part-specific effect of processing, modifications in the production and processing procedures of medicinal and aromatic plants could reduce their role in foodborne infections and intoxications and could help maintain or even enhance their health benefits.

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Revised paper received December 29, 2004 Accepted April 21, 2005

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FIRST ITALIAN AGENCY FOR FOOD SAFETY

ASCA, the first Italian Agency for Food Safety and Control, was inaugurated on June 27 and 28 in Ispica (province of Ragusa, Sicily). ASCA was created thanks to

a joint agreement signed by the councillorship of agriculture and forestry of the Region of Sicily, the research consortium called CoRiBia of Palermo, the Center for agricultural and food innovation called Agroinnova of Turin, the regional Province of Ragusa and the Commune of Ispica. Together they will guarantee clear and transparent production processes, certifying the safety of Sicilian agricultural and food products with a brand of quality called "Sicilia Agricoltura".

Food safety will be assured thanks to a network of specialized laboratories and to the presence of qualified auditors. They will check for the presence of substances that could be dangerous (such as GMO, pesticides, heavy metals, etc.) to consumers, and will be in charge of the voluntary compliance with the production specifications and the regulations regarding use of the quality brand.

ASCA has already started its own activities of defining techniques of production and specific processing methodologies and rigorous plans of control. The aim is to guarantee the quality and safety of Sicilian agricultural and food products, in order to launch products linked to the territory and to the cultural identity of the places of origin and to promote correct information and nutritional education.

27th IDF World Dairy Congress Shanghai 2006 20-23 October 2006

The Congress programme comprises a one-day conference on Dairy Science and Technology offering opportunity for dairy scientists to report on their latest findings irrespective of discipline or subject area in a series of oral presentations and guided poster discussions. It is meant to be a forum for open discussion of all aspects surrounding dairy chemistry and physics, physiology of lactation, processing and technology, microbiology and other areas of dairy science. The Programme Committee will select specific topic areas and presentations on the basis of abstracts submitted. Proposed contributions should preferably not overlap with the subject areas of other conferences to be held during the IDF World Dairy Congress 2006, for details see: http://www.idf2006shcn.com.

The Programme Committee is inviting submission of Abstracts of the proposed presentations expected to be 15-20 minutes in duration. The research work submitted, should be original, innovative and should not have been presented elsewhere. Submissions from young researchers are particularly encouraged. Presentations will be either in oral form or as posters.

The Programme Committee, which will evaluate all submitted abstracts, reserves the right to accept or reject any paper and make a final decision on the form of presentation. The Abstracts, not exceeding 300 words, should include sufficient information on the objectives, methodology, results obtained and contribution to the advancement of dairy-related knowledge. Routine analytical investigations, papers with clear commercial focus, or reviews of common knowledge will not be considered.

The Abstracts should be submitted in an electronic form to the attention of the Chair of the Programme Committee, Prof. Ulrich Kulozik, E-mail: Ulrich.Kulozik@wzw.tum.de with a deadline of 28 February 2006. Authors of submitted Abstracts will be informed about the PC decisions by 1 May 2006. Details, regarding the requirements for final manuscript submission and presentation, will be forwarded later to all authors of accepted Abstracts.

Members of the Programme Committee include: Ulrich Kulozik (DE), Sun Xin (CN), Nagendra Shah (AU), Paul Jelen (CA), Rafael Jimenez-Flores (US), David Everett (NZ), Stefan Ziajka (PL), Yoshitaka Tamura (JP), Petr Dejmek (SE).

BOOKS

La dieta mediterranea, anzi: italiana

Andrea Ghiselli and Lavinia Guffanti
238 pages, 2005
ISBN 88-200-3832-3
Sperling & Kupfer Editori, S.p.A., Milan, Italy
Price: 16.00 euro

Since the 1940s scientific research on nutrition has developed greatly, with all findings, old and new indicating the Mediterranean diet as being the most successful in preserving good health. The book "La Dieta Mediterranea, Anzi: Italiana", written by Andrea Ghiselli, a researcher at the Italian National Institute of Research for Food and Nutrition (INRAN) and a journalist, Lavinia Guffanti, explores the distinctive features of the Mediterranean diet and lifestyle, analyzing, one by one, the different groups of food as well as the effects the diet as a whole has on the body. From its ability to reduce inflammation to its low glycemic index, the combination of nutrients "Mediterranean style" reduces the risks of cardiovascular diseases, obesity and much more. After the first theoretical section, the book focuses on how to construct a healthy routine both at the table and during the rest of the day, with respect to the most recent findings and with the goal of maintaining good health and a sound weight.

International Dairy Federation Protecting dairy products and the consumer against fraud

"The Codex General Standard for the Use of Dairy Terms – Its nature, intent and implications" provides information on the nature, the intent and possible implications in the context of national, regional and international trade, including its status in relation to the WTO Technical Barriers to Trade Agreement (TBT). The Codex General Standard for the Use of Dairy Terms (GSUDT) was developed in order to clarify and provide guidance on the correct use of terms that are universally identified with dairy products so consumers around the world will have a clear understanding of the type of product they are purchasing and consuming.

Milk products have achieved a significant market position. This position makes cheaper imitations very lucrative and consequently it is tempting to take advantage of misleading practices. In the course of recent years this fact is mirrored in the number and nature of practices that occur and that could be considered as misleading in a number of countries, depending on the local context.

Bulletin IDF n. 397/2005 is intended for legislators working on labelling matters in the national context, the regional context and the international context as well as for legal specialists in food companies and in trade associations concerned with these issues. It can be ordered in electronic form via the IDF Website at http://www.fil-idf.org

IDF research highlights need for global standards

The increase in international trade must be matched by an increasing commitment to the implementation of uni-

form standards in milk and milk products.

The IDF has published "Method Standardization in the Third Millennium" a comprehensive research document, which presents the latest scientific thinking on performance improvement.

Jim Begg, president of the IDF, said: "The liberalization of trade will trigger more trade. More trade means more requirement to check compliance and this means standard methods of analysis". The IDF and the International Organization for Standardization (ISO) together play a leading role in providing authoritative internationally agreed methods that are recognized and applied all over the world. "The number of standard methods of analysis and sampling for milk and milk products is impressive in proportion to the size of the dairy sector. This reflects the importance of precise analytical results in determining milk quality, in trade and legal enforcement and the variety of uses to which the results are applied, as well as the relatively wide range of products that are derived from milk as raw material".

The symposium organized during the IDF/ISO/AOAC International Analytical Week 2004, in May in Parma (Italy), covered the performance of methods, the reliability of the results and the never ceasing quest for improvement. This bulletin features presentations delivered at the Symposium.

Bulletin IDF n. 395/2005 can be ordered in electronic form via the IDF Website at http: www.fil-idf.org

For further information please contact Marylène Tucci, IDF Communications and Public Affairs Assistant at IDF at: Tel. + 32 2 706 86 44, Fax +32 2 7330413, e-mail: MTucci@fil-idf.org.

Olive Production Manual 2nd Edition

University of California, Agriculture and Natural Resources Publication 3353

Available for $35.00 (US) by logging onto http://anrcatalog.ucdavis.edu.

Shipping and handling is added to the cost of each order.

First released in 1994, the University of California's "Olive Production Manual" quickly became a bestseller as the definitive guide to olive production in California. Now completely revised, and updated, growers will want to update their libraries with this much-anticipated 2nd edition. The 180-page manual is fully illustrated with 40 tables, 19 line drawings, and 36 charts, and 100 color and black and white photos. The most notable additions include a new chapter on deficit irrigation, a greatly expanded chapter on olive oil production, and coverage of four new pests, including the olive fly.

Includes production techniques for commercial growers worldwide - from orchard planning and maintenance to harvesting and postharvest processing.

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- Pollination
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- Deficit irrigation
- Irrigation
- New pests including olive fly, oleander scale, olive mite, and black vine weevil
- Mechanical harvesting methods including trunk-shaking and canopy contact harvesters
- Postharvest handling and processing methods
- Olive oil production.

Beer - Health and Nutrition

C.W. Bamforth

200 pages, illustrated, hardback, 2004

ISBN 0-632-06446-3

Price: £ 69.50/$ 139.99
Publisher: Blackwell Publishing Professional

This important and extremely interesting book is a serious scientific and authoritative overview of the implications of drinking beer as part of the human diet. Coverage includes a history of beer in the diet, an overview of beer production and beer compositional analysis, the impact of raw materials, the desirable and undesirable components in beer and the contribution of beer to health, and social issues.

Brewing yeast fermentation performance
Third Edition

Edited by K.A. Smart
336 pages, illustrated, hardback, Publishing in 2006
ISBN 1-4051-1908-X
Price: £ 99.00/ $ 144.99
Publisher: Blackwell Publishing Professional

The Brewing Yeast Fermentation Conferences are held every 2 years, organised by Katherine Smart of the School of Biological an Molecular Sciences at Oxford Brookes University, UK. This 3rd volume provides the proceedings of the third conference.

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656 pages, illustrated, hardback, 2001
ISBN 0-632-05475-1
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This unique volume provides a definitive review of modern and traditional brewing fermentation. Written by two experts with unrivalled experience from years with a leading international brewer, coverage includes all aspects of brewing fermentation and the biochemistry, physiology and genetics of brewers' yeast.

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GUIDE FOR AUTHORS

ITALIAN JOURNAL OF FOOD SCIENCE - IJFS

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(1) Manuscripts must be typed, double-spaced and **two** 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 must seek help from a person fluent in scientific English. The Assistant Editor reserves the right to make literary corrections and to make suggestions to improve brevity, but **the paper must be revised by a native English speaker** before submission.

Pages and lines on all pages, including those pages for "References" and figure legends, **must be electronically numbered in the left margin**, beginning with number one at the top of the page.

The paper must also be submitted by e-mail or on a digital support (cd-rom or 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 at 300 dpi in TIF, JPEG, EPS 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 (corresponding to the English) must also be included.

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.

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parentheses (only the initials in capital letters). If there are more than two authors, mention the first author and add *et al.*

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

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

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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, 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.

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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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CONTRIBUTORS

Gratitude is expressed to the following entities for contributing to the realization of the Journal by being supporting subscribers for 2005.

Si ringraziano i seguenti Enti, Ditte ed Istituti per aver voluto contribuire fattivamente alla realizzazione della Rivista, sottoscrivendo un abbonamento sostenitore per il 2005.

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ITALIAN JOURNAL OF FOOD SCIENCE
Rivista Italiana di Scienza degli Alimenti
DIRETTORE RESPONSABILE: Giovanni Chiriotti
AUTORIZZAZIONE: n. 3/89 in data 31/1/1989
del Tribunale di Perugia
Proprietà dell'Università di Perugia
TIPOGRAFIA Giuseppini - Pinerolo
Una copia € 6.00

ISSN 1120-1770 © 2005

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