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PAPER

EFFECT OF FLUID WHEY INCORPORATION ON QUALITY OF CHEVON NUGGETS

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ABSTRACT

Utilizing fluid whey to replace ice flakes in chevon nugget formulation was studied and the physicochemical, technological, microbiological and sensory quality of the product was evaluated. Results showed no statistically significant differences in most of the sensory parameters determined. Even 100% replacement of fluid whey did not produce any adverse effect in sensory properties. However, emulsion stability rate was significantly increased with fluid whey addition to the formulation and 100% replacement of fluid whey showed the most satisfactory results. A slight increase in protein, fat content and cooking yield and decrease in pH value was observed with liquid whey addition. Microbiological analysis of the chevon nuggets on the zero day showed a decrease in total plate count and yeast mold count with increasing levels of liquid whey. These results showed that chilled liquid whey can be added to chevon nugget formulations to improve the product quality at minimal cost.

- Keywords: whey, chevon, goat meat, nuggets, quality -

INTRODUCTION

Whey is a byproduct of cheese and paneer manufacture. It is nutritionally rich but is considered an expensive disposal problem. Currently out of the total fluid whey generated annually, only 62% is used as food or for feed production. Whey contains 20% of the original milk proteins. Fresh whey contains approximately 93% water and 7% solids: 5% lactose, 1% protein and 1% minerals (KOSIKOWSKI, 1982). It has long been recognized that whey proteins like lactalbumin, lactoglobulin, lactoferrin, lactoperoxidase and immunoglobulins confer protection to the neonate against disease. Whey protein has an exceptional biological value (BV) that exceeds that of egg protein by about 15%, the former benchmark, and a range of other common edible proteins and is the protein of choice for body builders, elite athletes, and those whose health is compromised (BUCKLEY *et al.*, 1998). Whey protein is a rich source of the essential amino acids when compared with other typical food proteins and is also thought to play a role as metabolic regulators in protein and glucose homoeostasis, and in lipid metabolism, and as such may play a role in weight control (ZEMEL, 2004; SMILOWITZ *et al.*, 2005). Whey protein is a rich and balanced source of the sulphur amino acids like methionine, and cysteine. These amino acids serve a critical role as antioxidants, and as precursors to the potent intracellular antioxidant glutathione (SHOVELLER *et al.*, 2005). Evidence is growing that specific whey proteins and peptides have potential anti-cancer effects against certain tumours (BOUNOUS *et al.*, 1991; GILL and CROSS, 2000).

Whey is often treated as a wasteful byproduct, due primarily to the cost of concentrating or drying. Liquid whey could be used directly in many food formulations, and could also be converted into a variety of protein concentrates, through electro dialysis, ion-exchange, gel filtration, metaphosphate complexing, reverse osmosis or ultra filtration techniques (ENSOR *et al.*, 1987). Increasing the utilization of nonmeat additives as ingredients in emulsion-type meat products has been investigated to produce less expensive, more stable and highly nutritious products. In this connection, milk proteins play a pivotal role as source of non meat ingredient in emulsion based meat products (DEMOTT *et al.*, 1977). Milk proteins are good moisture binders when used in meat processing and possess a good emulsifying capacity (MITTAL and USBORNE, 1985; ZORBA *et al.*, 1995). Whey proteins showed excellent nutritional and functional properties in low-fat meat products (PEREZ-GAGO and KROCHTA, 2001). Whey proteins have long been reported as potential antioxidants in emulsion type meat products even in comparison to other natural proteins like soy proteins (DECKER and CRUM, 1993). Whey is also reported as beneficial in manufacturing emulsion based products, resulting in a more balanced amino acid composition than with full meat controls (HOLSINGER *et al.*, 1971).

Emulsion based meat products are rich source of protein. Ice flakes are one of the key ingredients in meat emulsion. It aids in the extraction of salt soluble proteins and uniform dispersion of polyphosphates, salt and nitrites in meat emulsion. This process is highly required for emulsification of fat and formation of meat emulsion with non meat ingredients. But ice flakes contributes nothing to the nutritive value of the meat product. Any substitute for ice flakes that can add nutritive value to the product is a welcome measure in processing of meat products. Whey in fluid form is a good choice for this owing to its rich nutritional value. But little research was undertaken to study the effect of fluid whey incorporation on quality of emulsion based meat products. Therefore this experiment aims at incorporating chilled fluid whey as a complete or partial substitution of ice flakes. The study also aims at the effect of fluid whey on the technological, physicochemical, microbiological and sensory properties of chevon nuggets.

MATERIALS AND METHODS

Chevon nugget processing

Deboned lean chevon was obtained from a male goat slaughtered at the age of one year. The remaining fat, connective tissue was removed. The basic formulations of the meat emulsion are tabulated in Table 1 for a total mix of 1 kg. All ingredients including spice and curing ingredients were identical for all batches, except for ice flakes and liquid whey. The fluid whey was prepared in the laboratory from mixed milk containing 5.8% dry matter, of which 0.25% was fat, 0.75% was protein, 0.55% was ash and 4.25% was lactose. Liquid whey was used to substitute 0, 25, 50, 75 and 100% of the ice flakes (Table 1), after being cooled to 0°-1°C before adding to the batter formulation.

In the processing of chevon nuggets, deboned lean chevon was minced first. Then salt, polyphosphate, nitrite, condiments, ice flakes/ fluid whey were added and chopped in bowl chopper for 4-6 minutes. Following this, vegetable oil was added and chopped till oily appearance disappeared. Then maida and spice mix were added and chopped for 2-4 minutes. After that the batter was stuffed in a stainless steel box smeared with oil inside and steam cooked for 45 minutes.

Emulsion stability determination

Emulsion stability of the batters was evaluated by the method of TOWNSEND *et al.* (1968)

Table 1 - Formulation for fluid whey incorporation in chevon nuggets.

	Percent fluid whey substituted for ice flakes				
Ingredients (gm)	**0**	**25**	**50**	**75**	**100**
Deboned lean Chevon (70%)	700	700	700	700	700
Fat (10%)	100	100	100	100	100
Ice flake	100	75	50	25	0
Fluid whey	0	25	50	75	100
Condiment paste (3.5%)	35	35	35	35	35
Spice mix (1%)	10	10	10	10	10
Salt (1.7%)	17	17	17	17	17
Maida (3.3%)	33	33	33	33	33
STPP (0.5%)	5	5	5	5	5
Sodium nitrite (0.015%)	0.15	0.15	0.15	0.15	0.15
Total	**1,000**	**1,000**	**1,000**	**1,000**	**1,000**

with some modifications. About 25 g each of emulsion was placed in polythene bags and heated in a thermostatically controlled water bath maintained at 80°C for 20 minutes. After cooling and draining the exudates, the cooked emulsion mass was weighed and yield was expressed as emulsion stability in percentage.

Physicochemical evaluation

These analyses were performed the day after production, except for cooking yield which was determined by weighing the nuggets before and immediately after cooking. Proximate composition such as % moisture, fat, protein was determined as per AOAC (1995) and pH was determined by the standard method as outlined by TROUT *et al.* (1992). The product yield was determined as mentioned below.

Product yield (%) = Weight of cooked meat block/ weight of emulsion × 100

Microbiological analysis

Microbiological analysis of samples was done on the day of preparation of product for standard plate count and yeast and mold count as per standardized methods established by APHA (1984).

Sensory evaluation

Sensory characteristics of the chevon nuggets were evaluated using 8-point sensory scale (KEETON, 1983) for appearance (8=Bright brownish pink with smooth surface, 1=Very light colour with unattractive surface), binding (8=Excellent binding, 1=Very poor binding), flavor (8=Typical meaty flavor, 1= Off-flavor), texture (8=Very fine, 1=Very coarse) and juiciness (8=Optimum juiciness, 1=Very dry). Panel tests were conducted in an appropriately designed sensory panel room. About 10 panelists (average 5 years meat product sensory evaluation experience) have participated as judge for the evaluation of chevon nuggets in each experiment.

Statistical analysis

The results were analyzed statistically for variance and least significance difference test as per SNEDECOR and COCHRAN (1995). Statistically analyzed results were tabulated and the results were interpreted in Tables 2, 3 and 4.

RESULTS AND DISCUSSION

Emulsion stability

Emulsion stability of the batter was increased as the incorporation of fluid whey increased. Whey replacement of 100% ice flakes gave the most satisfactory results. YETIM *et al.* (2001) indicated that whey proteins improved emulsion stability and resulted in lower chewiness and elasticity.

Physicochemical analysis of the chevon nuggets

A slight variation was detected in proximate composition between the treatments. Numerically an increase in protein, fat content and cooking yield and decrease in moisture and pH value was observed with increasing liquid whey addition. HONGSPRABHAS and BARBUT (1999) reported that whey protein utilization in raw and cooked poultry meat batter resulted in increased water holding capacity, improved rheological properties, and reduced cooking loss. No significant ($P>0.05$) differences were noted in protein and pH, but significantly ($P<0.05$) higher fat, cooking yield and lower moisture content were observed with increase in liquid whey addition. The statistically significant increase in fat content of the higher whey replaced treatments

Table 2 - Proximate compositions, pH and cooking yield of cold whey substituted nuggets.

% Whey in nuggets	Cooking yield%	% Moisture	% Fat	% Protein	pH
0	83.6±0.11^{c}	63.44±0.06^{a}	10.50±0.09^{ab}	19.12±0.03	6.18±0.01
25	83.2±0.09^{c}	63.32±0.08^{a}	10.6±0.10^{c}	19.47±0.08	6.20±0.02
50	84.4±0.13^{ab}	63.27±0.07^{b}	10.62±0.06^{bc}	19.52±0.07	6.15±0.01
75	85.2±0.08^{b}	62.94±0.17^{c}	11.6±0.14^{b}	19.6±0.05	6.14±0.02
100	86.4±0.05^{a}	61.45±0.12^{d}	12.3±0.13^{a}	19.6±0.02	6.13±0.03

Means with different superscripts in the same column differ significantly ($p<0.05$).

Table 3 - Sensory quality of control and fluid whey substituted chevon nuggets.

%Whey in nuggets	Appearance	Binding	Flavor	Texture	Juiciness
0	7.84±0.08	7.00±0.04	7.25±0.03	7.15±0.04	7.05±0.02
25	7.77±0.09	7.15±0.08	7.20±0.07	7.17±0.06	7.15±0.03
50	7.60±0.08	7.20±0.09	7.13±0.05	7.22±0.08	6.95±0.07
75	7.62±0.07	7.23±0.04	7.15±0.04	7.26±0.06	7.35±0.09
100	7.58±0.05	7.25±0.03	7.10±0.09	7.38±0.08	7.50±0.01

Table 4 - Microbiological parameters of control and fluid whey substituted chevon nuggets.

% Whey in nuggets	Standard plate count (log_{10} cfu/g)	Yeast and mold count (log_{10} cfu/g)
0	3.71±0.01	2.46±0.04
25	3.47±0.03	2.94±0.06
50	3.42±0.05	2.94±0.09
75	3.01±0.02	2.97±0.08
100	3.01±0.08	2.89±0.07

may be due to slight contribution from the liquid whey which contained 0.25% milk fat and better emulsification of fat by whey proteins in addition to water soluble meat proteins. Slight increase in protein might be due to addition of whey proteins. Decrease in moisture values owed to increase in total solid content and the reduced pH values might be due to lower pH of liquid whey.

Microbiological analysis

Microbiological analysis of the samples showed that there was a considerable difference in standard plate count with increase in fluid whey substitution in chevon nuggets but yeast and mold count showed no statistically significant difference among the products as shown in Table 4. Similarly it was reported by SHON and CHIN (2008) that the growth of aerobic bacteria and *Listeria monocytogenes* were inhibited in sausages with whey protein coating.

Sensory evaluation

The chevon nuggets in the control and liquid whey added treatments generally had a desirable pinkish brown color though scores for treatments were little bit less than the control. The texture of the nuggets was normal and elastic when sliced, and texture gradually improved with addition of fluid whey. Similar findings were reported by BAARDSETH *et al.* (1992), BEUSCHEL *et al.* (1992) and KER and TOLEDO (1992). The present study indicated no statistically significant ($P>0.05$) differences in appearance, binding, texture, flavor and juiciness between treatments. ELLEKJAER *et al.* (1996) reported lowest off-flavor development and stickiness in whey protein containing cooked sausages, and MARRIOT *et al.* (1998) reported liquid whey incorporated boneless hams did not have any whey flavor.

CONCLUSIONS

It is concluded from the experiment that the fluid whey can replace ice flakes up to 100% in chevon nuggets. Incorporation of fluid whey in emulsion based nuggets provides more desirable emulsion stability, higher fat and protein content, lower moisture content, better pH value and lower microbial load. It improves cooking yield of the product without affecting any of the sensory properties in the treatments in comparison to the control. This experiment shows that fluid whey can be utilized as a massive food source to fortify processed meat prod-

ucts and thus reducing environmental concerns over dumping wastage of a valuable nutritious product.

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PAPER

EVALUATION OF CHEMICAL COMPOSITION AND NUTRITIONAL QUALITY OF BUCKWHEAT GROAT, BRAN AND HULL (*FAGOPYRUM ESCULENTUM* MÖENCH L.)

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ABSTRACT

We analyzed samples of buckwheat groat, bran and hull. Technological processing significantly affected the protein and fat content in buckwheat, and was the highest level in bran (respectively 183.4 g and 39.7 $g \cdot kg^{-1}$ DM). The examined products also significantly differed in the level of crude fiber and dietary fiber fractions. Total AA did not significantly differ between the varieties and between the analyzed products, and averaged 93 g per 16 g N. Isoleucine was the first limiting amino acid. The high level of EAA was reflected in EAAI. The highest PER_1-PER_3 was obtained for protein in buckwheat hull (on average from 2.11 to 2.92).

- Keywords: amino acids, buckwheat products, chemical composition, nutritional quality -

INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Möench L.) is a pseudocereal grown mainly in Japan, the U.S., China, and South Africa. In recent years there has been a renewed interest in this plant in Europe, including Poland, as an alternative crop for organic farming and as a raw material for the production of functional foods. The increased interest in the cultivation of buckwheat is related to its specific characteristics that can be used in crop rotation, its great ability to retrieve nutrients and beneficial due to the favorable chemical composition of grains (MORITA *et al.*, 2006).

Buckwheat is a good source of non-gluten protein with a well-balanced amino acid composition, and a high biological value compared with other plants used in nutrition (IKEDA, 2002). Buckwheat protein contains relatively large amounts of lysine, a limiting amino acid this amino acid for many cereals (KRKOŠKOWA and MRÁZOWÁ, 2005). According to TOMOTAKE *et al.* (2000) buckwheat protein is more effective in preventing the formation of gallstones than soy protein isolates. Its high fiber content is also highly beneficial, as fiber reduces the feeling of hunger and acts as a filler in the gastrointestinal tract. Fiber also binds cholesterol and thus indirectly reduces its level of blood. In addition, fiber absorbs harmful substances from food, such as heavy metals, toxic components of plant protection products and their metabolites. Currently, in Poland, buckwheat is cultivated in two varieties registered in 1990s.

The aim of this work was to evaluate the chemical composition of buckwheat products (groat, bran and hull), especially the nutritional quality of protein.

MATERIALS AND METHODS

The experimental material was common buckwheat (*Fagopyrum esculentum* Möench L.), Kora and Panda varieties, used in an experiment conducted at the Plant Breeding Station in Palikije in 2010. Chemical analysis concerned groat, bran and hull. The material was obtained from the Grain and Milling Plant, Białystok, Poland.

The chemical compositions of all samples were determined by the following AOAC (1990) procedures: dry matter, by drying in an oven at 105°C until a constant weight was obtained; ether extract, by Soxhlet extraction with diethyl ether; crude ash, by incineration in a muffle furnace at 580°C for 8 h; crude protein (N×6.25), by the Kjeldahl method. Nitrogen-free extract (NFE) calculated as 100-%(moisture + crude protein +lipid + ash + crude fibre). The fibre components were determined using the detergent method according to VAN SOEST *et al.* (1991) performed with a fibre analyzer ANCOM 220. Determination of neutral detergent fibre (NDF) was on an ash-free basis and included sodium dodecyl sulphate (Merc 822050). Determination of acid detergent fibre (ADF) included hexadecyl-trimethyl-ammonium bromide (Merc 102342), while acid detergent lignin (ADL) was determined by hydrolysis of ADF sample in 72% sulfuric acid. Hemicellulose was calculated as the difference between NDF and ADF, while cellulose – as the difference between ADF and ADL. A flame photometer (Flapho-4) was used to determine calcium, potassium and sodium. After oven-drying of samples, phosphorus content was determined by a colorymetric method. Results were expressed as gram (g) per kilogram (kg) of dry matter (DM).

Amino acids were determined using an AAA 400 automatic amino acid analyzer (INGOS, Czech Republic). Samples were subjected to acid hydrolysis in the presence of 6 M HCl at 105°C for 24 hours. Sulfur-containing amino acids were determined separately in 6 M HCl after oxidative hydrolysis (formic acid + hydrogen peroxide, 9:1 v/v, 20 h at 4°C). Tryptophan was determined according to the method described in AOAC (1990). Amino acid composition was displayed as g per 16 g of nitrogen.

All results are given as means of double analysis.

The quality of protein was estimated by determination of total amino acids (AA), as well as the fractions of the essential amino acids (EAA). The contents of different amino acids are presented as g/16 g of nitrogen and are compared with two standards: amino acids of food protein composition appropriate for a mature human (MH) (FAO/WHO, 1991) and amino acid composition of the of whole egg protein (WE) (HIDVÉGI and BÉKÉS, 1984).

The chemical score (CS) was calculated on the basis of procedure described previously by BLOCK and MITCHELL (1946), based on comparison of the concentration ratio of the amino acid having the shortest supply a_i (receive amino acid) to concentration of this amino acid in the standard a_s ($CS = (a_i/a_s) \times 100$).

The essential amino acid (EAAI) was calculated according to the method of OSER (1951).

The predicted protein efficiency ratio (PER) was calculated from their amino acid composition based on three equations developed by ALSMEYER *et al.* (1974):

$$PER_1 = -0{,}684 + 0{,}456 \times Leu - 0{,}047 \times Pro$$

$$PER_2 = -0{,}468 + 0{,}454 \times Leu - 0{,}105 \times Tyr$$

$$PER_3 = -1{,}816 + 0{,}435 \times Met + 0{,}780 \times Leu + 0{,}211 \times His - 0{,}944 \times Tyr$$

The results were analyzed statistically using analysis of variance for the two-way classification. Multiple comparisons of means were performed based on Duncan's procedure for $\alpha = 0.05$.

RESULTS AND DISCUSSION

The basic chemical composition is shown in Table 1. The greatest buckwheat protein content was found in bran (183.4 g·kg $^{-1}$ DM). The protein in groat was 148.3 g·kg $^{-1}$ DM, which confirms the results of WEI *et al.* (2003).

In our study, fat, an ingredient much more significant for the total energy content of buckwheat than protein, carbohydrates and fibre, averaged 26 g·kg $^{-1}$ DM for all the materials taken together. Fat content was the lowest in the hull and the highest in bran (respectively 10.8 and 39.7 g·kg $^{-1}$ DM), which is consistent with data provided by STEADMAN *et al.* (2001) and TAHIR and FAROOQ (1985).

The content and composition of crude fiber is crucial for the quality of buckwheat products. Many studies have shown that due to its characteristics, dietary fiber is very important in the prevention and treatment of diabetes, obesity, atherosclerosis, heart disease, and the cancer of colon and large intestine (FERGUSON and HARRIS, 2003; WANG *et al.*, 2002). However, the content and proportions of various carbohydrates in buckwheat may change during hydrothermal treatment. In our study, the significantly lowest content of crude fiber was found in groat and the highest in hull (average of 10.7 and 511 g·kg $^{-1}$ DM) (Table 1). The highest content of a neutral detergent fiber (NDF) was found in hull (834 g·kg $^{-1}$ DM), while the smallest in whole groats (56 g·kg $^{-1}$ DM) (Table 2). The varieties differed in content of NDF and its fractions. Panda variety contained significantly more NDF, hemicellulose and ADL fractions than Kora variety. Low affinity of acid detergent lignin (ADL) to water – and its large hydrophobicity – limits both access to water and the possibility of hydrolysis by amylolytic enzymes in animals. A high content of free hydroxyl groups (OH) results in the lignin forming bonds with other types of compounds (polysaccharides, peptides, proteins). It also binds large amount of bile acids (17-52%), which enhances cholesterol reduction (HILLMAN *et al.*, 1985). ADL content was the highest in hull and lowest in groat (respectively 353 and 5.6 g·kg $^{-1}$ DM), which is consistent with the results obtained by GÓRECKA *et al.* (2010).

Hemicelluloses and pectins are components that are potent absorbants of heavy metals (ANDERSEN *et al.*, 2004). Although cellulose is only slightly digested in the small intestine, it supports peristalsis. The highest content of the cellulose and hemicellulose fractions, similar to other fiber fractions, was found in hull, while the smallest in groats (Table 2). Small lignin and cellulose content is due to the removal of lignin-rich outer parts of grain during groat production (NYMAN *et al.*, 1984). Depending on the type of technology used in groat production, the content and fractional

Table 1 - Chemical composition of common buckwheat products (g·kg $^{-1}$ DM).

Trait	Variety	Products			Mean
		Groat	Bran	Hull	
Dry matter (g·kg^{-1})	Kora	696 a	856 a	855 a *	802 A**
	Panda	846 a	883 a	853 a	861A
	Mean	771 a	869 a	854 a	831
Ash	Kora	21.1 b	31.9 a	19.4 c	24.1 B
	Panda	23.6 b	30.9 a	22.6 b	25.7 A
	Mean	22.4 b	31.4 a	21.0 b	24.9
Crude protein (N×6.25)	Kora	137.4 b	177.9 a	62.2 c	125.8 B
	Panda	159.2 b	189.0 a	52.8 c	133.6 A
	Mean	148.3 b	183.4 a	57.5 c	129.7
Ether extract	Kora	26.3 b	40.1 a	13.0 c	26.5 A
	Panda	29.4 b	39.3 a	8.6 c	25.7 A
	Mean	27.8 b	39.7 a	10.8 c	26.1
Crude fiber	Kora	12.7 c	53.4 b	488.4 a	184.8 B
	Panda	8.7 c	51.0 b	533.9 a	197.9 A
	Mean	10.7 c	52.2 b	511.1 a	191.3
NFE	Kora	809 a	697 b	417 c	641 A
	Panda	779 a	690 b	382 c	617 B
	Mean	794 a	693 b	400 c	629

*Means in the lines for each trait that are denoted with the same lowercase letter do not differ significantly in the Duncan test at α =0.05.
**Means in the columns for each trait that are denoted with the same capital letter do not differ significantly in the Duncan test at α =0.05.

Table 2 - Fiber fraction (g·kg $^{-1}$ DM) of common buckwheat products.

Trait	Variety	Products			Mean
		Groat	Bran	Hull	
NDF	Kora	57.1 c	154.2 b	817.8 a*	343.0B**
	Panda	54.4 c	153.7 b	849.7 a	352.6 A
	Mean	55.7 c	153.9 b	833.7 a	347.8
ADF	Kora	26.3 c	91.8 b	713.0 a	277.0 A
	Panda	25.4 c	95.9 b	725.8 a	282.4 A
	Mean	25.9 c	93.8 b	719.4 a	279.7
ADL	Kora	5.1 c	35.8 b	348.0 a	129.6 B
	Panda	6.1 c	41.6 b	358.2 a	135.3 A
	Mean	5.6 c	38.7 b	353.1 a	132.5
Cellulose	Kora	21.2 c	56.0 b	365.0 a	147.4 A
	Panda	19.3 c	54.3 b	367.7 a	147.1 A
	Mean	20.3 c	55.2 b	366.3 a	147.2
Hemicellulose	Kora	30.8 c	62.4 b	104.8 a	66.0 B
	Panda	29.0 c	57.8 b	123.9 a	70.2 A
	Mean	29.9 c	60.1 b	114.3 a	68.1

* and ** as in Table 1.

Table 3 - The mineral composition of (g·kg $^{-1}$ DM) of common buckwheat products.

Trait	Variety	Products			Mean
		Groat	Bran	Hull	
Calcium	Kora	0.39 c	0.71 b	1.30 a*	0.80 A**
	Panda	0.46 b	0.66 b	1.31 a	0.81 A
	Mean	0.42 c	0.68 b	1.30 a	0.80
Phosphorus	Kora	4.47 b	5.66 a	1.50 c	3.88 A
	Panda	4.94 b	5.75 a	1.26 c	3.98 A
	Mean	4.70 b	5.70 a	1.38 c	3.93
Potassium	Kora	2.04 b	3.40 a	1.92 b	2.45 B
	Panda	2.53 c	3.81 b	4.61 a	3.65 A
	Mean	2.28 b	3.60 a	3.26 a	3.05
Sodium	Kora	0.232 a	0.228 a	0.236 a	0.232 A
	Panda	0.229 a	0.172 b	0.254 a	0.218 A
	Mean	0.231ab	0.200 b	0.245a	0.225

* and ** as in Table 1.

composition of fiber, and thus the functional properties may vary (GÓRECKA *et al.*, 2009).

Buckwheat is also a source of minerals, valuable both for nutritional and technological reasons (IKEDA and YAMASHITA, 1994). Mineral content, expressed as total ash, averaged 25 g·kg $^{-1}$ DM (Table 1). Varieties differed in the content of main components. Panda variety contained significantly more ash, protein, crude fiber in comparison to Kora variety. Phosphorus had the largest share in the composition of ash, followed by potassium (Table 3), which consistent with the results by CHRISTA and SORAL-ŚMIETANA (2008). These two elements were the most abundant in bran. However, calcium and sodium contents were the highest in hull.

Another parameter tested in our experiment was amino acids composition (Table 4) which determines the nutritional value of protein, and more specifically - the amount of essential amino acids. Many studies have shown that from the view of nutritional value the amino acid composition of buckwheat is the most favorable among all cereals. The sum of all amino acids (total AA) was not different between the examined varieties and three examined products and averaged 93 g per 16 g N (Table 4). Buckwheat products have a well-balanced composition of amino acids, with abundant lysine - a limiting amino acid for most cereals (BIEL *et al.*, 2009) – at 5.5 g per 16 g N, which is confirmed by the data presented by BONAFACCIA *et al.* (1994) and BONAFACCIA *et al.* (2003). The content of lysine in buckwheat protein is more than double in wheat protein (WEI *et al.*, 1995). Low lys/arg and met/arg ratios may suggest that buckwheat products have blood cholesterol-lowering properties. As

Table 4 - Amino acid profiles of common buckwheat products (g per 16 g N).

Trait	Variety	Products			Mean
		Groat	Bran	Hull	
Lys	Kora	5.89 a	5.72 a	4.89 a*	5.50 A**
	Panda	5.64 a	5.76 a	4.98 a	5.46 A
	Mean	5.76 a	5.74 a	4.93 a	5.48
Met+Cys	Kora	4.12 a	3.72 a	4.00 a	3.94 A
	Panda	3.95 a	3.71 a	2.90 b	3.52 B
	Mean	4.03 a	3.71 ab	3.45 b	3.73
Cys	Kora	2.60 a	2.38 a	2.16 a	2.38 A
	Panda	2.48 a	2.30 a	1.58 b	2.12 B
	Mean	2.54 a	2.34 a	1.87 b	2.25
Thr	Kora	3.76 a	3.68 a	4.71 a	4.05 A
	Panda	3.65 a	3.60 a	4.02 a	3.75 A
	Mean	3.70 a	3.64 a	4.36 a	3.90
Ile***	Kora	2.93 a	2.63 a	2.81 a	2.79 A
	Panda	2.56 a	2.97 a	3.02 a	2.85 A
	Mean	2.74 a	2.80 a	2.91 a	2.82
Trp	Kora	1.44 a	1.40 a	0.63 b	1.15 A
	Panda	1.47 a	1.42 a	0.61 b	1.16 A
	Mean	1.45 a	1.41 a	0.62 b	1.16
Val	Kora	4.07 a	4.05 a	4.62 a	4.25 A
	Panda	4.20 a	4.19 a	4.38 a	4.25 A
	Mean	4.13 a	4.12 a	4.50 a	4.25
Leu	Kora	6.45 a	6.32 a	6.84 a	6.54 A
	Panda	6.27 a	6.09 a	6.22 a	6.19 A
	Mean	6.36 a	6.20 a	6.53 a	6.36
His	Kora	2.72 b	2.47 b	3.66 a	2.95 A
	Panda	2.91 b	2.60 b	3.82 a	3.11 A
	Mean	2.81 b	2.53 b	3.74 a	3.03
Phe+Tyr	Kora	7.32 a	6.78 a	6.23 a	6.77 A
	Panda	7.09 a	6.29 a	6.05 a	6.48 A
	Mean	7.21 a	6.53 a	6.14 a	6.63
Tyr	Kora	2.73 a	2.17 a	2.04 a	2.31 A
	Panda	2.61 a	2.33 a	1.86 a	2.27 A
	Mean	2.67 a	2.25 b	1.95 b	2.29
Arg	Kora	7.98 a	8.05 a	6.97 b	7.66 A
	Panda	7.64 a	6.99 b	4.57 c	6.40 B
	Mean	7.81 a	7.52 a	5.77 b	7.03
Asp	Kora	9.97 a	9.96 a	9.54 a	9.82 A
	Panda	10.08 a	10.24 a	9.44 a	9.92 A
	Mean	10.02 a	10.10 a	9.49 a	9.87
Ser	Kora	5.15 a	5.12 a	5.65 a	5.31 A
	Panda	5.07 a	5.06 a	4.89 a	5.01 A
	Mean	5.11 a	5.09 a	5.27 a	5.16
Glu	Kora	21.6 a	21.2 a	16.3 b	19.7 A
	Panda	20.9 a	20.5 a	16.2 b	19.2 B
	Mean	21.3 a	20.8 a	16.2 b	19.4
Pro	Kora	3.72 a	3.61 a	3.91 a	3.75 A
	Panda	3.63 a	3.27 a	3.71 a	3.53 B
	Mean	3.68 a	3.44 b	3.81 a	3.64
Gly	Kora	6.69 a	6.92 a	7.07 a	6.89 A
	Panda	6.47 a	6.57 a	6.33 a	6.45 A
	Mean	6.58 a	6.75 a	6.70 a	6.67
Ala	Kora	3.81 a	4.22 a	4.13 a	4.05 A
	Panda	3.76 a	3.73 a	3.75 a	3.75 B
	Mean	3.78 a	3.97 a	3.94 a	3.90
Sum / Total AA	Kora	97.9 a	95.8 a	91.9 a	95.2 A
	Panda	95.3 a	93.0 a	84.8 a	91.0 A
	Mean	96.6 a	94.4 a	88.4 a	93.1

* and ** as in Table 1.
*** The most limiting essential amino acid (CS-chemical score).

shown by KAYASHITA *et al.* (1995) the use of buckwheat protein preparations in the hamster diet significantly contributed to the reduction in cholesterol in the blood, liver and gall bladder, and also inhibited the formation of gall stones as a result of changes in cholesterol metabolism. It is believed that protein buckwheat extracts reduce the level of LDL and VLDL, and prevent the development of colorectal cancer by reducing the proliferation of tumor cells. Buckwheat is an excellent source of tryptophan - in our study the most abundant in groat and bran, respectively 1.45 and 1.41 g per 16 g N, but the least in hull (0.62 g per 16 g N). Buckwheat products are also rich in sulfur amino acids (mean 3.73 g per 16 g N). They may therefore be a perfect complementation of legumes, in which the quality of protein is limited by methionine and cystine (SUJAK *et al.*, 2006). The amino acid composition of buckwheat protein, rich in lysine, methionine and cystine, is therefore much more desirable than in most other cereals.

Both the quantity and quality of protein are very important in nutrition. The proportion of essential amino acids to total amino acids (E/T, %), chemical score (CS), essential amino acids index (EAAI) both for MH and WE standards are shown in Table 5. In our examination, the ratio of essential amino acids to total amino acids was high and averaged 37% for MH and 40% for WE. The examined protein products, as in other cereals, had a deficit of isoleucine. Isoleucine was the first limiting amino acid (CS), both according to WE standard and MH. The high level of essential amino acids was reflected in the EAAI that averaged 90% (MH) or 71.4% (WE). The predicted protein efficiency ratio (PER) exceeded on average 2.00, suggesting that protein in buckwheat products has a high quality (FRIEDMAN, 1996). The highest values of PER_{1-3} were obtained for hull (an average of 2.11-2.92). The predicted PER values were rather satisfactory compared to casein (standard protein), equal 2.5 (FRIEDMAN, 1996). PER values obtained in our study for buckwheat exceed obtained by other authors for other vegetables: sorghum (0.27), rice (1.2) and millet (1.62) (OYAREKUA and ELEYINMI, 2004; PADHYE and SALUNKHE, 1979).

Table 5 - Nutritional evaluation of common buckwheat products.

Trait	Variety	Products			Mean
		Groat	Bran	Hull	
EAA_{MH} (g/16 g N)	Kora	36.0 a	34.3 a	34.7 a*	35.0 A**
	Panda	34.8 a	34.0 a	32.2 a	33.7 A
	Mean	35.4 a	34.1 a	33.4 a	34.3
E_{MH}/T, %	Kora	37.1 a	35.8 a	37.7 a	36.8 A
	Panda	36.5 a	36.6 a	37.9 a	37.0 A
	Mean	36.8 a	36.2 a	37.8 a	36.9
CS_{MH}	Kora	73.3 a	65.8 a	60.6 a	66.5 A
	Panda	63.9 a	74.1 a	61.1 a	66.4 A
	Mean	68.6 a	69.9 a	60.8 a	66.4
$EAAI_{MH}$	Kora	92.0 a	90.1 a	86.9 a	89.7 A
	Panda	90.0 a	91.1 a	84.3 a	88.5 A
	Mean	91.0 a	90.6 a	85.6 a	89.1
EAA_{WE} (g/16g N)	Kora	39.0 a	36.8 a	38.4 a	38.0 A
	Panda	37.7 a	36.6 a	36.0 a	36.8 A
	Mean	38.4 a	36.7 a	37.2 a	37.4
E_{WE}/T, %	Kora	39.8 a	38.3 a	41.7 a	39.9 A
	Panda	39.6 a	39.4 a	42.4 a	40.4 A
	Mean	39.7 ab	38.9 b	42.0 a	40.2
CS_{WE}	Kora	54.3 a	48.7 a	36.8 a	46.6 A
	Panda	47.3 a	54.9 a	35.8 a	46.0 A
	Mean	50.8 a	51.8 a	36.3 b	46.3
$EAAI_{WE}$	Kora	75.6 a	72.4 a	69.6 a	72.5 A
	Panda	73.4 a	72.7 a	64.5 a	70.2 A
	Mean	74.5 a	72.5 a	67.1 a	71.4
PER_1	Kora	2.09 a	2.03 a	2.25 a	2.12 A
	Panda	2.01 a	1.94 a	1.98 a	1.97 A
	Mean	2.05 a	1.99 a	2.11 a	2.05
PER_2	Kora	2.18 a	2.18 a	2.43 a	2.26 A
	Panda	2.11 a	2.05 a	2.16 a	2.11 A
	Mean	2.14 a	2.11 a	2.29 a	2.18
PER_3	Kora	1.88 c	2.17 b	3.17 a	2.40 A
	Panda	1.86 c	1.89 b	2.67 a	2.14 A
	Mean	1.87 b	2.03 b	2.92 a	2.27

[a]E/T, proportion of essential amino acids (E) to total amino acids (T).
* and ** as in Table 1.

CONCLUSIONS

Buckwheat is a valuable source of substances with nutritional or physiological importance. The technological process of groat production significantly affected the protein and fat content. The highest content of these compounds was found buckwheat bran (respectively 183.4 and 39.7 $g \cdot kg^{-1}$ DM). Groat, bran and hull had a significantly different level of crude fiber and dietary fiber fractions. The content of mineral compounds in total ash was 25 $g \cdot kg^{-1}$ DM. The largest share was found for phosphor and then potassium. There were no significant differences in amino acid content and nutritional value of protein in the examined products. Grain of the tested varieties had similar chemical composition and nutritional value. It is worth noting that, despite the ever-increasing interest in buckwheat, it is still relatively unpopular. The high nutritional value of buckwheat, as shown in our paper, is a strong rationale for the wider introduction of this plant in the diet.

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PAPER

MEAT QUALITY IN DONKEY FOALS

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ABSTRACT

A study to evaluate quality attributes in muscles Semimembranosus (SM) and Semitendinosus (ST) muscles was performed on 10 entire donkey foals slaughtered at 10 months of age and mean final body weight of 126 kg. Fat content was significantly ($P<0.05$) higher (1.77%) in SM muscle compared to ST (1.61%); protein, cholesterol and glycogen content were not statistically different between the two muscles examined. Collagen concentration in ST muscle (44.2 μg/mg) was significantly higher ($P<0.01$) compared to SM muscle (32.1 μg/mg). Polyunsaturated fatty acids were significantly higher ($P<0.05$) in SM muscle (22.11%) compared to ST muscle (20.61%).

- Keywords: chemical composition, cholesterol content, collagen concentration, donkey meat quality, fatty acid composition -

INTRODUCTION

Donkey (*Equus asinus, Perissodactyla*) can be considered the most useful domesticated animal in the history; in ancient Egypt, donkeys were once highly regarded, even worshipped (SMITH and PEARSON, 2005). Unfortunately, in the following Centuries this elevated status did not endure, and starting from the Middle Age donkeys have continued to be associated with the poorest and most marginalized in human societies. Even today, donkeys continue to be used for innumerable tasks, and are considered to be one of the best draught animals in developing nations (AGANGA *et al.*, 2003). Donkey can be also considered an important meat-producing animal in many countries of the world, especially in arid and semi-arid areas, but the potential of donkey breeding for meat production has received little attention compared to other species. Normally donkey meat produced in Italy comes from animals at the end of their career or slaughtered for health problems, and the meat obtained by these animals are normally considered unacceptably tough (POLIDORI *et al.*, 2009).

The dramatic decrease of donkey population has been recently stopped in the south of Europe, due to interest in the nutritional properties of donkey milk for infant nutrition (VINCENZETTI *et al.*, 2008), to the possible use of donkeys for leisure riding and also in pet-therapy with children affected by Down Syndrome (MILONIS, 2010). Donkeys are mainly raised in an extensive system and are able to survive and produce in harsh environmental conditions, and to convert low-quality forages to high quality protein (POLIDORI *et al.*, 2008). This agrees with recent European Union policy, to de-intensify animal production and to encourage a sustainable development of otherwise unproductive marginal Mediterranean areas by rearing and valorisation of local breeds and populations (MAIORANO *et al.*, 2001). Donkey meat is characterised by as low intra-muscular fat content and could be used as a valid alternative to other red meat species such as beef and lamb, together with horse meat (LANZA *et al.*, 2009). Nevertheless, little is known about the quality of young foals meat and, to our knowledge, no studies have been conducted in order to determine the intramuscular collagen amount, a very important attribute for evaluating meat quality.

The present study was carried out to evaluate the quality attributes of donkey meat obtained from two specific muscles, Semimembranosus and Semitendinosus, taken by young male entire foals.

MATERIALS AND METHODS

The study was performed using 10 entire crossbred donkey males, slaughtered at 10 months of age and a mean fasted final body weight of 126±13 kg. The foals were reared with their mothers on a natural Mediterranean pasture, with a higher grass availability from autumn to spring. The animals were fasted (only water was available) for about 18 h before slaughtering. All the carcasses were skinned and eviscerated, then stored in a cold room at a temperature of about 2°C, suspended by the hind legs. After 24 h storage in the cold room (2°C), from the right side of each chilled carcass, semimembranosus (SM) and semitendinosus (ST) muscle samples (300 g/sample) were collected. Meat samples were put in zipped plastic bags and transported for 3 h to the laboratory for chemical analysis.

Chemical composition and cholesterol content of the ST and SM muscle of the animals were determined using the methods described by BABIKER *et al.* (1990). Glycogen was determined spectrophotometrically (340 nm) using a commercial kit from Sigma (Glucose HK 16-50) which is based on a NAD-linked assay catalysed by hexokinase and glucose-6-phosphate deydrogenase, as described by IMMONEN *et al.* (2000).

Muscle samples were trimmed of fat and epimysium, lyophilised for 48 h, weighed and hydrolysed for determination of hydroxyproline (ETHERINGTON and SIMS, 1981). Collagen content was calculated assuming that collagen weighed 7.25 times the measured hydroxyproline weight (MAIORANO *et al.*, 1999).

Fatty acid composition was determined in both the muscles. Total lipids were extracted from the meat as described by BLIGH and DYER (1959); for the preparation of fatty acids methyl esters the lipid sample (20 mg) was dissolved in 0.1 mL of tetrahydrofuran in a test tube and 10% methanolic hydrogen chloride (2 mL) were added (SUKHIJA and PALMQUIST, 1988). The sample was sealed and heated at 100°C for 1 h. To each sample 2 mL of 1 M potassium carbonate solution was added. The fatty acids methyl esters were extracted with 2x2 mL of hexane and 1 µL was injected into a gas-chromatograph. Fatty acid analysis was performed on a Chrompack (model CP 9003) gas chromatograph with a flame ionization detector and a fused-silica capillary column, film thickness 0.2 µm, packed with CP Sil 88 (50 m x 0.25 mm i.d.). Helium was used as the carrier gas and the column temperature was held at 80°C for 4 min, and then increased at 10°C/min from 80° to 140°C, then at 5°C/min from 140°C to a final temperature of 210°C (held for 14 min). Fatty acid identification was made by comparing gas chromatographic retention times with the anti-oxidant standard butylated hydroxytoluene (BHT). Fatty acids were expressed as g/100 g identified total fatty acid methyl esters.

Data were analysed by the method of least squares using the general linear model procedures of SAS (2001) and results were expressed

as least square means. Significant differences between means were indicated when P<0.05.

RESULTS

Meat chemical composition is shown in Table 1. Fat content was significantly (P<0.05) higher in SM muscle (1.77 g/100 g) compared to ST muscle (1.61 g/100 g). Both the values obtained in this study were lower compared to fat content determined in donkey meat samples (POLIDORI *et al.*, 2011) taken from animals slaughtered at older age, namely 12 months (2.41 g/100 g) and 18 months (3.71 g/100 g). Protein content was very similar in both muscles examined in the present study, respectively 20.2 g/100 g in SM, 19.9 g/100 g in ST; these values were very similar to those obtained in a study conducted on Galician Mountains horse foals slaughtered at the age of nine and twelve months (FRANCO *et al.*, 2011). Cholesterol content was not statistically different in SM (57.6 mg/100 g) and in ST (55.8 mg/100 g) muscles; the values determined in this study on donkey foals are lower to those normally obtained in beef muscles (LAWRIE, 1991) and similar to those obtained in farmed red deer stags (STEVENSON *et al.*, 1992) and also in ST muscle of young Marchigiana bulls (54.1 mg/100 g) by CUTRIGNELLI *et al.* (2008). Glycogen content determined in both muscles, 0.38 g/100 g in ST and 0.46 g/100 g in SM, was not statistically different and very close to the values obtained in many studies conducted on horse meat, as reviewed by BADIANI and MANFREDINI (1994).

Collagen content was significantly (P<0.01) higher in ST muscle (44.2 μg/mg) compared to SM muscle (32.1 μg/mg), according with previous experiments conducted on goat (MAIORANO *et al.*, 2001) and beef muscles (DRANSFIELD, 1977). Previous studies conducted on male and female horse foals showed higher values in total collagen amount (SARRIÉS and BERIAIN, 2005; FRANCO *et al.*, 2011) compared to donkey foals. The results obtained by TATEO *et al.* (2008) confirmed the significantly higher statistical difference between total collagen content determined in ST muscle compared to SM muscle taken from Italian Heavy Draft horses. The intramuscular fatty acid composition is shown in Table 2. The foal meat fatty acids in this study are predominated by saturated fatty acids (SFA), followed by monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). These results are in agreement with those obtained by SARRIÉS *et al.* (2006) on horse foals slaughtered at ages of 16 and 24 months, and also with results indicated by LORENZO *et al.* (2010) on Galician Mountain foals breed slaughtered at 9 and 12 months. Palmitic acid (C16:0) was the most abundant fatty acid in both the muscles examined, followed by oleic acid (C18:1); this result agrees with that one obtained by TATEO *et al.* (2008) in a study conducted on horses slaughtered at the age of 11 months. Within PUFA, linoleic acid (C18:2) was predominant in both muscles, according with the results determined on horse meat obtained by two Spanish local breeds by JUÁREZ *et al.* (2009). The total PUFA content was significantly higher (P<0.05) in SM muscle (22.11% of total fatty acids) compared to ST (20.61% of total fatty acids) muscle. Meat samples examined in the present study showed an interesting content of C18:2 and C18:3, that is considered a positive characteristics from a nutritional point of view, in line with health recommendations concerning fatty acids and lipids consumption (WILLIAMS, 2000). Concerning this aspect, the favourable SFA/UFA ratio, similar to that found for buffalo meat (INFASCELLI *et al.*, 2004), has to be underlined.

Table 1 - Chemical characteristics (means±s.e.) of the *Semitendinosus* (ST) and *Semimembranosus* (SM) muscles of donkeys foals (n = 10).

	ST	SM
Moisture (%)	77.3±2.01	76.7±1.57
Fat (%)	1.61±0.37[a]	1.77±0.48[b]
Protein (%)	19.9±1.33	20.2±1.05
Ash (%)	0.82±0.21	0.89±0.18
Energy value (kcal/100 g)	108.50	111.44
Glycogen (%)	0.38±0.08	0.46±0.12
Cholesterol (mg/100 g)	55.8±1.43	57.6±2.77
Collagen concentration (μg/mg)	44.2±2.34[a]	32.1±1.08[c]

Different letters in the same row indicate a significant difference (b: P<0.05; c: P<0.01).

Table 2 - Fatty acid composition (% total identified fatty acids) determined in donkey *Semitendinosus* (ST) and *Semimembranosus* (SM) muscles (means±s.e.).

Fatty acid	ST (n = 10)	SM (n = 10)
C12:0	0.28±0.04	0.36±0.03
C14:0	3.88±0.56	3.79±0.61
C16:0	31.85±1.64	30.76±1.92
C16:1	6.21±0.44	5.93±0.77
C18:0	7.81±0.62	7.26±0.91
C18:1	28.77±2.13	29.18±2.08
C18:2	17.81±1.68	18.40±1.98
C18:3	1.22±0.34	1.30±0.28
C20:1	0.65±0.18	0.63±0.10
C20:4	1.58±0.46	2.41±0.59
SFA	43.82±3.11	42.17±2.89
MUFA	35.63±2.18	35.74±1.75
PUFA	20.61±1.44[a]	22.11±1.31[b]
SFA/MUFA	1.23	1.18
SFA/PUFA	2.13	1.91
SFA/UFA	0.78	0.73

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: total unsaturated fatty acids.
Different letters in the same row indicate a significant difference (b: P<0.05).

CONCLUSIONS

Donkey males are not always useful in the farm, and the possibility of finding new strategies to increase the income of local farmers selling meat obtained by young male foals slaughtered before a year of age can be a good opportunity. Donkey meat had low fat and cholesterol contents, together with a good protein content. Although in many western countries donkey breeding is often only a complementary activity, it has great potential for development, considering the increased requirement of alternative red meats. The quality parameters of meat obtained from donkey foals slaughtered at 10 months of age were very interesting, considering the low fat and cholesterol content, the good protein content and the low collagen concentration in these young animals. The fatty acid composition in donkey foals showed an important level of unsaturation of the intramuscular fat, giving a valid dietetic value to donkey meat, as indicated also by the ratio between total SFA and total UFA. The results obtained in the present study can represent a starting point for the promotion of donkey meat, that can be considered a suitable meat source for those seeking an alternative to beef.

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PAPER

APPLICATION OF PULSED ELECTRIC FIELD FOR ENRICHMENT OF *SACCHAROMYCES CEREVISIAE* CELLS WITH CALCIUM IONS

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ABSTRACT

Cultures of *Saccharomyces cerevisiae* were treated with PEF to improve accumulation of calcium in the biomass. Under optimized conditions, this is, on 20 min exposure of the 20 h grown culture to PEF of the 5.0 kV/cm and 20 μs pulse width, accumulation of calcium in the yeast biomass reached maximum 2.98 mg/g d.m. It constituted 30% of the total calcium in the medium. That accumulation is significantly correlated against calcium concentration in the medium. Neither multiple exposure of the cultures to PEF nor intermittent supplementation of the cultures with calcium increased the calcium accumulation. The intermittent supplementation of the cultures with calcium reduces the accumulation efficiency by 57%.

- Keywords: biomass, calcium, pulsed electric fields, *Saccharomyces cerevisiae* -

INTRODUCTION

Yeast demand for calcium ions is the matter of the numerous scientific reports. It has been stated, among others, that calcium has the influence on specific enzymatic reactions, facilitates synthesis of cell's material and prevents its leakage, and allows to regulate pH of wort. Because of its high ability to connect with carboxyl groups, calcium stabilizes cell walls and some of the intracellular enzymes e.g. α-amylase, endopeptidase and nitrogenase (STRATFORD, 1989; WALKER and MAYNARD, 1996; WALKER *et al.*, 1996). Calcium plays an important role in regulation of the nitrogen metabolism in mitochondria and activates albuminous components responsible for yeast ability to flocculate (MOCHABA *et al.*, 1996a; REES and STEWART, 1997). In the human organism, it determines first of all correct conduction of neural impulses, and liberation, activation or action of enzymes and hormones. It also participates in muscle contraction, including cardiac muscle, and its ions are essential for blood coagulation and intracellular transfer of signals. Moreover, calcium is indispensable for detoxification, mainly of heavy metals. Thanks to reduction of cell membrane permeability, calcium is considered to play an important role in alleviating allergy (PASTERNAKIEWICZ and TUSZYŃSKI, 1997; REES and STEWART, 1997). This element is provided mainly by the food, but only 20-30% calcium included in diet can be effectively assimilated and utilized by human organism. On the one hand it is caused by the chemical character of calcium – because of its high reactivity calcium does not exist in the ionized form but in complexes with other compounds which reduces its availability in the alimentary canal. Moreover, nutritional habits may decrease it. Carbonated beverages, coffee, sugars and carbohydrates, phytates and fats lower calcium assimilation.

For that reason, supplementation of the diet with calcium could become an optional procedure. Yeast biomass appeared to be a suitable source of deficient elements and vitamins.

Organisms better tolerate and assimilate bioelements in the form of metaloproteins than as components of pharmaceuticals. In the future, foodstuffs containing either magnesium enriched yeast or yeast preparations containing calcium biocomplexes could become additional sources of that element (BŁAŻEJAK *et al.*, 2004; GNIEWOSZ *et al.*, 2006).

Yeasts are known for their ability to accumulate metal ions from aqueous solutions by different physico-chemical interactions, e.g. by adsorption and absorption, or by a metabolism-dependent mechanism (GADD, 1990; BRADY and DUNCAN, 1994). Sorption processes are dependent on disposable functional groups on the cell surface and on the nature of metal ions. Thus, the concentration of free ions, ligand electronegativity, metal cation, ligand charge and the cavity size have a great influence on the selectivity of metal uptake (FRAUSTO DA SILVA and WILLIAMS, 1976). Furthermore, the composition of nutritive medium to which the microorganism is exposed affects the amount of metal uptake, because of the cell wall structure and the metabolic state of the cell (NORRIS and KELLY, 1977; GADD, 1990; STEHLIK-TOMAS *et al.*, 2004).

Pulsed electric field electro-technology (PEF) is an emerging technology in the field of food preservation. PEF has the potential to pasteurize several foods non-thermally via exposure to high-voltage short pulses while the material is between the electrodes of a treatment chamber. The electric field affects the cell membranes (BARSOTTI and CHEFTEL, 1999), and may cause irreversible membrane breakage (ZIMMERMANN, 1986; POTHAKAMURY *et al.*, 1997; CALDERON-MIRANDA *et al.*, 1999), alternation in transport of ions (KIM *et al.*, 2001) and changes in enzyme structure (VEGA-MERCADO *et al.*, 1995; FERNANDEZ-DIAZ *et al.*, 2000). In a cell subjected to PEF induced trans-membrane tension facilitates the formation of pores in the membrane and leads to an increase in its permeability (HÜLSHEGER *et al.*, 1983; ZHANG *et al.*, 1994a; EVRENDILEK *et al.*, 1999; WOUTERS *et al.*, 1999; ARONSSON *et al.*, 2005). Electroporation temporarily eliminates integrity of the plasmatic membrane, i.e. produces small pores which close back with a time (BARBOSA-CANOVAS *et al.*, 1999; ARONSSON and RÖNNER, 2001; ARONSSON *et al.*, 2005).

The aim of this paper was to test whether PEF is a suitable technique for the calcium enrichment of *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Culture maintenance and inoculum preparation

Saccharomyces cerevisiae 11 B_1 (industrial strain) from the Yeast Plant (Lublin, Poland) was used. Medium for agar slants and inoculum growth (g/L): sucrose (20); NH_4Cl (3.2); KH_2PO_4 (2.5); Na_2SO_4 (2.0); $MgCl_2$ $6H_2O$ (1.5) (POCH, Gliwice, Poland); yeast extract (YE) (5.0); agar (15) (DIFCO, Detroit, MI, USA); and unhoped wort (40.0 mL) (Lublin Breweries S.A., Lublin, Poland) had pH 5. Experimental medium for *S. cerevisiae* contained (g/L): peptone (10) (Sigma-Aldrich CO, St. Louis, MI, USA); YE (5); glucose (10) (POCH, Gliwice, Poland) (BLACKWELL *et al.*, 1997).

Biomass cultivation

Yeast was passaged three times for agar slants, grown for 48 h in a thermostat at 30°C and finally an inoculum was prepared. Cells from a single slant were used to inoculate 150 mL sterile medium in an Erlenmeyer flask. Cul-

tures were grown on a rotary shaker with water bath, at amplitude 4 and at 220 rpm for 48 h at 30°C. The fermented medium was centrifuged, washed three times with sterile water, and the remaining washed biomass of cells from three Erlenmeyer flasks was diluted with sterile water to a final volume of 300 mL. The inoculum was made of 48 h yeast cultures with OD_{400} of 2.2 (Spekol 11, Carl Zeiss, Jena, Germany). Biomass inoculum with washing was 12.2 g d.m./L. The inoculum prepared in this manner was used for inoculation of submerged grown in 500 mL Erlenmeyer flasks, each containing 100 mL medium inoculated with 10 mL inoculum. Calcium at specified concentration was added to the medium before starting the culture. After 20 hours (in the phase of logarithmic growth) the culture was stopped, treated with PEF and then continued for the next 22 hours. The culturing conditions were identical to those applied for the inoculum preparation. After 42 h culturing, the mycelium was centrifuged, washed several times with distilled water, and then lyophilised in a Labconco freeze dryer (Model 64132, Kansas City, MO, USA).

PEF treatment and enrichment with calcium

S. cerevisiae cultures grown in flasks were agitated for 20 h then treated with PEF for 15 min (electroporator ECM 830, BTX Harvard Apparatus, USA) at the field frequency of 1 Hz and 20 μs pulse width. PEF treatment chamber consists of four parallel plexiglas plates which have stainless steel electrodes of area equal 4 cm^2, facing each other with a gap of 5 mm. The culture was agitated in a chamber during PEF treatment with a magnetic stirrer. The electrical conductivity measured for the treated samples was between 4.2 mS/cm - 8.22 mS/cm. Simultaneously, voltage was optimized on the field exposition of 50, 100, 150, 200, 250, 500, 1000, 1500, 2000, 2500 and 3000 V; respectively 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 kV/cm.

At optimal electric field strength, fixed earlier, pulse width was optimized. The PEF pulse width at 10, 20, 50, 75, 100, 125 and 150 μs was selected at 5 kV/cm and the 15 min exposition, after 20 h of culturing, at the field frequency of 1 Hz.

Cells biomass from 42-hour culture carried out at Ca^{++} concentration of 100 μg/mL was treated with PEF after 20 hours of culturing at optimum electric field strength of 5 kV/cm, optimum pulse width of 20 μs and the field frequency of 1 Hz. These conditions were then used to optimize the field exposure time for 5, 10, 15, 20 and 25 min.

Concentration of calcium in the nutrient broth was estimated for PEF treated cells cultured at optimum field parameters (electric field strength 5.0 kV/cm, pulse width 20 μs, and optimum time of PEF exposition (20 min) after 20 h of culturing. Subsequent culturing of *S. cerevisiae* were carried out in the medium containing 10, 100, 200, 400, 500, 750 and 1,000 μg/mL calcium. Optimization of the time interval after which yeast cells were 20 min. treated with PEF was performed after 8, 12, 16, 20 and 24 h culturing at optimum 5 kV/cm and 20 μs pulse width, at the fixed optimal concentration 100 μg/mL calcium.

At optimum PEF parameters and 100 μg/mL calcium concentration in the medium was additionally enriched with subsequent 25 μg/mL doses of calcium after 8, 12, 16 and 20 h culturing. Subsequent culturing was performed under optimized conditions and these cultures were PEF treated several times after 8, 12, 16 and 20 h. The results allowed to check the role of enrichment with calcium and number of expositions to PEF upon the calcium accumulation in the yeast cells. Samples not treated with PEF and without calcium in the medium (K1) or with 100 μg Ca^{2+}/mL (K2) served as controls.

Determination of the calcium concentration

Mineralization of yeast for the determination of the calcium concentration using the technique of flame atomic absorption spectrophotometry (F-AAS, VARIAN AA 280 FS) was conducted as follows: 250 mg samples of lyophilized calcium-enriched yeast were weighed into glass thimbles, 3 mL HNO_3 was added. Determination by the F-AAS method was carried out according to the Norm PN-EN 14082:2004. Standard curve has been plotted at following Ca^{++} concentrations: 5, 10, 20, 40, 60, 80, 100 mg/L and determination coefficient was equal 1. Samples were digested in a MARS5 (CEM Corporation) microwave oven at 200°C for 30 minutes. After cooling, solutions were transferred to 50 mL measuring flasks and topped up with deionised water.

Determination of the yeast cell viability

Cell viability was determined in the Thoma chamber, dyeing necrotic yeast cells with the 0.01% methylene blue solution. The percentage of necrotic cells was the mean of 16 fields calculated according to the formula: % necrotic cells = (number of necrotic cells/sum of necrotic and living cells) x100%.

Determination of the crop of yeast biomass

Biomass was estimated from optical density at 400 nm. Dry mass was calculated by referring a standard curve of cell mass versus absorbance. The fermented medium from culturing (2 mL) was centrifuged (3,000 rpm), supernatant was discarded, cells were rinsed with deionized water and brought to the original volume of 2 mL. Nephelometric measurements were run against

pure water in 2 mm measurement cell. Amount of dry residue was calculated using equation for the standard curve: $A_p = 0.4476c$, where A_p and c were apparent absorbance and concentration (mg/mL), respectively.

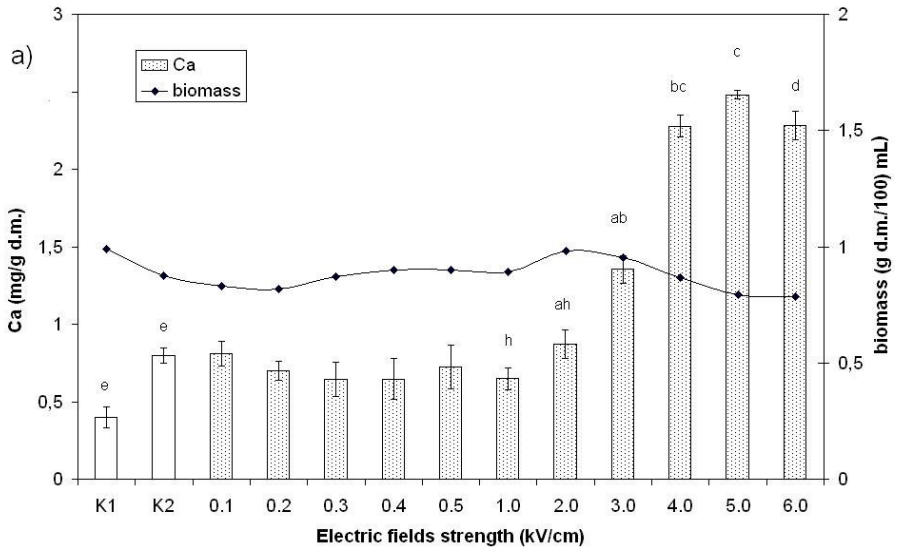
Data analysis

Significant differences between particular groups were found involving the Student t-test applied to compare independent samples in pairs, and variance analysis (ANOVA) was used for more than two groups. Statistical processing of results was performed using Statistica 6.0 software.

RESULTS AND DISCUSSION

As expected, low electric field strength ~~in~~ within the range of 0.1 - 1 kV/cm did not influence the calcium accumulation in the yeast cells maintaining it on the level of 0.7 mg/g d.m. which is not significantly different to control sample K2 (Fig. 1a). In the control culture K1 calcium accumulation was 0.4 mg/g d.m. Statistically essential changes in accumulation appeared just when electric field strength of above 1 kV/cm was applied and that of 5 kV/cm provided maximum accumulation reaching 2.5 mg/g d.m., that is, threefold as much as in the K2 sample. The electric field strength exceeding 5 kV/cm reduced the calcium accumulation. Voltage applied had an essential influence on the cell vitality (Fig. 1b). An elevation of electric field strength in the range from 1.0 to 6.0 kV/cm resulted in a gradual increase in the count of necrotic cells. At optimum 5 kV/cm there were 17% necrotic cells in the culture, and at 6.0 kV/cm the count of necrotic cells was by three times as high as at 1.0 kV/cm.

Treatment time is defined as the product of the number pulses and the pulse duration. An increase in any of these variables increas-



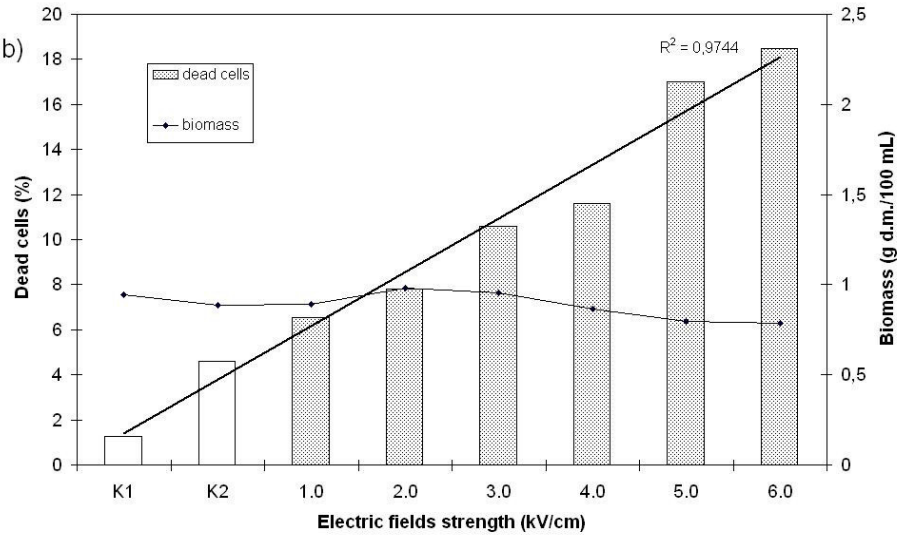


Fig. 1 - Effect of electric field strength PEF treatment upon: a) calcium accumulation in *S. cerevisiae*, b) the vitality of *S. cerevisiae* cells (electric field strength between 0.1-6 kV/cm, pulse width 20 μs, at the field frequency of 1 Hz, the time of exposure to PEF 15 min. after 20 h culturing, calcium concentration in the culture medium 100 μg/mL). Means with the same letters are highly significantly different ($P<0.01$; n=6).

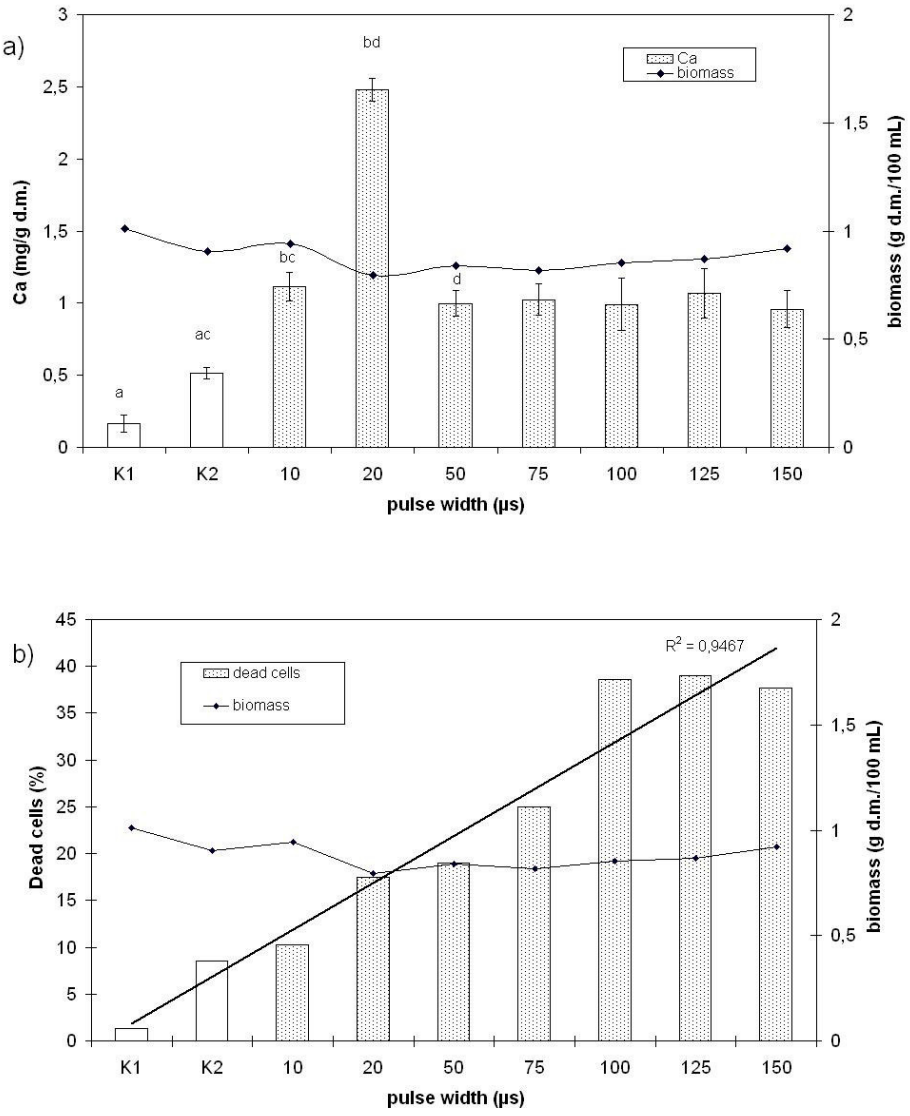


Fig. 2 - Effect of the PEF pulse width upon: a) accumulation of calcium in *S. cerevisiae*, b) the vitality of the *S. cerevisiae* cells (pulse width between 10-150 µs, electric field strength 5 kV/cm, at the field frequency of 1 Hz, the time of exposure to PEF 15 min after 20 h culturing, calcium concentration in the culture medium 100 µg/mL). Means with the same letters are highly significantly different (P<0.01; n=6).

es microbial inactivation (SALE and HAMILTON, 1967). The critical electric field Ec (electric field intensity below which inactivation does not occur) increases with the transmembrane potential of the cell. Transmembrane potentials, and consequently Ec, are larger for larger cells (JEYAMKONDAN *et al.*, 1999). Pulse width also influences the critical electric field; for instance, with pulse widths greater than 50 µs, Ec is 4.9 kV/cm. With pulse widths less than 2 µs, Ec is 40 kV/cm (SCHOENBACH *et al.*, 1997). As noted above, pulse width influences microbial reduction by affecting Ec. Longer widths decrease Ec, which results in higher inactivation. We suppose that yeast cells were in logarithmic phase of growth which is characterized by a high proportion of cells undergoing division, during which the cell membrane is more susceptible to the applied PEF. In these conditions permeabilization is reversible and the membrane can return to its initial state.

The pulse width also appeared to be an essential factor. The 20 µs pulse width provided superior calcium accumulation of 2.5 mg/g d.m. (Fig. 2a). This value was by 100% higher then that acquired with the 10 µs pulse width. The pulse width in the range from 50 to 150 µs affected the statistically significant decrease of calcium accumulation in the cells. It was on the invariable level of calcium 1 mg/g d.m. and was 2.5-fold lower from the accumulation at the optimal pulse width of 20 µs (Fig. 2a). The pulse width affected also the cell vitality (Fig. 2b). The 10 µs pulse width did not practically affect their vitality which was the same as in the control K2 sample not treated with PEF. At optimum calcium concentration in yeast (2.5 mg/g d.m.) and the 20 µs pulse width, the culture contained 17% necrotic cells. Increase in the pulse width to 100 µs doubled amount of necrotic cells. Further increase in the pulse width up to 150 µs had no statistically significant effect on the cell vitality, which was on the level of 37% necrotic cells. HÜLSHEGER *et al.* (1983) reported over 99.99% inactivation of microorganisms exposed to peak electric fields of 20 kV/cm and pulses of several milliseconds.

Prolonged PEF treatment increased the calci-

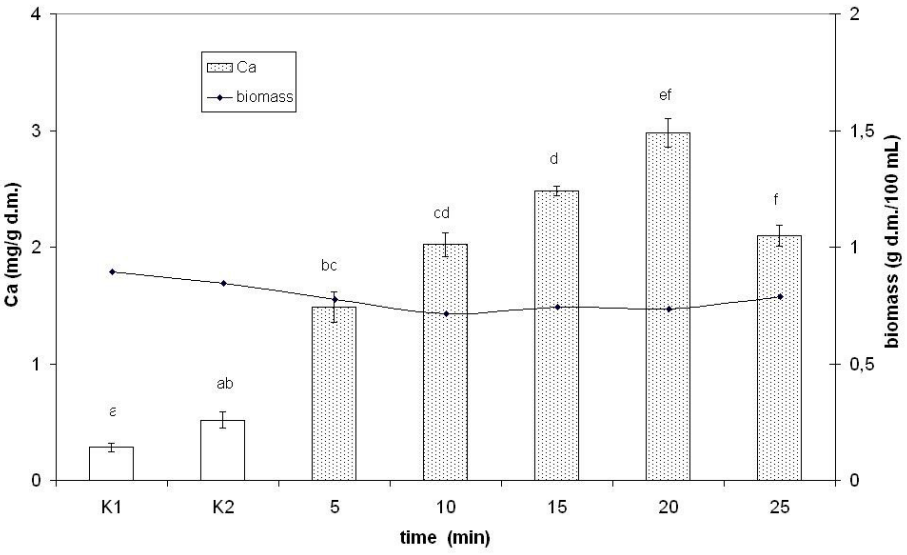


Fig. 3 - Accumulation of calcium in the cell biomass dependent on the treatment time of exposure to PEF (the time of exposure to PEF between 5-25 min after 20 h culturing, pulse width 20 μs, electric field strength 5 kV/cm, at the field frequency of 1 Hz, calcium concentration in the culture medium 100 μg/mL). Means with the same letters are highly significantly different (P<0.01; n=6).

um content in the cell biomass (Fig. 3). The 20 min. exposure provided the highest accumulation of calcium reaching 3 mg/g d.m. i.e. by 100 and 20% higher concentration in respect to 5 and 15 min. exposure, respectively. Extending of the PEF exposition time from 20 to 25 minutes caused the 32% decrease of the calcium accumulation. An increase of calcium concentration in the cells may be a result of larger pore formations during culture after PEF exposure. After 25 min of PEF exposure the integrity of cell's membrane is probably destroyed irreversibly. It might lead to high leakage of calcium ions outside the cell and this is the reason why the concentration of this element in the cells was lower.

Control culture K1 without calcium supplementation and PEF treatment accumulated calcium on the level of 0.16 mg/g d.m. The supplementation with calcium without the PEF treatment (control K2) provided an increase in accumulated calcium up to 0.5 mg/g d.m.

Accumulation of calcium in the cells increased with the increase of the calcium concentration in the nutrient medium and that increase was not proportional (Fig. 4a). Intensity of uptake and level of ions biosorption by yeast depend on their concentration in medium and bioavailability. Yeast cells can bind some of the metal cations forming stable bonds. In the majority of microorganisms, absorption of metals takes place through their passive accumulation of the surface, binding in the cell wall (carboxyl, hydroxyl, amine, hydrogen sulphate, phosphate groups), and then in the next phase, active transport of the ion through the cell membrane to the cytoplasm (KAPOOR and VIRARAGHAVAN, 1995). According to BRADY and DUNCAN (1994), cell wall carboxyl groups of peptide glucans are the main sites for binding of divalent metals. The composition of nutritive medium to which the microorganism is exposed affects the amount of metal uptake (STEHLIK-TOMAS and GULAN, 2004), because of the cell wall structure and the metabolic state of the cell. At the optimum 100 μg/ml calcium concentration in the medium the accumulation was six fold higher when compared to that in the control sample. It reached 3 mg Ca^{2+}/g d.m., that is, about 30% additional calcium was accumulated. At 1,000 μg Ca^{2+}/mL medium cells accumulated only 4.4 mg/g d.m. calcium i.e. only 4.4% supplemented calcium. Increase in the calcium concentration in the medium from 200 to 750 μg Ca^{2+}/mL resulted in the 26% increase in the accumulation of that element. However, such increase in the level of calcium in the medium without PEF treatment had only a slight effect upon the accumulation. It was 0.36 and 0.9 mg/g d.m. for the 10 and 1,000 μg Ca^{2+}/mL medium, respectively. On the basis of the results it can be stated that calcium concentration in the medium did not significantly influence yeast biomass production. Although the differences were not statistically significant one should notice (Fig. 4a) that the lowest biomass with washing (7.8 g d.m./L) was available at the 100 and 750 μg/mL calcium concentration in the medium whereas 8.5 g d.m./L biomass was obtained at the concentration of 10 μg/mL. In the cultures enriched with Mg Cl_2 $6H_2O$ (1.25 g Mg^{2+} /L) after 48 h BŁAŻEJAK *et al.* (2002) collected 5.77 g d.m./L biomass with washing. PANKIEWICZ and JAMROZ (2011) reported a significant decrease of biomass, from 0.35 to 0.15 g d.m/100 mL, for Zn concentration range from 200 to 1,000 μg/mL.

It was revealed that the number of necrotic cells increased together with the increase of calcium concentration in the culture medium. At the optimum concentration of 100 μg Ca^{2+}/mL medium the contribution of necrotic cells was equal 17%. At the concentration of 1,000 μg Ca^{2+}/mL medium it was observed that the number of nectrotic cells doubled (Fig. 4b). The biomass growth in the whole range of applied concentrations reached

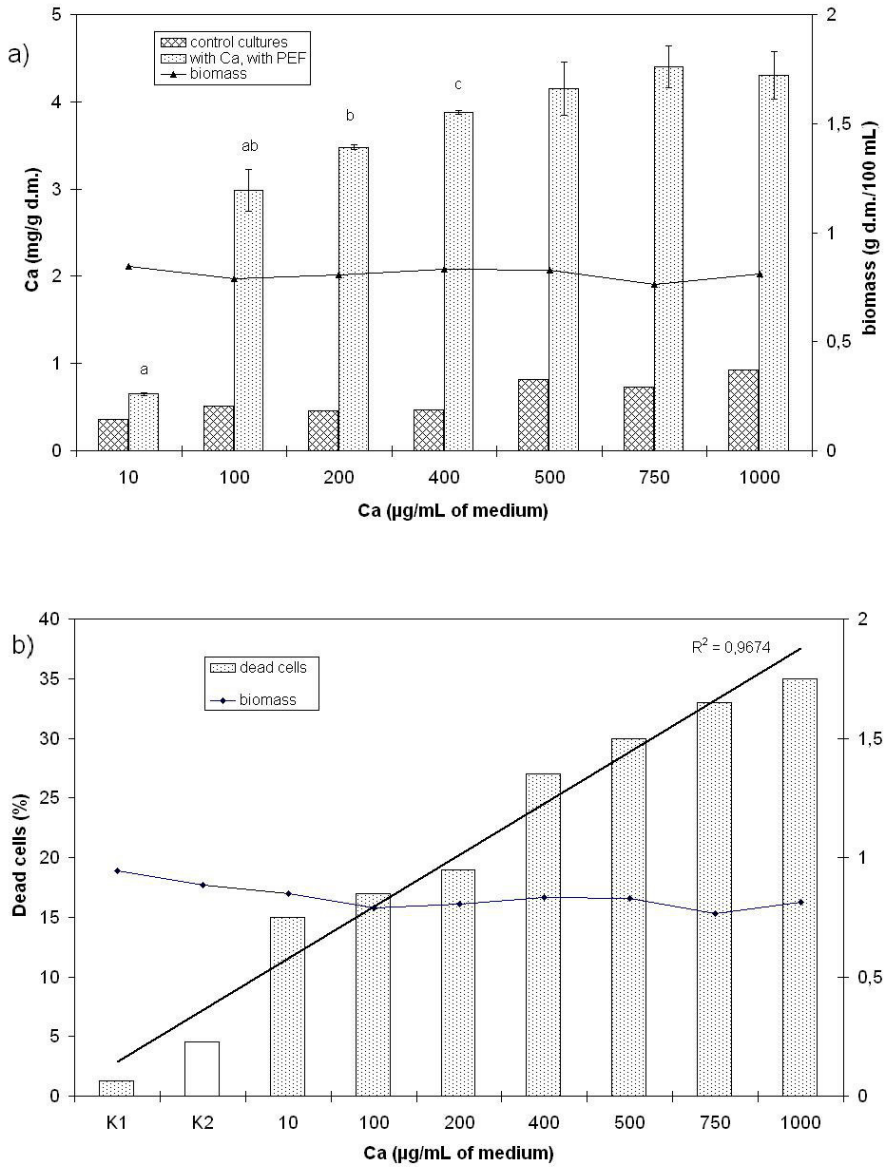


Fig. 4 - Effect of the calcium concentration in the medium upon: a) calcium accumulation in *S. cerevisiae*, b) the vitality of the *S. cerevisiae* cells (pulse width 20 µs, electric field strength 5 kV/cm, at the field frequency of 1 Hz, the time of exposure to PEF 20 min after 20 h culturing, calcium concentration in the culture medium 100 µg/mL). Control cultures: no PEF, supplemented with Ca^{2+}. Means with the same letters are highly significantly different ($P<0.01$; $n=6$).

from 7.6 to 8.4 g d.m./L biomass with washing, respectively, for 750 and 10 µg Ca^{2+}/mL medium. BONIN and ŚLUSARSKA (2007) obtained the highest increase of biomass (9.2 g d.m./L) for the sample enriched with 40 mg Ca^{2+}/L after 72 hours of culturing *Saccharomyces bayanus* yeasts, and the lowest (7.7 g d.m./L) – for the culture supplemented with 400 mg Ca^{2+}/L.

Duration of the culturing after the PEF treatment had no effect upon the calcium accumulation (Fig. 5). Under applied culturing conditions the maximum calcium concentration in the cells reached 3 mg/g d.m after 20 h which was by 150 and 8% higher than that after 8 and 24 h culturing, respectively.

LOTAN *et al.* (1976) demonstrated that cells of *Saccharomyces carlsbergensis* accumulated relatively low amounts of calcium isotope. Other investigations (MOCHABA *et al.*, 1996; MOCHABA *et al.*, 1996a) demonstrated the ability of yeast cell walls to bind Ca^{2+} in the amount of 2.5 to 7.5 mol per 100 g of dry matter. The role of calcium ions in the microorganism growth has been widely discussed (JONES and GREENFIELD, 1986; WILLIAMS, 1986; YOUATT, 1993; WALKER and MAYNARD, 1996). The role of this ion, especially in the first stages of the microorganism growth, their transport to the cells, their binding to the cell components and their toxicity was dependent, among others, on pH of the environment. PASTERNAKIEWICZ and TUSZYŃSKI (1997) experiments confirmed the beneficial effect of calcium ions on yeast growth. However, the studied strains differed in their sensitivities to these ions. For instance, the *Mautner* strain growth was reduced by the calcium concentrations higher than 20 mmol/L after 16 h cultivation whereas the YT strains did not negatively respond even to 50 mmol/L concentrations within the whole culturing period. PANKIEWICZ

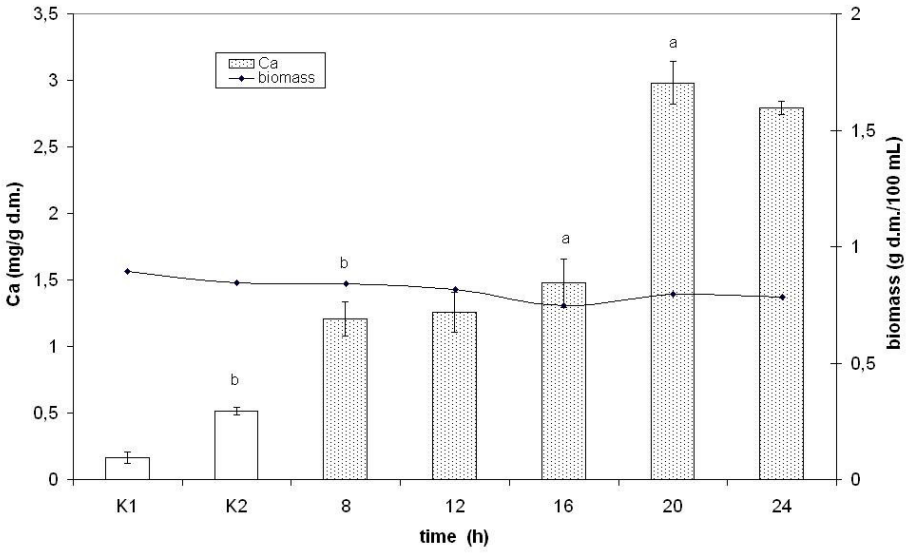


Fig. 5 - Effect of the after PEF treatment culturing duration upon the calcium concentration in *S. cerevisiae* cells (pulse width 20 μs, electric field strength 5 kV/cm, at the field frequency of 1 Hz, the time of exposure to PEF 20 min after 8, 12, 16, 20 or 24 h culturing, calcium concentration in the culture medium 100 μg/mL). Means with the same letters are highly significantly different (P<0.01; n=6).

and JAMROZ (2010) obtained under optimized conditions, this is, on 15 min exposure of the 20 h grown culture to PEF of the 4.0 kV/cm and 20 μs pulse width, accumulation of magnesium in the *S. cerevisiae* biomass reached maximum 3.98 mg/g d.m. It constituted 40% of the total magnesium in the medium. PANKIEWICZ and JAMROZ (2011) investigated the influence of PEF on accumulation of zinc in *S. cerevisiae*. Under optimized conditions, that is: 15 min exposure of the 20 h grown culture to PEF at 3.0 kV/cm and pulse width of 10 μs, accumulation of zinc in the *S. cerevisiae* biomass reached maximum 15.5 mg/g d.m. CHA and CHO (2009) observed the highest accumulation of zinc in a medium containing 2.0% (w/v) glucose, 5.0% yeast extract, and 0.05% $ZnSO_4$ after cultivation at 110 rpm, 30°C for 72 h. Under these conditions, the 31,932 ppm zinc represented a 93-fold increase over the 339 ppm in the basal medium.

This study revealed that multiple exposition of the yeast culture to PEF did not increase the accumulation in the yeast cells. Four subsequent 20 min exposures to PEF of 8, 12, 16 and 20 h lasting culturing provided accumulation of 1.32.±0,13 mg/g d.m calcium which was only 125% lower than that after single PEF treatment (2.98 mg/g d.m.). Supplementation of total calcium in four subsequent doses to cultures lasting 8, 12, 16 and 20 h had no effect upon the accumulation. A portionwise supplementation provided accumulation of 1.89±0.13 mg/g d.m. of calcium in the cells. Thus, it was by 57% lower than that after the single addition of calcium at the beginning of the culturing.

CONCLUSIONS

Optimized electric field strength (5 kV/cm), exposure time (20 min), the pulse width (20 μs) and the point of treatment in course of the growth of cultures (20 h) provide the highest level of the calcium accumulation in *Saccharomyces cerevisiae*. The calcium accumulation is correlated with its concentration in the medium. At 100 μg Ca/mL 30% accumulation was achieved.

ACKNOWLEDGMENTS

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PAPER

EFFECT OF PRE-TREATMENT CONDITIONS ON STRUCTURE AND MECHANICAL PROPERTIES OF FREEZE-DRIED PUMPKIN

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ABSTRACT

In this work the effect of blanching, osmotic dehydration, freezing method and time of frozen storage on the structure and mechanical properties of freeze-dried pumpkin was shown.

Osmotic dehydration limited shrinkage and porosity of freeze-dried material, made the structure stronger and caused an increase in hardness and the value of compression work, but the time of osmotic pre-treatment did not have significant influence. Blanching caused a statistically insignificant increase in hardness and compression work and a decrease in shrinkage. Quick freezing caused an insignificant increase in shrinkage and porosity. Frozen storage time did not have an influence on the mechanical and physical properties of freeze-dried pumpkin.

- Keywords: pre-treatment, freeze-drying, mechanical properties, porosity, pumpkin, shrinkage -

INTRODUCTION

Pumpkin is one of many vegetables used as functional food, and also as a raw material to produce medicines because it contains many valuable phytocomponents. Many studies concerning the health-promoting properties of pumpkin and its products have been conducted in the last few years (CAILI *et al.*, 2006; ADAMS *et al.*, 2011). The short consumption suitability period of pumpkin in its raw state makes it advisable to conduct research on the effects of processes prolonging the life of the product, such as freezing or drying.

Freeze-drying is one of the most modern drying methods. The essence of freeze-drying is direct water evaporation from frozen material through sublimation under low pressure (CIURZYN'SKA and LENART, 2010a). The freezing stage, being the first stage, is critical to the overall success of the freeze-drying process (STAPLEY, 2008) because it sets the structure of the ice crystals (shape and size) which affect the heat and mass transfer rates and, therefore, the subsequent sublimation (drying) stages (CEBALLOS *et al.*, 2012).

Freezing can preserve the taste, texture and nutritional value of foods better than most other preservation methods. However, such qualities depend upon the careful choice of food materials, use of appropriate pre-treatments, the choice of freezer and frozen storage options and the use of packaging (GEORGE, 2000). When ice crystals form, a uniform network is created throughout the product that after sublimation yields a dense porous matrix (CEBALLOS *et al.*, 2012).

The crystallization of water to form pure ice crystals is a two-step process, requiring crystals first to nucleate and then grow. Generally, rapid freezing results in better quality frozen products when compared with slow freezing. If freezing is instantaneous, there will be more locations within the food where crystallization begins. In contrast, if freezing is slow, the crystal growth will be slower with few nucleation sites, resulting in larger ice crystals. Large ice crystals are known to cause mechanical damage to cell walls in addition to cell dehydration. Thus, the rate of freezing for plant tissues is extremely important, due to the effect of the freezing rate on the size of ice crystals, cell hydration, and damage to cell walls (OETJEN, 1999; RAHMAN, 1999).

Following the freezing operation, frozen products are maintained in frozen storage, which is also an important step in the quality retention of frozen food products. During frozen storage, the amount of ice in the system generally remains constant for any given temperature, where the number of ice crystals decreases due to an increase in average crystal size (BLANSHARD and FRANKS, 1987). Fluctuations in storage temperature and temperature gradient within the product cause moisture migration, relocation of the water within the product and undesirable recrystallization which may affect texture and general quality. Therefore, it is important to maintain and monitor the efficiency of frozen storage for retention of high quality within the frozen products (ZARITZKY, 2000; BARBOSA-CÁNOVAS *et al.*, 2005).

Some endogenous enzymes are responsible for undesirable changes, such as bad flavours and odours and colour and nutritive alterations during frozen storage. Blanching is a thermal treatment designed to inactivate a target enzyme to a given extent. Practically every vegetable (except herbs and green peppers) needs to be blanched and promptly cooled prior to freezing since heating slows or stops the enzyme action which causes vegetables to grow and mature (BARBOSA-CÁNOVAS *et al.*, 2005). For this purpose, blanching of food material was used as a pre-treatment operation before osmotic dehydration (AMAMI *et al.*, 2006).

Research done in recent years has proved that application of osmotic dehydration to fruit and vegetable pre-treatment yields very good results prior to freezing, since the treatment has the effect of excluding oxygen from the fruit, which helps to retain colour and appearance. The influence of some sugars on changes in the structure of osmotically dehydrated plant material can be determined on the basis of texture analysis, particularly tissue damage and volume change (BARAT *et al.*, 1998; KOWALSKA and LENART, 2001). Osmotic dehydration also causes deformation and porosity of the cellular surface, which influence the shape and volume of intercellular spaces. As a consequence, the structure of osmotically dehydrated apple tissue is less regular than that of fresh fruit (LEWICKI and PORZECKA-PAWLAK, 2005).

Among the most important physical properties of food are mechanical properties significant to consumers' perception of the quality of food products. The measurement of mechanical properties can also be used to characterise food texture, which is probably the most important sensory property of fruit and vegetables. Structure changes directly influence food texture changes, therefore it is important to control the change of mechanical properties influenced by processes which cause destruction, preservation or creation of a new food structure.

It was shown that texture is related to hardness to the greatest extent, in contrast to other discriminants, correlation of which depends on such factors as the type of product (SURMACKA-SZCZEŚNIAK, 2002; RAO and QUINTERO, 2005).

Freeze-dried vegetables are characterised by low mechanical resistance and elasticity in comparison to vegetables dried by other methods (STĘPIEŃ, 2009), e.g. freeze-dried parsley has two times less resistance to cutting in comparison to raw material. The use of pre-treatment before freeze-drying can significantly influence the structure and mechanical properties of the obtained dried materials, e.g. blanching and

osmotic dehydration significantly change mechanical properties of parsley, but osmotic dehydration has a greater effect on changes in values of compression work and cutting of parsley (STĘPIEŃ and MICHALSKI, 2006). Mechanical properties are modified by the presence of osmoactive substance, i.e. sugars. However, mechanical properties of plant tissue dehydrated by osmosis and subsequently dried by convection have not been the subject of thorough studies (LEWICKI and ŁUKASZUK, 2000).

The aim of this work was to show the influence of different pre-treatment methods and their conditions on the structure or mechanical properties of freeze-dried pumpkin. The range of this work included analysis of the effect of blanching, time of osmotic dehydration, freezing conditions and time of frozen storage of pumpkin on shrinkage, porosity and mechanical properties of freeze-dried pumpkin after pre-treatment.

The raw material included fresh pumpkin from the cultivar belonging to Cucurbita Maxima, bought in Warsaw, harvested in a state of harvest maturity and cut into cubes of a 10 mm side. Table 1 presents the changes in the parameters of the processes conducted in order to obtain the freeze-dried pumpkin:

- Sample 1 was not subjected to any pre-treatment.
- Samples 4, 6, 8 and 10 were subjected to blanching in distilled water at a temperature of 100°C for 5 minutes, with a raw material/water mass ratio of 1:10. The duration of the process was 1 and 8 minutes.
- Samples 2-10 were osmotically dehydrated in a 66.3% starch syrup solution at a temperature of 20°C for 3 or 20 hours, with a raw material/water mass ratio of 1:4 in static conditions (PĘKOSŁAWSKA and LENART, 2008).

Blanched and not blanched material was frozen at a temperature of -18°C for 24 hours in a Gorenje freezer and at a temperature of -70°C for 2 hours in a NationalLab ProfiMaster freezer. Frozen material was stored at a temperature of -18°C for 72 hours or 60 days in a Gorenje freezer before freeze-drying.

The freeze-drying process was conducted under pressure 63 Pa and safety pressure 103 Pa on heating shelves at a temperature of 10°C for 24 hours, in a Christ Company ALPHA1-4 LDC-1m freeze-dryer. After freeze-drying, the material was stored in jars in a dark place at a temperature of 20°-25°C.

Shrinkage was measured with a volumetric method (using toluene displacement) for pumpkin cubes before and after freeze-drying in three iterations (MAZZA, 1983). Initially, eight pumpkin cubes, weighed on analytical scales, were placed in a graduated cylinder. Then toluene was poured onto the samples from a burette of the same volume as the cylinder until the lower meniscus of liquid reached a value equal to the burette volume. The volume of pumpkin cubes was calculated as the difference between the nominal cylinder volume and the volume of the toluene residue in the burette.

The shrinkage was calculated on the basis of dependency:

$$S = \left(1 - \frac{V_o}{V_1}\right) \times 100\% \qquad [1]$$

where:

S - shrinkage [%];
V_1 - volume of cubes after freeze-drying [cm^3];
V_0 - volume of cubes before freeze-drying [cm^3].

The cubes of freeze-dried pumpkin used for shrinkage determination were previously subjected to coating with pectin. The solution of 2% low-methylated pectin was used. In addition, a 2% calcium chloride solution was prepared. Pumpkin cubes, using laboratory tweezers, were plunged consecutively for one second, first in a beaker with a 2% pectin solution, and then in a 2% calcium chloride solution. The cubes were left for 24 hours at a room temperature of 20°-25°C for drying. The determination of shrinkage was performed the next day.

Porosity of the raw material was measured with a Quantachrome helium pycnometer according to the producer's instruction. The research material of a known mass and unknown volume was introduced to a large measurement cell of a known volume. Next, it was placed in an apparatus. Helium flowing by the samples filled all the interspaces and pores between the cubes, which enabled the measurement of the volume of the studied sample. Each sample was rinsed three times with gas. The helium pycnometer determined the value of pressure. After introduction of the obtained values to the Pycnometer software version 2.7, the apparent density of the investigated pumpkin samples was determined.

Volume of samples

$$V_p = \frac{V_c + V_a}{1 - \frac{P_1}{P_2}} \qquad [2]$$

where:

V_p - volume of sample [cm^3];
V_c - volume of cell [cm^3];
V_a - references volume [cm^3];
P_1, P_2 - pressure, which was read from the measurement equipment [Pa].

Apparent density

$$\rho_S = \frac{m}{V_p} \qquad [3]$$

where:

ρ_s - apparent density of particles [g/cm^3];
m - particle mass [g].

Porosity

$$\varepsilon = \left(1 - \frac{\rho_b}{\rho_S}\right) \times 100\% \qquad [4]$$

where:

ε - porosity [%]

ρ_b - density of particles [g/m^3]

The mathematical and statistical analyses involved the use of StatGraphics Plus (Manugistics), with the use of variance analysis in the Tukey test. The residual parameters were determined with the use of MS Excel 2003. Corresponding standard deviations were calculated for the averaged results obtained. In the course of analysis, the Tukey test for verification of the hypothesis concerning the equality of means for the analysed coefficients in the scrutinised samples was employed.

RESULTS AND DISCUSSION

The influence of osmotic dehydration time

It was shown that osmotic dehydration, as a pre-treatment before freeze-drying (samples 2 and 3) has an effect on a statistically significant decrease in dried pumpkin shrinkage in comparison to samples without osmotic pre-treatment (sample 1) (Fig. 1). Similar dependency was obtained by CIURZYŃSKA and LENART (2010b) who investigated the effect of osmotic dehydration in different sugar solutions on shrinkage of freeze-dried strawberries, and also by PARK *et al.* (2002) for conventionally dried pears. Also, LEWICKI and LENART (2007) obtained similar results for conventionally dried apples, where osmotic dehydration significantly decreased shrinkage and deformation of fruit cubes.

Extension of osmotic dehydration time in starch syrup solution from 3 hours (sample 3) to 20 hours (sample 2) caused a gradual increase in shrinkage of freeze-dried pumpkin, but differences were not statistically significant (Fig. 1).

PĘKOSŁAWSKA and LENART (2008), who osmotically dehydrated pumpkin, also did not show pumpkin mass changes as an effect of extension of osmotic pre-treatment time, which confirms the lack of significant changes in freeze-dried pumpkin shrinkage. SALVATORI and ALZAMORA (2000) observed that at the beginning of the glucose osmotic process, cell walls of deformed apple tissue remained bound to plasmalemma during the cell shrinkage caused by water loss. However, after 200 min of osmosis, original arrangement of cells was quite well maintained and cells looked rounded similarly to the fresh ones. The effect of osmotic dehydration on a decrease in shrinkage is related mostly to an increase of dry mass, and to a lesser extent to water loss. It is compatible with the observations of DOMIAN *et al.* (2008) during osmo-convective drying of apples.

As a result of osmotic dehydration, there was a statistically significant decrease in the porosity of freeze-dried pumpkin (samples 2 and 3) in comparison to material without osmotic pre-treatment (sample 1) (Fig. 2). Observed changes are also related mostly to sugar saturation of pumpkin tissue. CIURZYŃSKA and LENART (2010b) who investigated the structure of osmotically dehydrated freeze-dried strawberries showed that during osmotic dehydration sugar penetrated to the surface layer and cells situated deeper, changing the thickness of cell walls and, therefore, decreasing the porosity of freeze-dried material. Observed changes were visible in microscopy photos of freeze-dried strawberry tissue. KOWALSKA *et al.* (2008) also showed surface penetration of sugar into pumpkin tissue during osmotic dehydration before freezing.

However, there was no significant influence of osmotic dehydration extension time from 3 hours (sample 3) to 20 hours (sample 2) on material porosity (Fig. 2).

Osmotic dehydration before freeze-drying causes decrease in the water content of fruit. This phenomenon can have an influence on the

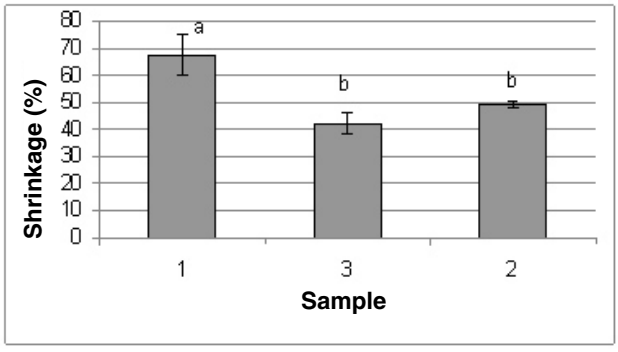


Fig. 1 - Shrinkage of osmotically pre-treated freeze-dried pumpkin in relation to osmotic dehydration time. Freezing temperature -18°C. Statistically insignificant differences (significant level 0.05) are indicated by the same letters (ab). Designations of investigated material samples. Tab. 1.

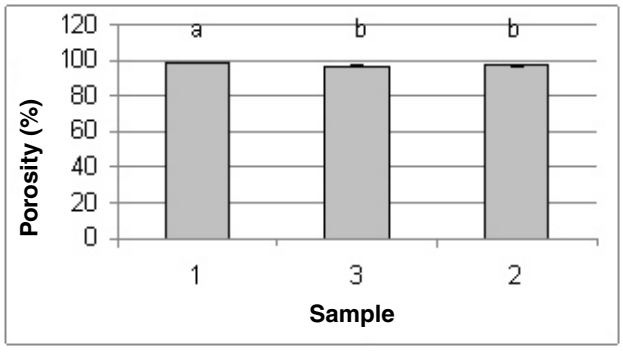


Fig. 2 - Porosity of osmotically pre-treated freeze-dried pumpkin in relation to time of osmotic dehydration. Freezing temperature -18°C. Statistically insignificant differences (significant level 0.05) are indicated by the same letters (ab). Designations of investigated material samples. Tab. 1.

Table 1 - Parameters of technological processes of freeze-dried pumpkin samples preparation.

Sample symbols	Blanching	Osmotic dehydration	Freezing	Storage (-18°C)	Freeze-drying
1	-	-	-18°C, 24 h	24-72 h	10°C 24 h
2	-	20 h	-18°C, 24 h	24-72 h	10°C 24 h
3	-	3 h	-18°C, 24 h	24-72 h	10°C 24 h
4	+	3 h	-18°C, 24 h	24-72 h	10°C 24 h
5	-	3 h	-18°C, 24 h	50-60 days	10°C 24 h
6	+	3 h	-18°C, 24 h	50-60 days	10°C 24 h
7	-	3 h	-70°C, 2 h	24-72 h	10°C 24 h
8	+	3 h	-70°C, 2 h	24-72 h	10°C 24 h
9	-	3 h	-70°C, 2 h	50-60 days	10°C 24 h
10	+	3 h	-70°C, 2 h	50-60 days	10°C 24 h

mechanical properties of freeze-dried pumpkin samples. It was shown that an increase in the moisture content of pumpkin slices influences decrease in the force required for cutting them, which is connected with the less compact texture of the pumpkin flesh (NAWIRSKA *et al.* 2009).

Osmotic dehydration before freeze-drying caused statistically significant increase in the hardness of obtained material (samples 2 and 3) in comparison to pumpkin not subjected to pre-treatment (sample 1) (Fig. 3). Achieved results confirmed previous observations of CIURZYŃSKA and LENART (2010b) for freeze-dried strawberries, where osmotic dehydration significantly increased the hardness of dried samples after pre-treatment and it was necessary to use greater deformation force.

Extension of osmotic dehydration time from 3 to 20 hours did not significantly influence the changes in the hardness of freeze-dried pumpkin (samples 3 and 2) (Fig. 3).

Osmotic dehydration (samples 2 and 3) had effect on greater compression work during deformation of freeze-dried pumpkin samples, but differences were not statistically significant in comparison to the material without osmotic pre-treatment (sample 1) (Fig. 4). Insignificant differences probably resulted from heterogeneity of the pumpkin. In the case of freeze-dried strawberries, osmotic dehydration in solutions of glucose, sucrose and starch syrup contributed to the increase of compression work values in comparison to material not subjected to pre-treatment, and differences were statistically significant (CIURZYŃSKA and LENART, 2010b).

There was no effect of osmotic dehydration time extension (from 3 to 20 hours) on the compression work (samples 3 and 2) (Fig. 4). However, the tendency towards increase of the maximum compressive force with prolonged time of osmotic dehydration was shown by PIOTROWSKI *et al.* (2008) for freeze-dried strawberries.

The influence of blanching, temperature of freezing and time of frozen storage

It was observed that the use of blanching (samples 4, 8, 6 and 10) (Tab. 1) preceding osmotic dehydration of pumpkin samples before freezing and freeze-drying caused a decrease in shrinkage of lyophilised material in comparison to unblanched samples (samples 3, 7, 5 and 9), but in most cases differences were statistically insignificant (Fig. 5). Obtained dependences were

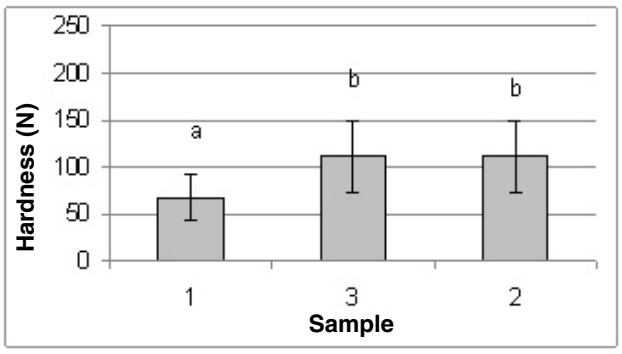


Fig. 3 - Hardness of osmotically pre-treated freeze-dried pumpkin in relation to the time of osmotic dehydration. Freezing temperature -18°C. Statistically insignificant differences (significant level 0.05) are indicated by the same letters (ab). Designations of investigated material samples. Tab. 1.

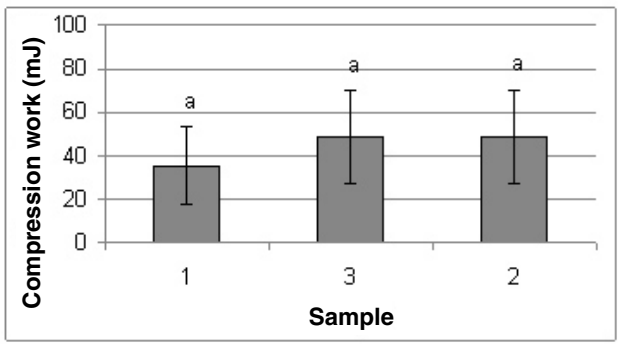


Fig. 4 - Compression work of osmotically pre-treated freeze-dried pumpkin in relation to time of osmotic dehydration. Freezing temperature -18°C. Statistically insignificant differences (significant level 0.05) are indicated by the same letters (ab). Designations of investigated material samples. Tab. 1.

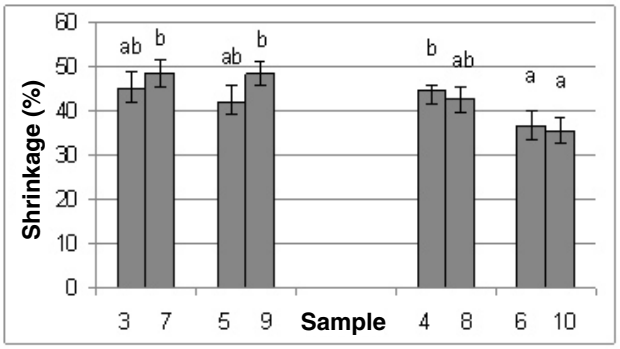


Fig. 5 - Shrinkage of freeze-dried pumpkin. Unblanched and blanched samples. Osmotic dehydration 3 h. Freezing temperature: -18° and -70°C. Statistically insignificant differences (significant level 0.05) are indicated by the same letters (ab). Designations of investigated material samples. Tab. 1.

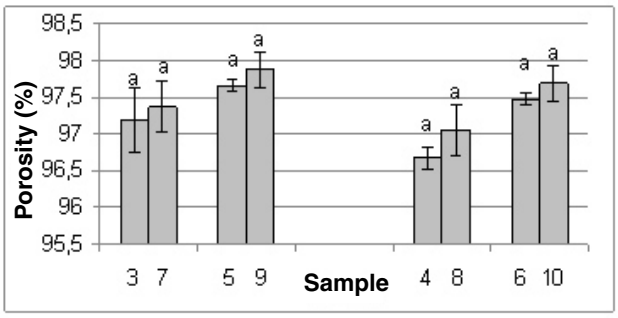


Fig. 6 - Porosity of freeze-dried pumpkin. Unblanched and blanched samples. Osmotic dehydration 3 h. Freezing temperature: -18° and -70°C. Statistically insignificant differences (significant level 0.05) are indicated by the same letters (a). Designations of investigated material samples. Tab. 1.

similar to those achieved during microwave drying of preliminarily osmotically dehydrated celery root. Blanching of celery root caused the decrease of the shrinkage of dried material (SZARYCZ *et al.*, 2005).

It was shown that the decrease of freezing temperature of pumpkin not subjected to blanching, from -18°C (sample 3 and 5) to -70°C (sample 7 and 9), caused the increase in shrinkage of the investigated freeze-dried samples, both for samples stored for a short (72 hours) and long time (60 days), but considering the wide variety of investigated material, the obtained results were statistically insignificant (Fig. 5). The quality of frozen products is usually dependent on the rate of freezing. Rapid freezing is advantageous for freezing of many kinds of food, however, some products are susceptible to cracking when exposed to extremely low temperature. Several mechanisms, e.g. volume expansion, contraction and expansion, and building of internal pressure, are explanations of the mechanisms of product damage during freezing proposed in literature (HUNG and KIM, 1996). The process of slow freezing of tissues allows sufficient time for water migration from the inner region of the tissue to the freeze-concentrated intercellular region, due to osmotic forces. This can result in cell desiccation and cell wall disruption, loss of turgor and crushing of the dried cell by large intercellular ice masses. These phenomena have an effect on the texture of the frozen product, and significant leaching during thawing and cooking can occur, leading to a loss of nutrients (ZARITZKY, 2008).

For pumpkin samples blanched before freezing, it was found that the application of the heat treatment resulted in the reduction of shrinkage caused by changes in the shock-freezing (-70°C) (sample 8 and 10) in relation to the slowly frozen pumpkin at -18°C (sample 4 and 6). In this case, however, the differences were not statistically significant.

Analysis of the impact of frozen storage time of unblanched samples showed that extension of storage time at a temperature of - 18°C from 72 hours (samples 3 and 7) to 60 days (samples 5 and 9) resulted in a slight reduction in shrinkage of the freeze-dried pumpkin (Fig. 5). For blanched samples (4, 6, 8 and 10), the reduction in shrinkage was observed for the samples frozen at -18° and -70°C with increased frozen storage time from 72 hours (samples 4 and 8) to 60 days (samples 6 and 10). The largest (statistically significant) difference in shrinkage as a result of extension of storage time in the frozen state was observed in the case of pumpkin blanched before freezing, and frozen at -18°C (samples 4 and 6).

It was shown that the use of a blanching pretreatment before freeze-drying of pumpkin (samples 4, 6, 8 and 10) reduces the porosity of lyophilised material, but the differences were not statistically significant compared to unblanched samples (sample (3, 5, 7 and 9) (Fig. 6).

For blanched and unblanched freeze-dried pumpkin, an increase in porosity was achieved with a decrease in temperature of freezing from (-18°C) (samples 3, 5, 4 and 6) to (-70°C) (samples 7, 9, 8 and 10), but differences were not statistically significant (Fig. 6). The reason for diversification of the obtained results can be the heterogeneity of the pumpkin.

It was shown that extension of storage time in a frozen state from 72 hours to 60 days caused an increase in porosity of freeze-dried pumpkin, irrespective of blanching and freezing conditions (Fig. 6), but differences were not statistically significant. These findings are related to changes in pumpkin tissue structure during frozen storage. Main physical changes during storage of frozen food are moisture migration and ice recrystallization. Both phenomena are related to the stability of frozen water inside the product and on its surface (ZARITZKY, 2000). Slow freezing results in a low rate of nucleation and the production of a small number of large ice crystals, whereas fast freezing causes a high rate of nucleation, leading to the formation of a large number of small ice crystals.

However, during frozen storage, ice crystals

undergo metamorphic changes. Recrystallization reduces the advantages of fast freezing and involves changes in number, size, shape, orientation, and perfection of crystals following the completion of initial solidification (FENNEMA *et al.*, 1973). In frozen aqueous solutions, recrystallization is the process by which the average ice crystal size increases with time. Small ice crystals are thermodynamically unstable, having a high surface/volume ratio and, therefore, a large excess of surface free energy. The net result of minimising free energy is that the number of crystals decreases at constant ice phase volume, but their mean size increases (SUTTON *et al.*, 1996). Recrystallization basically involves small crystals disappearing, large crystals growing and crystals fusing together, and affects the quality (MACKENZIE and LUYET, 1967; REID *et al.*, 1987).

KAMIŃSKA and GAUKEL (2009), studying the recrystallization phenomenon in ice cream during storage, showed that the change in temperature significantly accelerated the growth of crystals during storage for a month. The recrystallization phenomenon may also cause changes in the porosity of freeze-dried pumpkin. A large increase of porosity, which is directly caused by the ice crystals formed during the freezing process, after the long-term storage of unblanched pumpkin frozen at -70°C, may be due to the acceleration of the crystal growth after changes of temperature from -70° to -18°C (samples 5 and 9). A similar relationship was observed in the pumpkin frozen at -70°C and blanched, but the differences are smaller and, therefore, it can be assumed that the change in the structure due to blanching modifies the rate of recrystallization during storage in freezing conditions.

It has been shown that blanching pumpkin before its osmotic dehydration (samples 4, 8, 6 and 10) increases the hardness of freeze-dried samples, compared to the unblanched lyophilised material (samples 3, 7, 5 and 9), regardless of the temperature of freezing (-18° and -70°C) or frozen storage time (72 hours and 60 days). The differences were not statistically significant, due to large standard deviations (Fig. 7). Similar relationships were obtained for convectively dried celery root, where the hardness was higher when preceded by blanching. This phenomenon may be the result of chemical composition and physical properties that occur during blanching, where leaching of dry matter and hydration of raw material takes place. Water transport through the relaxed internal structure is faster in blanched material, whereas hardening occurs in the solid phase, caused by chemical changes (ŁAPCZYŃSKA-KORDON and FRANCIK, 2006).

It has been shown that decreasing the freezing temperature from -18°C (samples 3, 5, 4 and 6) resulted in a statistically significant change in the hardness of the freeze-dried material in comparison to samples shock-frozen at -70°C (samples 7, 9, 8, and 10) for samples both blanched and unblanched before freezing (Fig. 7).

Increase of frozen storage time from 72 hours (samples 3, 7, 4 and 8) to 60 days (sample (5, 9, 6 and 10) resulted in the increase of hardness of the freeze-dried pumpkin, regardless of blanching and freezing temperatures. This trend, however, has not been confirmed in the statistical analysis ($\alpha = 0.05$) (Fig. 7).

For frozen dough which was stored in different periods of time in the frozen state, MEZIANI *et al.* (2012) showed that with extension of frozen storage time there was also an increase in the hardness of samples, but it was statistically significant.

It was shown that the use of blanching before osmotic dehydration of pumpkin caused an increase in compression work necessary to deform freeze-dried pumpkin (Fig. 8). For samples frozen at temperatures of -18° and -70°C, stored for 72 hours, differences were not statistically significant (samples 3 and 4, 7 and 8). The obtained results were characterised by high standard deviation, which had an impact on the outcome of statistical analysis. In the case where the raw material before freeze-drying was stored in a frozen state either at - 18° or - 70°C (sam-

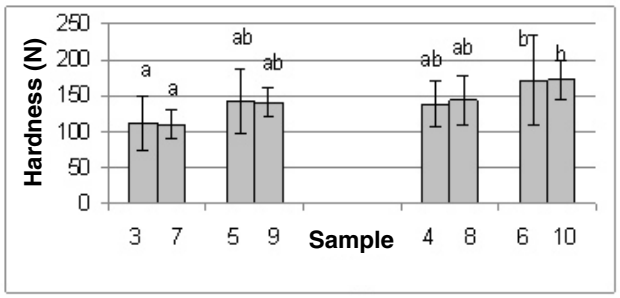


Fig. 7 - Hardness of freeze-dried pumpkin. Samples unblanched and blanched. Osmotic dehydration 3 h. Freezing temperature: -18° and -70°C. Statistically insignificant differences (significant level 0.05) are indicated by the same letters (ab). Designations of investigated material samples. Tab. 1.

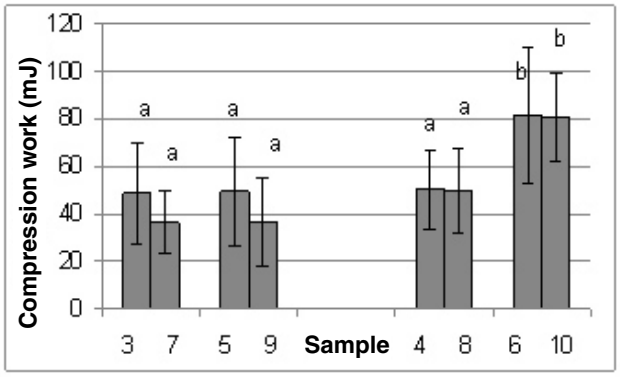


Fig. 8 - Compression work of freeze-dried pumpkin. Samples unblanched and blanched. Osmotic dehydration 3 h. Freezing temperature: -18° and -70°C. Statistically insignificant differences (significant level 0.05) are indicated by the same letters (ab). Designations of investigated material samples. Tab. 1.

ples 5 and 6 and 9 and 10), blanching of pumpkin had a significant effect on compression work (Fig. 8). Similar correlations were also observed for freeze-dried mushrooms, where blanching significantly increased the compression work necessary to deform samples, compared to those that were not pre-treated (LENTAS *et al.*, 2011). For vacuum-microwave dried carrot, blanching contributed to increasing the compression work almost twofold compared to unblanched carrot (STĘPIEŃ, 2008).

Lowering the freezing temperature from -18°C (samples 3, 5, 4 and 6) to -70°C (samples 7, 9, 8 and 10) resulted in the reduction of compression force required for 50% deformation of the sample. For unblanched samples (3 and 7, 5 and 9) differences were higher, but not statistically significant, as in the case of blanched samples (4 and 8, 6 and 10) (Fig. 8). The smallest change was observed for the pumpkin blanched before freezing (samples 4 and 8 and 6 and 10), which confirms the protective effect of pre-treatment. This is probably due to the greater porosity of samples frozen at -70°C, whose structure is more delicate. However, LENATAS *et al.* (2011) showed that the freezing temperature has a significant impact on the value of breaking work for freeze-dried mushrooms. They attributed that to the fact that at the higher temperature (-18°C) larger ice crystals are formed, which in turn leads to the formation of larger pores.

For unblanched pumpkin, frozen at temperature -18° and -70°C, the extension of frozen storage time from 72 hours (samples 3 and 7) to 60 days (samples 5 and 9) resulted in the reduction of compression work, which, however, was not statistically significant (Fig. 8). Whereas when the material was blanched prior to technological processes, there was a statistically significant increase in this parameter, as a result of extension of frozen storage time before freeze-drying to 60 days (samples 6 and 10) in relation to the pumpkin stored for 72 hours (samples 4 and 8) (Fig. 8).

CONCLUSIONS

The use of osmotic dehydration reduces drying shrinkage and porosity of freeze-dried material, stiffens the structure, causes an increase in hardness and compression work for dried samples, however, the time of osmotic dehydration is not a significant factor influencing the mechanical properties of freeze-dried pumpkin.

Changes in the structure and physical and chemical properties while blanching affect the physical and chemical properties of osmotically pre-treated freeze-dried pumpkin. The use of pre-treatment increases the hardness and the compression work, which may be caused by changes in the structure and the chemical composition of pumpkin, but the differences are not significant. Reduction of shrinkage of dried material which was blanched before drying was also insignificant, which resulted in the lack of major changes in the porosity of the samples.

The method of freezing influences the physical properties of freeze-dried pumpkin. The use of shock freezing (-70°C, 2 hours), in comparison to slow freezing (-18°C, 24 hours), increased shrinkage and porosity, but the differences were insignificant, with a large scatter of the results. Also, the mechanical properties of freeze-dried pumpkin were not significantly changed.

Extension of frozen storage time from 72 hours to 60 days did not have a significant effect on the mechanical and physical properties of freeze-dried pumpkin. Increase in porosity and hardness of dried material, as well as lower compression work values were noticed, but the differences were not significant.

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PAPER

PHYSICOCHEMICAL, MICROBIOLOGICAL, RHEOLOGICAL AND SENSORY PROPERTIES OF SET-TYPE YOGURT PRODUCED WITH TWO DIFFERENT ORIGINS: WILD *LACTOBACILLUS DELBRUECKII* SUBSP. *BULGARICUS* AND *STREPTOCOCCUS THERMOPHILUS* STRAINS

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ABSTRACT

In this study, different origins of yogurt bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) produced set-type yogurt, and we investigated their effects on yogurt quality. We selected these strains according to their proteolytic activities and their ability to produce lactic acid, aroma compounds, and exopolysaccharides (EPS). Appropriate strains were combined as a starter culture. We used five different combinations and one commercial starter. The pH of all yogurts decreased, but the decrease was relatively low in some samples. In addition, titratable acidity revealed that treatments and storage period had affected the yogurts significantly. Viscosity appeared different in all samples but increased in all during the storage period. The mean values of yogurts' flavor, texture, and acceptability were different in all yogurt samples, but yogurts produced by the wild starter culture had an especially high sensory score.

- Keywords: yogurt bacteria, traditional yogurt production, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, wild lactic acid bacteria -

INTRODUCTION

Lactic acid bacteria (LAB) have been extensively studied in the food industry, due how they not only acidify and preserve food products, but also contribute to the sensory properties of the fermented food. Among lactic acid bacteria, a combination of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are widely used as starter cultures for the production of yoghurt (AMOROS *et al.*, 1989; TAMIME and MARSHAL, 1997; ROBINSON, 2002; DE VUYST, 2002; VANINGELGEM *et al.*, 2004; DURLU-OZKAYA *et al.*, 2007; GURSOY *et al.*, 2010; TARAKCI, 2010). These two species grow synergistically in milk, increase acidity by secreting lactic acid, coagulate milk proteins, and produce yogurt aroma compounds.

It is well documented that yogurt containing these species alleviate lactose intolerance, modulate the immune system and alleviate diarrhea. The bacteria protocooperate, which speeds up acidification, but the mechanisms involved are not completely understood (VAN DE GUCHTE *et al.*, 2006). The combined "thermophilic" starter cultures, at an optimal temperature of approximately 42°C, grow and acidify quickly, creating the desired sensory properties (HAO *et al.*, 2011). An overview of the bacterial species' interactions, including the exchange of CO_2, pyruvate, and folate, can be found in a recently published review by SIEUWERTS *al.* (2008). Examples of biochemical protocooperation between *L. bulgaricus* and *S. thermophilus* include the action of cell-wall-bound proteases on *S. thermophilus,* produced by *L. bulgaricus* strains, and formate, required for growth of *L. bulgaricus* and supplied by *S. thermophilus* (LIU *et al.*, 2009).

For thousands of years, yogurt has been produced throughout the Middle East. Although there is no record of its invention, yogurt most likely came from Middle Eastern nomads (ERKUS, 2007). It is now produced worldwide. "Yogurt" is a term used to describe a wide range of related products, classifiable according to legal standards of fat content (full-, medium- or low-fat), gel type (set or stirred), flavoring (natural, fruit, or flavored), and whether they are subjected to a further process (FERNANDES, 2008).

In the dairy industry, the selection criteria for lactic acid bacteria depends on the characteristics of raw materials, the type of applied technology, the desired characteristics of the final product, and the bacteria's metabolic activities (BUCKENHIISKES, 1993; TORR *et al.*, 2003; SODINI *et al.*, 2004). Early starter cultures were simply samples of fermented food, also_called artisanal or undefined cultures. They contained historically tested blends of starter culture organisms. Often the actual organisms in a mixed culture were unknown, and not characterized microbiologically or biochemically. The proportions of different organisms in a mixed culture were inconstant from one product to another (ERKUS, 2007).

Today, the dairy industry uses more controlled fermentations. The starter cultures contain several identified microorganisms. The lactic acid bacteria used in commercial starter cultures have such identified metabolic characteristics as acidifying activity, proteolytic activity, bacteriocin synthesis, bacteriophage resistance and exopolysaccharide production (FADELA *et al.*, 2008). All of these activities contribute to flavor, texture and, frequently, nutritional attributes. Dairy workers add bacteria directly to milk in concentrated, frozen or freeze-dried (direct inoculation) form (AYAD *et al.*, 2004; SODINI *et al.*, 2004).

This study aims to determine the microbiological characteristics of yogurt manufactured with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* isolated from homemade yogurt, to produce yogurt using cultures with appropriate levels of titration acidity, pH value, proteolytic activity, and flavor compound formation characteristics, and to evaluate the technological and microbiological properties of the products. These yogurts were stored at 4°C for 21 days.

MATERIALS AND METHODS

Materials

The pure strains of *L. bulgaricus* and *S. thermophilus* isolated from homemade yogurts were obtained from the Ege University Faculty of Agriculture Department of Dairy Technology (İzmir-Turkey). We used API 50 CH tests to control both systems phenotypically, using traditional methods on *L. bulgaricus* and the Vitec-II compact system on *S. thermophilus*. 26 *L. bulgaricus* and 18 *S. thermophilus* strains activated upon transfer to sterile, reconstituted skim milk (10% w/v, RSM) supplemented with 1% yeast extract, 1% casein peptone, and 0.1% K_2HPO_4. After that, cultures were transferred *L. bulgaricus* cultures to MRS broth, and *S. thermophilus* cultures to M17 broth. *L. bulgaricus* incubated in the RSM at 42°C for 24 hours, while *S. thermophilus* did so at 37°C.

Evaporated cow's milk was supplied by Ege University, Menemen Practise and Research Farm of Faculty of Agriculture (Izmir, Turkey).

Methods

Technological properties of bacterial strains

We monitored changes in pH at the 3rd, 6th, 9th, and 24th hours of incubation, using a pH meter (Hanna pH 211 Microprocessor, Instruments, Singapure). Inoculation revealed the strains' lactic acid production (10% w/v). At the outset, 3rd,

6th, 9th,12th and 24th hours, we measured acidity by titratiny with 0.1N NaOH, using phenolphtalein as the indicator (KILIC, 1986; MICHAYLOVA *et al.*, 2007).

We evaluated exopolysaccharide production as reported by SMITINONT *et al.* (1999). We planted lines of *L. bulgaricus* cultures onto double-strength-MRS and *S. thermophilus* onto double-strength-M17 agar plate. We analyzed ropy isolates by microscope, after incubating at 42°C for 72 h anaerobically and 37°C for 48 h aerobically, respectively.

To determine proteolytic activity, we subcultured *L. bulgaricus* and *S. thermophilus* twice in sterile RSM (12% w/v), for 24 h each time at 42° and 37°C, respectively (1% v/v inoculum). Then we applied the method of CITTI *et al.* (1965). Spectrophotometric analysis (Analytikjena Spekol-1300) measured concentration of free amino acids at 650 nm. Results are expressed as μg tyrosine/mL.

Active cultures were inoculated in sterile RSM (1% v/v inoculum) and held at 37°C for 24 h. Spectrophotometric analysis also measured acetaldehyde production At 650 nm according to ROBINSON *et al.* (1977) with minor modification. Diacetyl content was determined using the method described by COGAN (1972).

Bacteriophage controls of *L. bulgaricus* and *S. thermophilus* strains were determined according to KOSKA (2007).

In yoghurt production, selection of appropriate *L. bulgaricus* and *S. thermophilus* strains is very important. Such properties as lactic acid production, post-acidification effect, aroma compound and exopolysaccharide (EPS) production, and proteolytic activity play important roles in high-quality yogurt production. The type of starter culture can therefore change the final product's physical characteristics, such as texture and appearance.

Lactic acid plays a specific role in the aroma and flavor of yogurt. The acid-forming activity of the starter culture depends on its strains, and *Lactobacillus* strains in particular (BESHKOVA *et al.*, 1998). Therefore, we considered these criteria when selecting strains.

Preparation of set-type yogurt

Starter cultures included *L. bulgaricus* and *S. thermophilus* at a 1:1 ratio aseptically in the inoculation cabinet. When total bacteria count reached 3.2 x 10^{10} cfu/mL, incubation ended. We produced yogurt with raw milk standardized to contain 12% non-fat dry matter and 3.8% fat and was divided into six equal parts. The milk was pasteurized at 85°C for 10 min and cooled to 42°C. After that, milk was inoculated the milk with the starter culture and incubated until its pH reached 4.7. The yogurt was cooled and put in 200 g quantities into sterile beakers. To produce control yogurt, we used industrial freze-dried starter yogurt culture (Chr. Hansen, Copenhagen, Denmark) YF-3331, containing *L. bulgaricus* and *S. thermophilus.* The yogurt samples were stored at 4°C over 21 days

Characterization of yogurt

Total solids content was determined by drying duplicate samples at 105°C for 3 h according to OYSUN (1999). The pH values were measured at 20°C using a pH meter (Hanna pH 211 Microprocessor, Instruments, Singapure). Titratable acidity (lactic acid %) were determined also according to OYSUN (1996). Apparent viscosity of yogurt samples was measured at 8°C during the storage period, using a DVII digital viscometer with a LV-2 spindle (Brookfield Engineering Laboratories, Stoughtoni, MA, USA). At constant revolutions of 12 rpm, the viscometer gently stirred samples for 30 s (right-left) before analysis. All analyses were repeated twice (DONKOR *et al.*, 2007).

Bacterial enumerations were carried out on the 1st, 7th, 14th, and 21st days in triplicates of each batch. Samples (10 mL) were diluted with ringer solution (90 mL). Afterwards, serial dilutions were prepared, and counted colonies which grew at 37°C for 48 h and at 42°C for 72 h. We applied pour plate technique in microbiological analyses (BRACQUART, 1981). *L. bulgaricus* in MRS agar (pH 5.8 and anaerobic incubation) at 42°C for 48 h (DE MAN *et al.*, 1960) and *S. thermophilus* in M17 agar (pH 6.9 and aerobic incubation) at 37°C for 48 h (TERZAGHI and SANDINE, 1975).

Samples were evaluated for their sensory characteristics (taste, aroma, and consistency) and overall acceptability on a 9-point hedonic scale (9 excellent, 4 indifferent, and 1 unacceptable) performed by a panel of judges selected according to their accomplishment of a general sensory aptitude test. All sensory analyses were carried out in triplicate (BODYFELT, 1998).

The SPSS statistical program (SPSS Inc., ver. 11.5.) helped us analyze the effect of using different cultures from homemade yogurt samples on the characteristic microflora, pH, acidity, dry-matter, viscosity, and sensory properties of set-type yogurts during storage. Significance of difference was set at $P < 0.05$ in all cases.

RESULTS AND DISCUSSION

Technological properties of bacterial strains

The results for pH, EPS production, proteolysis, acetaldehyde, and diacetyl production of 26 strains of *Lb. bulgaricus* were given in Table 1. The initial pH of the milk was 6.60. While some *Lb. bulgaricus* strains (K1L2, G61, E1M4, and G71) showed low acidity, others showed high acidity after 9 h of incubation. Highest pH val-

ues were determined in the SL3, K1L2, G61, F5R, SL1, and G74 strains after 24 h incubation. Only four strains (G74, K2M4, K2M1, and E3M2) of *L. bulgaricus* were able to produce exopolysaccharides (EPS). While the highest proteolytic activity was found in the ML1 strain, the P6R, K1M1, OL2 and G61 strains ranked after ML1 in descending order, respectively. The highest acetaldehyde production appeared in the F5R, K2M1, OL2, P4R, G44 and G61 strains and the highest diacetyl production appeared in the SL3, G74, OL2, K1L2 and G61 strains in descending order.

The results for pH, EPS production, proteolysis, acetaldehyde, and diacetyl production of 18 strains of *S. thermophilus* strains were given in Table 2. While some *S. thermophilus* strains had high pH, others had low pH after 9 h of incubation. In *S. thermophilus* strains, the highest pH values were seen in the C5, C7, SY2, F1M6, and E3M2 strains after 9 h incubation. Only four strains (E2MN2, K2M4, E3M2, and K2M2) of *S. thermophilus* were able to produce exopolysaccharides (EPS). While the highest proteolytic activity was found in the K1M6 strain, the C7, SY73, C1M, and G11 strains ranked after in descending order, respectively. The highest acetaldehyde productions appeared in the E2MN2, K1M6, M3, C7 and C6 strains and the highest diacetyl productions appeared in the C1M, C7, SY73, K2M2 and K1M6 strains in descending order.

Selection of suitable strains for yogurt production

Strains of bacteria show pronounced differences in pH reduction, formation of milk acid and flavor ingredients, production of exopolysaccaride, proteolytic activity, and other areas. Selection of any microorganisms, including isolated bacteria, for mixing into pure cultures should be based on specific criteria (YAYGIN and KILIC, 1993).

In yogurt production, pH is important in fermentation and especially, the post-acidification effect on yogurt quality during storage. After incubation, bacterial enzymatic activity leads to more acid production while yogurt cools from 42° to 19-20°C. The lactic acid formed by starter cultures affects the flavor of yogurt. Using this acid production also gives yogurt a proper structure and texture.

EPS production by yogurt starter cultures affects the texture of yogurt and improves such sensory characteristics as mouthfeel, shininess, clean cut, ropiness and creaminess. However, the final textural characteristics of yoghurt strongly depend on the structural properties of EPS (PURWANDARI *et al.*, 2007; JAMALY, 2010).

Proteolytic activity is greatly relevant to nutrition and to interactions of yogurt bacteria. Some lactic acid bacteria cannot synthesize essential amino acids. Therefore, they need exogenous nitrogen, and use peptides and proteins in their

Table 1 - Some technological properties of *L. bulgaricus* strains.

Strains No	pH					EPS production	Proteolysis (mg/g tirozin)	Acetaldehyde (mg/kg)	Diacetyl (mg/kg)
	3 h	6 h	9 h	12 h	24 h				
Control	6.60	6.60	6.60	6.60	6.60				
SL3	6.41	6.37	6.33	5.40	4.30	-	0.190	20.60	12.42
ML1	6.46	6.44	6.40	5.50	6.30	-	0.215	22.97	3.33
K1L2	6.43	6.39	5.90	5.20	4.30	-	0.030	15.05	9.50
D6R	6.48	6.20	6.00	5.30	4.50	-	0.026	12.86	8.43
G61	6.43	6.00	5.90	5.20	4.15	-	0.195	23.07	9.41
G612	6.41	6.38	6.24	5.50	6.00	-	0.128	24.95	5.35
E1M4	6.42	6.10	5.00	4.60	4.40	-	0.130	8.71	6.59
E3M2	6.34	6.00	5.80	5.20	4.90	+	0.070	14.97	5.43
G53	6.39	6.16	5.90	5.50	5.10	-	0.040	13.25	9.20
OL4	6.39	6.14	5.80	5.10	4.40	-	0.023	37.62	7.86
P4R	6.40	6.27	6.03	5.30	5.45	-	0.180	26.33	8.49
F5R	6.32	6.00	5.82	5.00	4.30	-	0.032	34.51	3.33
G3R	6.35	6.04	5.59	4.90	4.40	-	0.157	14.75	8.14
Z4R	6.47	6.21	6.07	5.20	4.50	-	0.122	5.90	3.68
K1L4	6.40	6.22	5.82	5.10	4.60	-	0.081	2.26	2.77
FL2	6.27	5.88	5.48	4.90	4.55	-	0.058	6.36	5.14
SL1	6.27	6.14	5.84	5.15	4.30	-	0.112	14.85	4.46
OL2	6.28	6.00	5.47	4.85	4.50	-	0.201	31.68	9.54
G44	6.52	5.85	5.41	5.30	5.10	-	0.150	25.44	8.64
OL1	6.38	6.14	5.79	5.65	5.38	-	0.138	7.62	5.94
G74	6.07	5.90	4.70	4.60	4.30	+	0.148	18.84	10.20
P6R	6.25	6.10	5.90	4.90	4.50	-	0.210	20.20	6.95
Z4R	6.35	6.00	5.70	4.95	4.40	-	0.143	15.01	4.12
K2M4	6.40	6.20	6.00	5.60	5.00	+	0.124	15.25	3.03
F3M2	6.42	6.00	5.30	5.00	4.30	-	0.140	15.96	4.75
K2M1	6.45	6.10	5.90	5.60	5.05	+	0.205	33.65	8.61

Table 2 - Some technological properties of *S. thermophilus* strains.

Strains No	pH 3 h	pH 6 h	pH 9 h	pH 12 h	pH 24 h	EPS production	Proteolysis (mg/g tirozin)	Acetaldehyde (mg/kg)	Diacetyl (mg/kg)
Control	6.70	6.70	6.70	6.70	6.70				
E2MN2	6.42	6.20	5.80	5.30	5.05	+	0.028	16.26	10.30
SY72	6.40	6.22	5.10	4.90	4.62	-	0.008	5.92	17.03
K1M5	6.35	6.24	6.05	5.70	5.30	-	0.012	9.25	16.12
SY73	6.32	6.10	5.70	5.10	4.81	-	0.060	7.35	25.94
K1M6	6.22	6.05	5.15	4.80	4.62	-	0.065	14.85	20.45
C1M	6.35	6.15	6.05	5.80	5.50	-	0.055	8.51	27.52
M3	6.50	6.32	6.20	6.10	6.00	-	0.045	14.34	7.23
C6	6.28	5.57	5.40	5.20	4.65	-	0.009	10.79	4.95
SL4	6.37	6.08	5.95	5.25	4.60	-	0.007	5.21	11.29
SY2	6.12	5.87	5.45	5.30	4.87	-	0.042	3.52	8.51
ET6	6.34	6.11	5.85	5.55	4.72	-	0.040	7.31	18.39
K2M4	6.35	6.10	5.50	5.80	4.90	+	0.010	6.67	14.48
F1M6	6.04	5.87	5.68	5.58	5.40	-	0.032	9.25	16.12
E3M2	6.10	5.80	5.60	5.50	5.45	+	0.030	9.70	15.86
SY5	6.50	6.10	5.30	5.10	4.65	-	0.025	8.55	6.21
C7	6.30	5.60	5.20	4.95	4.35	-	0.064	12.02	26.33
K2M2	6.25	6.00	5.85	5.40	4.85	+	0.038	6.29	23.20
G11	6.30	6.10	5.80	5.50	4.80	-	0.050	5.01	17.82

growth medium. Yogurt bacteria exhibit less proteolytic activity than other lactic acid bacteria, as is desirable. *L. bulgaricus* is more proteolytic than *S. thermophilus* and enriches the medium with amino acids and peptides (DUTTA *et al.*, 1971). Incubation temperatures, pH of medium, and strain variety all help determine proteolysis.

Cultures should also be chosen for the taste and flavor they produce, which depends on the amount of acetaldehyde and diacetyl in the yogurt (YAYGIN and KILIC, 1993). Acetaldehyde is considered the major flavor component of yogurt (PETTE and LOLKEMA, 1950; DUMONT and ADDA, 1973; LAW, 1981) while high concentrations of it in cheese or cream lead to flavor defects described as "green" or "yogurt-like" (LINDSAY *et al.*, 1965).

The typical aroma of yogurt is characterized chiefly by acetaldehyde as well. PETTE and LOLKEMA (1950) were the first to suggest that acetaldehyde was the most important constituent of yogurt aroma. It was later suggested that "high concentration of acetaldehyde is necessary to produce a desirable flavor with yogurt" (ROUTRAY and MISHRA, 2011). RASIC and KURMANN (1978) suggested that *S. thermophilus* was only responsible for the availability of diacetyl in yogurt, whereas other authors reported that diacetyl was also largely produced by *Lactobacillus bulgaricus.*

We selected our strains based on properties such as different levels of proteolytic activity and production of acid, acetaldehyde, and diacetyl. Therefore, our yogurts ended up with different physicochemical properties. We coded names of the strains we used and their combinations as follows:

A: Commercial culture, **B:** *L. bulgaricus* G61 x *S. thermophilus* K2M4, **C:** *L. bulgaricus* G44 x *S. thermophilus* ET6, **D:** *L. bulgaricus* P4R x *S. thermophilus* E2MN2, **E:** *L. bulgaricus* G74 x *S. thermophilus* K1M6, **F:** *L. bulgaricus* G3R x *S. thermophilus* SY73ST.

The physicochemical composition of the raw milk used in yogurt production yogurts shows the following averages: lactic acid 0.135±0.02%, pH 6.65±0.01, fat 3.80±0.04%, and protein 3.48±0.10.

Physicochemical properties of yogurt

Total solids of yogurt samples A, B, C, D, E, and F were respectively 15.385, 15.387, 15.384, 15.375, 15.455, and 15.351% at the first day of storage period. The fat contents of the samples were found to be (3.50±0.14)%.

Some physicochemical properties of yogurt samples during the storage period are shown in Table 3. While pH values of yogurt samples decreased, lactic acid density increased. According to LUQUET (1990), lactic strains can ferment lactose to lactic acid by increasing acidity, decreasing pH. The analysis of variance showed that using a wild starter culture had a significant effect on pH reduction. Similar changes were observed in the level of titratable acidity in the yogurt samples during storage ($P<0.05$).

While the samples exhibit low pH, the slowest decrease in pH was in sample E. Therefore, we preferred strains with low pH in sample E's preparation. Hence, while the lowest average pH value was detected for sample A (the control sample), sample E had the highest. Put another way, the most lactic acid was in sample A, the least in sample E. These results are consistent with research by POURAHMAD and ASSADI (2007), PURWANDARI *et al.* (2007), GULER-AKIN *et al.* (2009) who used starter cultures with different properties in yogurt production.

Rheological properties of yogurt

Details of rheological properties of yoghurt samples were shown in Table 3. Using starter cultures with different origins had an effect on the viscosity of yoghurts ($p<0.05$). This fact was also reported by researchers like GUZEL-SEYDIM *et al.* (2005), JUMAH *et al.* (2006) studying the effects of starter cultures on the quality of yoghurt. It is thought that the increase in viscosity was caused by EPS production ability of *S. thermophilus* strains (B samples) ($p<0.05$). However, viscosity value of the yoghurt using EPS producing *L. bulgaricus* strain (G74) was lowest apparent viscosity. Researchers reported that using of strains producing EPS in fermented milk products had affected the viscosity characteristics (RAMCHANDRAN, 2009; GURSOY *et al.*, 2010).

RAWSON and MARSHALL (1997) suggested that *S. thermophilus* was the most germs incremented in the production of exocellular texturizing agents that might interact with the protein content of milk and increase the viscosity of yoghurts.

The ability of some strains of *S. thermophilus* and *L. bulgaricus* to produce exopolysaccharides (EPS) during fermentation and gel formation contributes to the improvement in yogurt texture. EPS may be bound to the cell as capsules, or may diffuse away from the cell as an extracellular slime (RAMCHANDRAN, 2009; GURSOY *et al.*, 2010). 50 among EPS-producing starters, one can distinguish encapsulated and unencapsulated bacteria. When EPS production significantly enhances the texture of yoghurt, EPS-producing strains become ropy, but sometimes also become viscous, viscosifying, or thickening starters. They will be compared to the acidifying starters used as a control (SODINI *et al.*, 2004).

SCHELLHAASS and MORRIS (1985) observed that yogurts made with ropy cultures (polysaccharide production) had more viscosity than yogurts made with nonropy cultures. Because EPS interacts with free water in the gel-like structure, the texture of GUZEL-SEYDIM *et al.*, 2005).

Viability of lactic acid bacteria

The microbiological attributes of yogurt samples during the storage period are shown in Table 4. Counts of *L. bulgaricus* and *S. thermophilus* decreased. Using starter cultures from different origins had an effect on the viability levels of bacteria ($p<0.05$). Furthermore, wild starters showed the highest level of viability. GAVIN (1968), SPECKMAN *et al.* (1974), EL-SADEK (1975), YANG and SANDINE (1974) also reported that species and strains of bacteria affected bacterial viability and activity, which verifies our findings show similarity our findings (KOSKA, 2007).

It is generally accepted that yogurt should contain at least 10^7 cfu/g or 10^7 cfu/mL of viable bacteria per gram (ANONYMOUS, 2009). Counts of *S. thermophilus* and *L. bulgaricus* were close to each other during the storage period. Yogurts produced with the wild culture contained more *Lb. bulgaricus* than the control yogurt, and its number of *S. thermophilus* was lower only than Sample C. This is a consequence of that wild cultures are more resistant than commercial cultures and they have a high viability.

Sensory properties

Sensory scores of the yogurts were given in Table 5. Using different types of starter cultures improved the taste and flavor of the samples ($P<0.05$). The samples showed similar viscosities.

Sample F had the lowest post-incubation acid-

Table 3 - Physico-chemical and rheological properties of yoghurt samples during storage at 4°C (n=3 ± sd).

	Days	A	B	C	D	E	F
pH	1	4.61±0.01bx	4.63±0.00ax	4.66±0.04bx	4.55±0.01bcx	4.70±0.00cx	4.63±0.04bx
	7	3.98±0.01ay	4.13±0.03by	4.13±0.03by	4.16±0.01by	4.59±0.01dy	4.29±0.01cy
	14	3.94±0.02az	4.07±0.02ayz	4.09±0.02byz	3.98±0.04bz	4.53±0.04dyz	4.24±0.04cyz
	21	3.87±0.01at	4.01±0.01az	4.05±0.02bz	3.93±0.04bz	4.48±0.04dz	4.17±0.02cz
	Av	4.10±0.32	4.20±0.26	4.23±0.27	4.14±0.27	4.57±0.09	4.33±0.19
Titratable acidity (°SH)	1	1.286±0.01ex	1.102±0.01ex	1.194±0.03cx	1.298±0.02dx	0.803±0.01ax	1.009±0.03bx
	7	1.417±0.01fy	1.245±0.01ey	1.292±0.01cy	1.380±0.01dy	0.830±0.02axy	1.015±0.01b x
	14	1.517±0.01ez	1.270±0.03dy	1.310±0.02cy	1.470±0.02cz	0.839±0.01ay	1.044±0.01bx
	21	1.615±0.05ct	1.321±0.05dy	1.339±0.01bcy	1.489±0.01z	0.968±0.01az	1.256±0.03by
	Av	1.459±0.13	1.234±0.09	1.284±0.06	1.409±0.08	0.860±0.07	1.081±0.11
Viscosity (mPas/s at 12 rpm)	1	6389±25.46cx	7118±40.75c	6389±25.46d	6009±14.90cx	4484±96.17ax	5226±41.01bx
	7	7622±14.90cy	7164±23.75bc	6551±94.30bc	7123±63.60by	4563±31.25axy	6405±113.50bxy
	14	7871±27.58dyz	7194±20.65cd	7260±14.60cd	7283±10.60bcy	5651±24.20ayz	6250±37.26bxy
	21	8101±18.53ez	7171±14.07e	7472±70.00c	7914±31.80dz	5712±14.75az	6548±29.70by
	Av	7496±712.48	7162±202.80	6663±701.09	7082±737.64	4977±555.00	5982±593.06

A: Commercial culture, *B: Lactobacillus delbrueckii* ssp. bulgaricus G61 x *Streptococcus thermophilus* K2M4, C: *Lactobacillus delbrueckii* ssp. bulgaricus G44 x *Streptococcus thermophilus* ET6, D: *Lactobacillus delbrueckii* ssp. bulgaricus P4R x *Streptococcus thermophilus* E2MN2, E: *Lactobacillus delbrueckii* ssp. *bulgaricus* G74 x *Streptococcus thermophilus* K1M6, F: *Lactobacillus delbrueckii* ssp. bulgaricus G3R x *Streptococcus thermophilus* SY73ST

Table 4 - Microbiological properties of yoghurt samples during storage at 4°C (log cfu/g, n=3 ± sd).

	Days	A	B	C	D	E	F
***Lactobacillus delbrueckii* ssp.**	1	9.46±0.01bx	9.45±0.21b	9.83±0.10bx	9.48±0.09c	8.91±0.07ax	10.18±0.04dx
	7	7.98±0.04ay	9.59±0.08d	9.75±0.04cdx	9.75±0.02cd	8.83±0.18bx	9.54±0.01cy
	14	6.52±0.01az	9.55±0.06bc	8.65±0.04cy	9.23±0.35bc	7.88±0.04aby	8.49±0.06bcyz
	21	6.42±0.05at	9.47±0.02c	8.61±0.01ey	8.34±0.11d	7.84±0.04by	8.45±0.06cz
	Av	7.59±1.33	9.51±0.11	9.21±0.62	8.95±0.89	8.36±0.55	9.16±0.78
Streptococcus thermophilus	1	9.45±0.02x	9.67±0.05	9.32±0.12x	9.98±0.02x	9.51±0.75	10.01±0.01x
	7	9.39±0.04xy	9.63±0.04	9.21±0.18x	9.95±0.01xy	9.48±0.70	9.97±0.03x
	14	9.35±0.04ay	9.52±0.01c	9.17±0.18bx	9.51±0.05ay	8.95±0.02c	9.85±0.01cx
	21	8.06±0.03az	8.49±0.03b	8.23±0.16by	8.93±0.01bz	8.92±0.01a	8.98±0.04by
	Av	9.06±0.62	9.47±0.38	8.98±0.49	9.70±0.47	9.47±0.55	9.73±0.46

A: Commercial culture, B: *Lactobacillus delbrueckii* ssp. bulgaricus G61 x *Streptococcus thermophilus* K2M4, C: *Lactobacillus delbrueckii* ssp. bulgaricus G44 x *Streptococcus thermophilus* ET6, D: *Lactobacillus delbrueckii* ssp. bulgaricus P4R x *Streptococcus thermophilus* E2MN2, E: *Lactobacillus delbrueckii* ssp. bulgaricus G74 x *Streptococcus thermophilus* K1M6, F: *Lactobacillus delbrueckii* ssp. bulgaricus G3R x *Streptococcus thermophilus* SY73ST.

Table 5 -Sensory properties of yoghurt samples during storage at 4°C (n=3 ± sd).

	Days	A	B	C	D	E	F
Taste-Aroma	1	7.50±0.70bx	6.60±0.57b	7.50±0.60ab	7.50±0.70b	5.70±0.42ax	7.50±0.70b
	7	6.40±0.14by	7.35±0.07e	7.50±0.00d	7.85±0.07d	5.80±0.00ax	6.90±0.14c
	14	5.90±0.14by	6.90±0.14c	6.95±0.20c	6.85±0.07c	4.35±0.20ay	7.00±0.00c
	21	6.00±0.14by	7.00±0.14c	6.95±0.07c	6.95±0.07c	4.50±0.28ay	7.00±0.14c
	Av	6.45±0.73	6.96±0.37	7.23±0.41	7.29±0.51	5.09±0.74	7.10±0.37
Consistence	1	8.00±0.00bx	7.55±0.20ab	7.75±0.35ab	6.85±0.20abx	7.10±0.55a	7.60±0.50ab
	7	7.50±0.00by	7.75±0.07d	7.85±0.07c	7.95±0.07cy	7.20±0.00a	7.75±0.07c
	14	7.25±0.07abz	7.30±0.00bc	7.70±0.29ab	7.55±0.07cy	7.20±0.00a	7.80±0.14c
	21	7.15±0.07az	7.25±0.07b	7.70±0.14a	7.50±0.00bcz	7.20±0.14a	7.60±0.00c
	Av	7.48±0.36	7.34±0.37	7.75±0.19	7.46±0.43	7.05±0.35	7.74±0.24
General Aceptable	1	7.85±0.20bx	6.75±0.35bx	7.75±0.35abx	7.60±0.30b	5.85±0.20a	7.50±0.70b
	7	6.30±0.14ay	7.55±0.07dy	7.70±0.00cx	8.00±0.00c	6.30±0.07a	6.90±0.14b
	14	6.35±0.21by	7.20±0.00bcxy	7.30±0.14bxy	7.25±0.07cd	6.10±0.14a	7.55±0.07d
	21	6.35±0.36ay	7.15±0.07bxy	7.15±0.07by	7.40±0.14b	6.25±0.21a	7.60±0.00b
	Av	6.71±0.72	7.16±0.33	7.48±0.31	7.56±0.37	6.13±0.23	7.39±0.41

A: Commercial culture, B: *Lactobacillus delbrueckii* ssp. bulgaricus G61 x *Streptococcus thermophilus* K2M4, C: *Lactobacillus delbrueckii* ssp. bulgaricus G44 x *Streptococcus thermophilus* ET6, D: *Lactobacillus delbrueckii* ssp. bulgaricus P4R x *Streptococcus thermophilus* E2MN2, E: *Lactobacillus delbrueckii* ssp. bulgaricus G74 x *Streptococcus thermophilus* K1M6, F: *Lactobacillus delbrueckii* ssp. bulgaricus G3R x *Streptococcus thermophilus* SY73ST

ity and less viscosity. Sample F was the most acceptable to the panelists, Sample E the least. Panelists stated that Samples C and D fit best the traditional definition of yogurt, and they preferred both to Sample A, which was produced with commercial culture.

In a sensory examination of yogurt samples produced from 10 different commercial cultures, KOSKA (2007) found statistically significant differences between all evaluation parameters except smell.

AKALIN and GONC (1999) produced yogurts using a non-viscous culture strain (A) and two types of viscous culture strains (B and C), both separately and in combination. They found the highest viscosity and lowest serum decomposition in yogurts containing viscous culture C, which showed more increase in acidity and more growth in starter bacteria. The same study revealed that the highest values of structure, texture, and appearance came from the yogurts produced with combined cultures, and did not decrease during storage.

CONCLUSIONS

The results of using wild yogurt bacteria (*L. bulgaricus* and *S. thermophilus*) to produce set-type yogurt appear satisfactory. In this study, we produced yogurt cultures, using isolates obtained from traditional milk products. Proper selection led to cultures with more favorable properties than commercial cultures. Strains with different properties in different combinations lead to differences between products. Physicochemical, microbiological and rheological properties of the products were better than those produced using commercial cultures; especially in terms of consumer observation, these products exhibited better sensory properties. Thus, advanced studies of yogurt-producing strains will yield positive results.

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PAPER

FILM TYPE AND MAP ON *CV.* HIMBO TOP RASPBERRY FRUIT QUALITY, COMPOSITION AND VOLATILES

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ABSTRACT

This study evaluates the effects of different MAP methods (active and passive) on the postharvest life of raspberry fruits using different plastic packaging films. In particular the comparison between biodegradable and conventional plastic films represent an important objective since not many studies are available on the effect of biodegradable films used for food packaging. Raspberry fruits were packaged using three different plastic films and were stored with different gas conditions for 24, 48, 72 and 96 hours. The gas concentration values of the boxes were monitored daily with a PBI Dansensor gas analyzer and expressed as percentages. Weight losses, skin color and qualitative and nutraceutical characteristics were monitored during the storage period to identify the best film-atmosphere combination for raspberry shelf life. Fruit volatile components were investigated. The results show that the evolution of the O_2 and CO_2 concentrations in the packed box was more influenced by the film characteristics rather than by the initial gas concentrations. The best maintenance of qualitative characteristics and the lowest weight decrease over 96 hours was obtained by storing fruits under a biodegradable film.

- Keywords: raspberry, storage, packaging, MAP, quality, total phenolic compounds, antioxidant activity, anthocyanins, organic acid, ascorbic acid, postharvest -

1. INTRODUCTION

Raspberries (*Rubus idaeus* L.) have a short postharvest life due to their high susceptibility to mechanical injury, loss of firmness, darkening of color, physiological deterioration, water loss and decay. The short shelf life of these fruits has limited their marketability, but appropriate preservation methods, such as controlled atmosphere (CA) techniques can extend their shelf life (AGAR *et al.*, 1991; ROBBINS and FELLMAN, 1993). In general, these storage practices maintain important quality characteristics, such as firmness, soluble solid content, acidity and freshness, and may reduce fungal decay (AGAR *et al.*, 1997). Furthermore, raspberries are valued by consumers for their flavor, nutritional properties and health benefits, as they are an excellent source of natural antioxidants, such as anthocyanins, other flavonoids and phenolic acids, as well as vitamins and minerals (KALT *et al.*,1999). While the effect of cold storage temperature on nutritional parameters such as anthocyanins, phenolics, ellagic acid, and ascorbic acid, as well as levels of antioxidant activity, of fresh strawberries is well documented (KALT *et al.*, 1999; CORDENUNSI *et al.*, 2002), only a few studies report on cold storage effects on quality and nutritional characteristics of fresh raspberry fruit under different temperature treatments or storage conditions including controlled atmosphere (KALT *et al.*, 1999; HAFFNER *et al.*, 2002; MULLEN *et al.*, 2002a). Really it is difficult to preserve all of these qualitative characteristics. Flavor tends to decline before the changes in appearance that indicate the end of acceptable postharvest life (PELAYO-ZALDIVAR *et al.*, 2007). In berry fruits acids, along with aromatic compounds, are the constituents that determine flavor. Storage conditions can influence the variability of these components (MAWELE and BRENT 1993, PENG *et al.*, 2012). The interactions of sugar, acids, anthocyanins, total phenolics including ellagitannins, ascorbic acid and the level of antioxidant activity within a species is strongly affected by cultivar but also by fruit maturity at picking (KRÜGER *et al.*, 2011).

A test of different storage treatments showed that storage in controlled atmosphere significantly suppressed rotting and maintained more attractive berry color compared with storage under a normal atmosphere (HAFFNER *et al.*, 2002). A few studies have also reported on the effects of cold storage on the quality and nutritional characteristics of fresh raspberry fruits under different temperature treatments or storage conditions, including CA (KALT *et al.*, 1999, HAFFNER *et al.*, 2002, MULLEN *et al.*, 2002b).

Shelf life extension also confers a number of commercial benefits, by decreasing waste in manufacturing, retail display and long distribution channels and by improving product value.

The use of reduced (1-5 kPa) O_2 and elevated (5-10 kPa) CO_2 levels balanced with N_2, in combination with storage temperatures at refrigeration levels, has been proposed by many researchers as the optimal storage strategy for several fresh fruit, fresh vegetable and fresh-cut vegetable products (AHVENAINEN, 1996; CHURCH, 1994; KADER *et al.*, 1989; OHLSSON, 1994; JACXSENS *et al.*, 1999; VAN DER STEEN, 2002).

The main objective of this study was to evaluate the effects of different (active and passive) modified atmosphere packaging (MAP) methods using biodegradable and conventional plastic packaging on the weight loss, respiration rates, volatile compound contents and nutritional properties of cv. Himbo Top raspberry fruit up to 96 hours.

2 MATERIALS AND METHODS

2.1 Plant material

Raspberries *cv.* Himbo Top were obtained from Agrifrutta Soc. Coop. S.R.L. (Piedmont, Italy) commercial orchard. The fruits were picked by hand on end of July at the red ripe stage of maturity, individually deposited in polypropylene baskets and immediately transferred to the laboratory under cold conditions. Samples were transferred, in a few minutes, to +1°C and analyses were carried out after 0, 24, 48, 72 and 96 hours.

2.2 Packaging and storage

2.2.1 Packaging materials and storage methods

The fruits were packed using three different films.

1 - Macro-Perforated Film (control): transparent commercial polypropylene film (pp) (Trepack, Italy) with a thickness of 20 microns and 6-mm-diameter holes, no modified atmosphere. The film has a typical isotactic structure (in which the substituents are arranged on one side) and high crystallinity;

2 - Film Polypropylene (pp): transparent, non perforated, flexible, thermo formable commercial (Trepack, Italy) film with a thickness of 30 microns (T° 38°C, R.H. 90%, O_2TR 2,834 $cm^3/m^2 \cdot 24$ h, CO_2TR 8,926 $cm^3/m^2 \cdot 24h$);

3 - Biodegradable film: non perforated and non-commercial film. The molecules in these materials are derived from starch and vegetable oils (T° 38°C, R.H. 90%, O_2TR 3,000 $cm^3/m^2 \cdot 24h$, CO_2TR 44,113 $cm^3/m^2 \cdot 24h$) biodegradable and compostable, with a thickness of 25 microns.

For the trials in modified atmosphere (MA), fruits were placed in polyethylene terephthalate (PET) baskets (150 g per basket) and wrapped with the different packaging films under different gas compositions according to the following scheme:

Film	N.A. (20,8% O_2, 0.03% CO_2, 78% N_2)	MA (10% O_2, 10% CO_2, 80% N_2)
macro-perforated (control)	X	
polypropylene (pp)	X	X
biodegradable	X	X

The gas addition was conducted using a Taurus 500 (Delphin, Italy) flowpack machine with a PBI Dansensor (Italy) gas mixer that allowed different gas mixtures (O_2, CO_2, N_2) to be used for each packed basket.

The recommended controlled atmosphere storage conditions for raspberry fruit are 15-20% CO_2 and 5-10% O_2 (KADER, 2001). We did some preliminary tests with different gas concentration, and improved concentration in our environment has been 10% for CO_2 and 10% for O_2, data not shown. Fruits were stored at +1°C and 85% R.H. for 24, 48, 72 and 96 h.

2.2.2 Sampling method for analyses

At the end of each storage period (24, 48, 72 or 96 h), 10 raspberry fruits (for each basket) were used to measure skin color, and 80 g of raspberries were squeezed with a juicer (Multiquick - Braun, Italy) to measure soluble solid content, titratable acidity and pH. Three replicates per measurement were used.

Three lots were used to determine the physicochemical properties at harvest (0 hours).

2.3 Weight loss and fruit color

The weight of each basket was recorded at harvest, and at the end of each storage period, the fruits were weighed again. Weight loss was calculated for each period and expressed as the percent loss from initial weight. Three lots were used to determine the weight loss per each film.

Color was measured on the first ten sound, non mouldy fruit from each basket. The color was evaluated just after harvest and again after each storage period (0, 24, 48, 72 and 96 h). The mean of the 10 fruit measurements was used for data analysis. Color was measured on the side of a slightly flattened whole fruit using a tristimulus color analyzer (Chroma Meter, Model CR-200b, Minolta, Germany) equipped with a measuring head with an 8-mm-diameter measuring area. The analyzer was calibrated to a standard white reflective plate and used CIE (Commission Internationate de l'Eclairage) Illuminant C.

Color was recorded using the CIE L*a*b* uniform color space (CIELab), where L* indicates lightness, a* indicates chromaticity on a green (–) to red (+) axis, and b* chromaticity on a blue (–) to yellow (+) axis. These values were used to calculate chroma ($C^*=[a^{*2}+b^{*2}]^{1/2}$), which indicates the intensity or color saturation, and hue angle (h ° = arctangent [b */a *]), where 0° = red – purple; 90° = yellow; 180° = bluish-green; and 270° = blue (MCGUIRE, 1992).

2.4 Headspace composition

Carbon dioxide and oxygen concentrations were measured with a CO_2 and O_2 analyzer (PBI Dansensor, Italy). The composition values were measured daily over the whole period of the trial and expressed as percentages. The same air volume (free volume 425 mL) was maintained in the packages across the trial period, as the analyzer introduced the same quantity of air that it removed for the analyses.

2.5 Chemical analysis

Chemical analyses were conducted on the filtered supernatant (0.45 mm acetate cellulose filter) of a centrifuged juice (Centurion Scientific Ltd, West Sussex, England) at 13,000 g for 15 min. Acetonitrile, methanol and formic acid were of HPLC grade and were purchased from Merck (Darmstadt, Germany). The other reagents were of analytical grade. Sodium carbonate was obtained from Merck (Germany). 2,2-Diphenyl-1-picrydazyl (DPPH), hydrochloric acid, potassium chloride, sodium acetate and Folin-Ciocalteu's phenol reagent were purchased from Fluka (Buchs, Switzerland). Glucose, fructose and sucrose were purchased from Sigma-Aldrich Co. (Milan, Italy), and cyanidin 3-glucoside was purchased from Extrasynthese (Genay, France). All analyses were performed in triplicate, and the results are expressed as the mean value.

2.6 Total soluble solids, pH, titratable acidity and carbohydrates

Total soluble solids (TSS) were measured using a digital refractometer (Mod. PR-101, Atago, Tokyo, Japan) and expressed as °Brix at 20°C. pH measurements were performed using an automatic titrator (Titritino 702, Metrohm, Swiss). The titratable acidity (TA) was determined potentiometrically, using 0.1 N NaOH to an end point of pH 8.1 in 5 mL of juice diluted in 50 mL of distilled water. The results were calculated as g 100 $mL^{-1}$of anhydrous citric acid.

Carbohydrates were analyzed according to the method of YANG and MING-YU (2000). A LaChrom Merck-Hitachi liquid chromatograph (Hitachi Ltd., Tokyo, Japan), consisting of a

D-7000 system manager, an L-7100 pump, and an L-7200 autosampler coupled with an evaporative light scattering detector (ELSD SEDEX 60LT, France), was used. A fast carbohydrate column (100 × 7.8 mm, lead form, 9 μm particle size, Biorad, Hercules, California) with a micro-guard carbo-P refill cartridge (30 × 4.6 mm i.d.), thermostated at 80°C, was employed. The isocratic mobile phase was ultrapure water with a flow rate of 0.8 mL/min. The ELSD detector was set with the following specifications: drift tube temperature, 45°C; nebulizer gas, air; pressure, 2.5 bar.

Stock standard solutions of each carbohydrate were prepared in ultrapure water, and their quantification in raspberry juice was calculated according to the linear calibration curves of standard compounds.

2.7 Total phenolic compounds, antioxidant activity, anthocyanins, organic acids and ascorbic acid

The total soluble phenolic content was analyzed according to the Folin-Ciocalteu colorimetric method (SINGLETON and ROSSI, 1965). Total phenols were expressed as gallic acid equivalents.

Antioxidant activity was assessed using a commercially available free radical DPPH (BONDET *et al.*, 1997), using 100 mL samples. The mixture, containing 3 mL of a methanol solution of 0.16 mM DPPH (2,2- diphenyl-1-picrylhydrazyl), was allowed to react for 15 min in a cuvette. The percentage inhibition of the absorbance of the DPPH solution added to the sample was calculated using the following equation:

$$\text{Inhibition \%} = \\ = [(\text{Abst} = 0 - \text{Abst} = 15\ \text{min})/\text{Abst} = 0] \times 100$$

The total anthocyanins content was determined by the pH-differential spectrophotometric method (RAPISARDA *et al.*, 1994) using a 2-buffer system at pH 1.0 and 4.5. An aliquot of juice (1 mL) was diluted up to 10 mL with a pH 1 buffered solution (25% 0.2M KCl and 75% 0.2M HCl), and a second aliquot (1 mL) was diluted up to 10 mL with a pH 4.5 buffered solution (40% 1M CH_3CO_2Na, 24% 1M HCl, and 36% H_2O). The absorbance of the solutions was measured at 510 nm. Anthocyanins concentration were calculated by the differences in absorbance at 510 nm between the pH 1 (colored oxonium or flavylium form) and pH 4.5 (colorless carbinol form) solutions; the results were expressed as cyanidin 3-glucoside.

Organic acid and ascorbic acid contents were determined using a Merck-Hitachi (Tokyo, Japan) liquid chromatography with an L-7455 photodiode detector (DAD), a D-7000 system manager, an L7200 autosampler and L-7100 pumps. Simultaneous separation and determination of the organic acids and ascorbic acid were achieved according to the procedure described by YUAN and CHEN (1999) and CHINNICI *et al.* (2005) using a Bioradcation guard column and a Bio-Rad Aminex HPX-87H hydrogen form cation exchange resin-based column (300 × 7.8 i.d.) at 40°C. The mobile phase consisted of a 0.005 M sulfuric acid aqueous solution, and the samples were isocratically separated at 0.6 mL/min. The peaks of organic acids and ascorbic acid were measured at wavelengths of 210 and 245 nm, respectively, and were identified by comparing the retention times with those of standards. Quantification was conducted using external standards.

2.8 Fruit volatile components

Volatile components were analyzed by the automatic headspace solid-phase dynamic extraction (HS-SPDE) technique combined with GC-MS and a CTC AnalitycsCombi Pal autosampler. The samples (three trays of 125 g for each preservation environment) were blended to a fine puree, and 3 g of puree was placed in a 20-mL headspace glass vial (Brown-chromatography supplies) with 5 g of sodium chloride to increase the recovery of the extraction.

After equilibration at 35°C for 15 min, the volatile components were extracted with a SPDE fiber (SPNdl-01/AC-50-56,PDMS + 10% active charcoal, 50 μm, 57 mm) exposed to the headspace for 30 min. The fiber was then introduced to the injector of the gas chromatograph (GC) for desorption at 250°C for 3 min in the splitless mode.

An analysis of volatile compounds was performed using an Agilent 6890 gas chromatograph equipped with a Restek Rxi-1MS column (30 m × 0.25 mmID × 0.25 μm thick film) and a mass-spectrometer detector (Agilent 5973). The initial oven temperature was held at 0°C for 2 min, increased by 5°C/min to 100°C, raised by 4°C/min to 230°C and held for 10 min.

Mass spectra were scanned from m/z 35 to 300, and volatile compounds were identified by comparing the mass spectral data with the Wiley library and retention times.

The system has been set up through injection of a standard mixture solution (terpenes/terpenoids, esters, aldehydes, hydrocarbons) in methanol at the concentration of 0.01 μg/μL for each component: hexanal, isoamyl acetate, camphene, beta-pinene, limonene, gamma-terpinene, delta-3-carene, dodecane, menthol, carvone, alpha-copaene, pentadecane, hexadecane, heptadecane (Sigma-Aldrich).

We processed increasing concentrations of the compounds and verified the system linearity under operation conditions, and good correlation coefficients ($r \geq 0.85$) were found.

The repeatability of the method was investigated by performing three different injections from each 100 g tray.

A semi-quantitative analysis was performed with external standard calibration, with a focus on checking the retention time of each single component identified by mass spectra.

For qualitative discussion and consideration, the concentrations of each compound in a chemical family (e.g.,aldehydes) were added together.

2.9 Data analysis

All statistics were performed using SPSS for Windows version 17.0. The data were analyzed with ANOVA, and the means were separated using the Duncan test ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 Weight loss and fruit color

According to CALLESEN and MØLLER HOLM (1989) and HAFFNER *et al.* (2002) raspberry weight losses cannot be considered as a limiting factor for berry quality during storage because fruits in all storage conditions showed marginal and low values (less than 1%) (Fig. 1). The raspberries *cv.* Himbo Top weight losses varied minimally for the several films used. The control (macro-perforated packages) exhibited higher fruit weight loss than other packaging system for all the storage time. The lowest weight loss was observed with the pp film, particularly in the baskets with gas, but the fruits could not be stored for the whole storage trial period (96 h) because the fruits were overripe and showed an high softness of pulp and for this they weren't commerciable.

The greatest loss was recorded for the control (0.35% after 48 h) and biodegradable film (0.43% after 24 h). For fruits packed using biodegradable film under 10% CO_2 and 10% O_2, a weight loss of 0.35% was recorded after 48 h of storage. The biodegradable film without added gas showed a mean loss of 0.30% for the whole storage period.

Color is one of the most important attribute associated with quality in raspberry berries and it is an important parameter of fruits ripeness and freshness. Red raspberries stored under regular and controlled atmospheres showed a variety of berry colors. According to HAFFNER (2002) raspberries *cv.* Himbo Top (Table 1) stored under normal atmosphere (control) showed darker colors, in contrast, MAP storage conditions, limit the change of color maintaining values similar at harvest. In fact this packaging condition showed at the end of storage time (96 h) the lowest chroma value (24.47), also for the b* color parameter was observed the lowest value (8.15). After 96 h, the biodegradable film was the packaging condition that maintained the highest chroma value (37.49) and the b value (15.55); fruits packed with the biodegradable film exhibited values very similar to those observed at harvest (C* 33.78, b* 14.99).

This trend was maintained during all the storage time.

3.2 Headspace composition

In Table 2, the O_2 and CO_2 levels detected on the sample package headspace during storage are reported. Despite the high metabolic activity of raspberries, the gas concentration in the packed baskets was more influenced by the film permeability than by the initial O_2 and CO_2 concentrations. Indeed, after 24 h of storage, the gas concentrations in the baskets packed with pp film (with initial concentrations of 20.8% O_2 and 0.03% CO_2) and with pp film + gas (with initial concentrations of 10% O_2 and 10% CO_2) were found to be equal. This result shows that the gas concentration of the package depends more on the film permeability than on the initial concentration values.

After 72 h, gas concentrations of 11.1% O_2

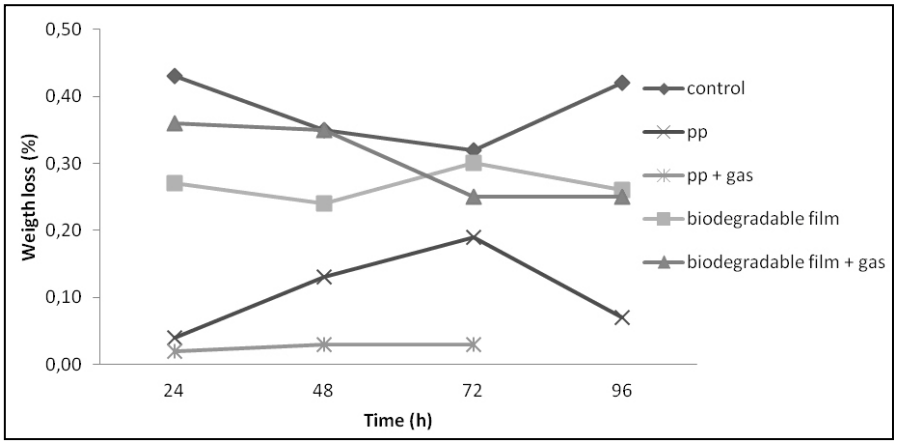


Fig. 1 - Changing in weight (%) of raspberries *cv.* Himbo Top packaged in different films and stored at +1°C (expressed as the percent loss from initial weight).

Table 1 - Changes in raspberry fruit *cv.* Himbo Top color value during storage at +1°C in different MAP conditions.

Variable	Treatment	Storage time (h)							
		24		48		72		96	
L*	Harvest				30.61±2.67				
	control	27.09	±2.34	26.82	±1.26	26.21	±1.25	25.38	±1.92
	pp	27.98	±2.20	27.18	±1.40	26.8	±1.39	25.43	±2.19
	pp + gas	29.19	±2.36	29.15	±2.01	29.02	±2.43	-	
	biodegradable film	29.83	±2.10	29.62	±1.79	29.1	±1.89	30.32	±2.50
	biodegradable film + gas	29.67	±2.29	29.55	±3.02	29.38	±3.07	30.36	±2.53
a*	Harvest				30.23±2.42				
	control	26.08	±3.47	25.81	±2.12	24.22	±2.39	23.06	±2.71
	pp	28.87	±3.37	28.52	±2.98	27.71	±3.04	24.95	±2.61
	pp + gas	29.42	±3.87	29.34	±3.62	29.34	±3.63	-	
	biodegradable film	33.35	±3.98	32.98	±2.98	30.66	±3.07	34.09	±4.12
	biodegradable film + gas	29.98	±3.28	29.98	±3.33	29.92	±3.49	31.88	±3.72
b*	Harvest				14.99±3.04				
	control	10.32	±1.93	9.32	±1.02	8.7	±1.15	8.15	±1.12
	pp	12.08	±1.87	11.21	±1.63	11.08	±1.82	9.54	±1.43
	pp + gas	12.43	±2.85	12.41	±2.77	12.35	±2.81	-	
	biodegradable film	14.92	±2.90	13.67	±1.98	13.37	±2.08	15.55	±2.92
	biodegradable film + gas	12.87	±2.38	13.01	±3.32	13.78	±3.52	14.52	±2.85
C*	Harvest				33.78±0.45				
	control	28.06	±3.90	27.60	±2.53	25.74	±2.61	24.47	±2.89
	pp	31.30	±3.78	30.00	±3.25	29.85	±3.46	26.72	±2.89
	pp + gas	31.97	±4.60	31.89	±4.21	31.86	±4.38	-	
	biodegradable film	36.56	±4.72	32.23	±3.80	33.47	±3.60	37.49	±4.88
	biodegradable film + gas	32.64	±3.91	30.25	±3.33	33.00	±4.56	35.05	±4.53
h*	Harvest				3.48±0.05				
	control	0.41	±0.03	0.37	±0.03	0.34	±0.02	0.34	±0.02
	pp	0.45	±0.03	0.40	±0.03	0.38	±0.03	0.36	±0.03
	pp + gas	0.39	±0.04	0.40	±0.04	0.39	±0.04	-	
	biodegradable film	0.43	±0.04	0.42	±0.04	0.41	±0.03	0.43	±0.04
	biodegradable film + gas	0.41	±0.04	0.40	±0.04	0.42	±0.06	0.42	±0.04

Average and S.D. s were calculated for 3 replicates.

Table 2 - O_2 and CO_2 concentrations in the headscape of packaged raspberry fruit *cv.* Himbo Top stored at +1°C in different MAP conditions.

Variable	Treatment	Storage time (h)							
		24		48		72		96	
O_2 (%)	pp	10.6	±0.1	11.3	±0.1	9.6	±0.1	9.5	±0.5
	pp + gas	10.1	±0.3	10.0	±0.4	9.4	±0.5	-	
	biodegradable film	9.3	±0.2	8.6	±0.2	11.1	±0.3	8.5	±0.3
	biodegradable film + gas	9.1	±0.3	9.5	±0.3	9.3	±0.1	9.9	±0.3
CO_2 (%)	pp	15.6	±0.6	13.0	±0.9	13.2	±0.4	12.1	±0.2
	pp + gas	15.9	±0.9	17.5	±0.2	15.0	±0.4	-	
	biodegradable film	12.8	±0.7	14.6	±0.1	12.4	±0.1	14.0	±0.2
	biodegradable film + gas	13.6	±0.4	14.6	±0.1	13.2	±0.3	14.3	±0.3

Average and S.D. s were calculated for 3 replicates.

and 12.4% CO_2 were observed in the baskets packed with biodegradable film without gas, close to the optimal 1:1 ratio for raspberry storage (HAFFNER *et al.*, 2002). In the baskets with a modified atmosphere, this ratio was not observed for either the biodegradable film or the pp film.

3.3 Fruit volatile components

Overall fruits of *cv.* Himbo Top sample have comparable compound types and numbers, and 50 of 75 identified volatiles are potent odorants as defined in other studies (MALOWICKI *et al.*, 2008a; KLESK *et al.*, 2004).

Table 3 - Major volatile compounds identified in *cv.* Himbo Top raspberry fruit in all storage conditions.

Aldehydes	Trans-2-hexenal Hexanal Ethyl hexanoate Ethyl butanoate Trans-2-hexenyl acetate 2-Hexenyl acetate Benzoic acid ethyl ester
Ketones	β-Ionone α-Ionone Nonanone Dihydroionone 2-Heptanone Acetophenone 2-Eptanone Damascenone Isophorone 4-Methylcyclohexanone
Furanones	Theaspirane 2-Amyl furan 5-Ethyl-2-furan
Terpenoids	α –Pinene α-Phellandrene Ocimene γ-Terpinene β-Pinene α-Terpinolene Junipene Isoledene δ-Cadinene Alfaphellandrene Linalool Beta-cyclocitral Piperitone
Alcohols	2-Heptanol 3-Hexenol Butyl alcohol Fenchyl alcohol

In this study, a total of 72 aromatic compounds were isolated in fruits of cv. Himbo Top over all storage condition. The most prevalent compound classes recovered were ketones (10 compounds), terpenoids (13 compounds, with 9 of them unidentified) and aldehydes (7 compounds), including many compounds recognized as the most significant in the aroma of raspberries (Table 3). The influence of storage conditions was evaluated by analyzing samples stored for several periods at +1°C and observing the changes in the various classes of compounds. Volatile compounds are secondary metabolites generated through numerous chemical pathways during the fruit ripening process, and several factors influence their kinetics, including cultivar, growing conditions, processing and storage conditions (MALOWICKI *et al.*, 2008b).

Storage temperatures and the concentration of oxygen influence the chemical reactions that occur during the ripening process, particularly the oxidative process.

Storage at +1°C significantly inhibits the chemical reactions and therefore the ripening process and production of volatile substances, and the tests at this temperature showed smaller amounts of some volatile compounds compared to similar tests conducted at 18°C (APREA *et al.*, 2009).

About the volatile patterns, while no statistically significant differences were observed between samples stored in the various types of MAP under the barrier films, much larger variations were observed between the samples stored in these types of packaging and the control film samples in an oxidizing environment. The evolution of volatile compounds in these samples shows that the oxidative processes of maturation take place more readily in these environments.

The main difference was observed in the increasing trend of aldehydes (represented mainly by hexenal and 2-hexenal) in the control packaging, which shows a regularly increase with values greater than those of the other types of packaging.

Aldehydes are generated from the oxidative degradation of fatty acids and are responsible for 'green' odor notes; their concentrations are typically related to fruit maturity. In the MAP samples tested, the aldehydes showed an irregular pattern associated with a more moderate increase; this pattern was most likely due to the effects of temperature and a storage environment with lower oxygen content.

Higher level of terpenes (particularly α-phellandrene and α-pinene) and alphain was observed in samples stored under MAP in comparison to trays stored under the control film (Fig. 2). The aroma of raspberries is strongly characterized by the presence of ketones such as α and β-Ionones. These compounds are generated from carotenoids, and their concentrations increase with maturity. For this chemical class, there were no significant differences between the various storage conditions, and in all cases, we observed an increase in ketones (24, 48, 72 and 96 h) during storage followed by a subsequent (7 days) decrease in their levels.

3.4 Total soluble solids, titratable acidity, pH, and carbohydrates

Changes in the balance between total soluble solid content and acidity during storage can affect the sensory quality of the fruit (NESTBY, 1978).

Fruits under biodegradable film at 72 h had the highest value (11.1°Brix) of total soluble solids (Table 4) while the minimal value was observed for those of the control (8.2°Brix). At 96 h significant statistically differences are shown for the soluble solid content between the different MAP conditions; particularly with the biodegradable film the soluble solid content (8.5°Brix)

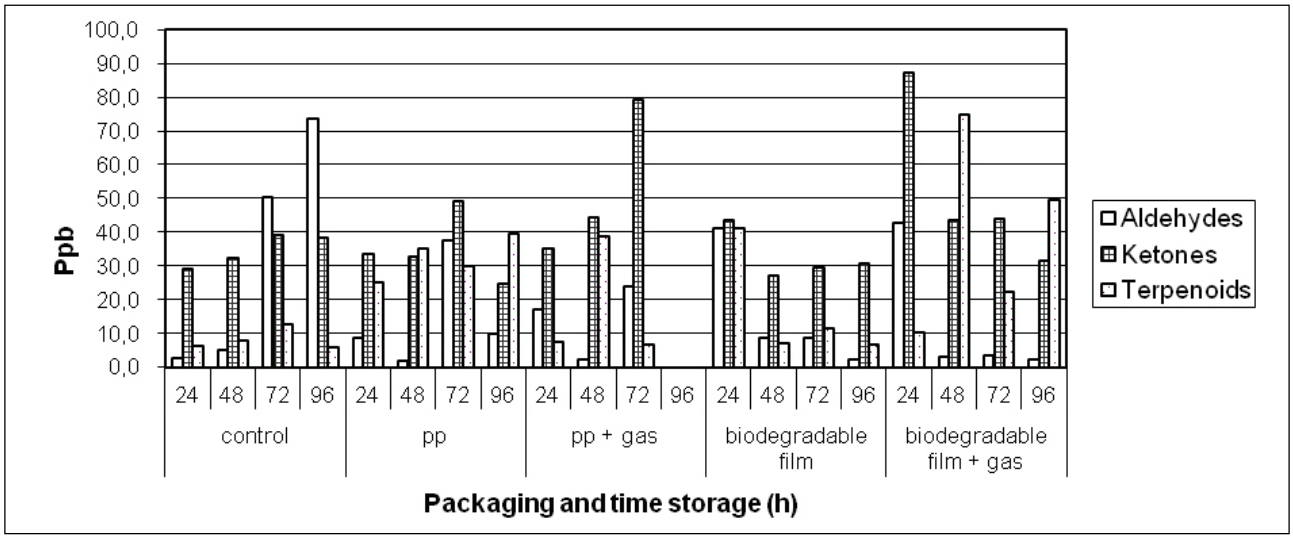


Fig. 2 - Effect of various MAP on volatile aroma concentrations (μg/kg) of the 3 main classes of compounds (aldehydes - ketones - terpenoids) of raspberry *cv.* Himbo Top stored at +1°C.

decreased due to weight loss, the highest value was for the biodegradable film + gas (9.9°Brix).

The titratable acidity of cv. Himbo Top raspberries decreased in the storage time and at 96 h the control and the pp film showed the lowest values (11.5 and 12.1 g/mL). When using the biodegradable film, fruit titratable acidity after 24 h was very similar to the control (Table 4), however the values of both biodegradable film (with and without gas) increased to similar values at the end of the time and were both in the range of fresh fruits.

The pH (Table 4) value, measured as 3.5 at harvest, was relatively stable, at each storage period and during the trials for all packaging methods. However, the last pH observa-

Table 4 - Changes in total soluble solids, titratable acidity and pH of the raspberry fruit *cv.* Himbo Top stored at +1°C in different MAP conditions.

Variable	Treatments	Storage time (h)			
		24	48	72	96
TSS (°Brix)					
	Harvest	10.6			
	control	9.5c[a]	9.3cd	8.2c	9.6a
	pp	10.6a	11.3a	9.6b	9.5a
	pp + gas	10.1b	10.0b	9.4b	-
	biodegradable film	9.3cd	8.6c	11.1a	8.5b
	biodegradable film + gas	9.1d	9.5bc	9.3b	9.9a
TA (g/mL)					
	Harvest	15.5			
	control	12.5b	11.8d	12.9bc	11.5b
	pp	15.6a	13.0c	13.2b	12.1b
	pp + gas	15.9a	17.5a	15.0a	-
	biodegradable film	12.8b	14.6b	12.4c	14.0a
	biodegradable film + gas	1.6b	14.6b	13.2b	14.3a
pH					
	Harvest	3.5			
	control	3.7 n.s.	3.3ab	3.5ab	6.5a
	pp	3.5	3.3ab	3.5a	3.3b
	pp + gas	3.6	3.2b	3.2b	-
	biodegradable film	3.5	3.5a	3.3ab	3.2b
	biodegradable film + gas	3.5	3.2ab	3.4ab	3.4b

[a]For each variable (TSS, TA, pH) means in column followed by different letters are significantly different at $P \leq 0.05$ according to the Duncan's test of the least significant difference.

Table 5 - Changes in total carbohydrates of the raspberry fruit *cv.* Himbo Top stored at +1°C in different MAP conditions.

Variables	Treatments	Storage time (h)			
		24	48	72	96
Sucrose (g/100 mL)					
	Harvest	3.58			
	control	2.45bc[a]	2.36b	2.17b	1.78a
	pp	2.87a	3.05a	2.75a	1.49b
	pp + gas	2.37bc	2.29b	2.09b	-
	biodegradable film	2.62ab	2.66ab	2.39b	0.89b
	biodegradable film + gas	2.20c	2.24b	2.14b	1.03b
Glucose (g/100 mL)					
	Harvest	2.02			
	control	1.82b	1.87bc	1.79b	1.72b
	pp	1.95ab	1.98ab	1.86b	1.75b
	pp + gas	1.83b	1.79c	1.81b	-
	biodegradable film	1.99a	2.01a	2.02a	1.96a
	biodegradable film + gas	1.80b	1.90abc	1.79b	1.82ab
Fructose (g/100 mL)					
	Harvest	2.96			
	control	2.71 n.s.	2.90ab	2.79bc	2.73n.s.
	pp	2.85	3.13a	2.90b	2.85
	pp + gas	2.65	2.80b	2.58c	-
	biodegradable film	2.91	3.00ab	3.19a	3.04
	biodegradable film + gas	2.61	3.04ab	2.91b	3.05
Total sugar (g/100 mL)					
	Harvest	8.56			
	control	6.97abc	7.12c	6.75b	6.24n.s.
	pp	7.68a	8.16a	7.51a	6.09
	pp + gas	6.85bc	6.88c	6.47b	-
	biodegradable film	7.53ab	7.67b	7.61a	5.89
	biodegradable film + gas	6.61c	7.18c	6.84b	5.90

[a]For each variable (Sucrose, glucose, fructose, total sugar) means in column followed by different letters are significantly different at $P \leq 0.05$ according to the Duncan's test of the least significant difference.

tion for the control film treatment showed a mean value of 6.5, compared to a value of approximately 3.4 for the other packages, probably due to mold.

The total sugar and the most important sugars (sucrose, glucose, fructose) found in raspberry cv. Himbo Top are reported in Table 5.

At harvest the most aboundant sugar was the sucrose (3.58 g/100 mL) but during the storage, as ripening raspberries progresses it was converted to glucose and fructose; in fact at 96 h for all packaging conditions the content of fructose is higher than content of sucrose and this can affect the sweetness of fruits (WANG *et al.*, 2009). Generally the total sugar content decreased during the storage time and no significant statistically differences are shown between the different MAP conditions after 96 h of storage. Sucrose contents at 72 h changes between 2.75 (pp) and 2.09 (pp + gas) g/100 mL. Glucose contents at 72 h changes between 1.79 (control) and 2.02 (biodegradable film) g/100 mL, fructose between 2.58 (pp + gas) and 3.19 (biodegradable film) g/100 mL. At end of the storage similar sugars values were observed in the biodegradable film (with and without gas).

3.5 Total phenolic compounds, antioxidant activity, anthocyanins, and organic acids

In Table 5 are reported values of the most important chemical constituents of quality of raspberry fruits cv. Himbo Top stored in different MAP conditions at +1°C.

Organic acids such as citric and malic counterbalance the total sugar content in berries; they are responsible of astringent flavors of raspberries and help to stabilize ascorbic acid and anthocyanins content (WANG *et al.*, 2009). The citric acid was the major organic acid found in fresh raspberry cv. Himbo Top (1.84 g/100 mL). This acid levels decreased with increasing maturity and after 96 h of storage fruits the control showed the lowest value (1.46 g/100 mL). No significant statistically differences are shown for the malic acid content between packaging con-

Table 6 - Organic acids, total phenolic compounds, anthocyanins, and antioxidant activity of the raspberry fruit *cv.* Himbo Top stored at +1°C in different MAP conditions.

Variable	Treatment	Storage time (h)			
		24	48	72	96
Citric acid g/100 mL					
	Harvest	1.84			
	control	1.57ca	1.50d	1.56d	1.46c
	pp	1.78b	1.74c	1.83b	1.73ab
	pp + gas	1.94a	1.92a	2.00a	-
	biodegradable film	1.91a	1.78bc	1.74c	1.72b
	biodegradable film + gas	1.73b	1.82c	1.76c	1.78ab
Malic acid mg/100 mL					
	Harvest	0.13			
	control	0.13 n.s.	0.13 n.s.	0.13 n.s.	0.13 n.s.
	pp	0.14	0.14	0.12	0.15
	pp + gas	0.13	0.13	0.12	-
	biodegradable film	0.13	0.13	0.13	0.13
	biodegradable film + gas	0.14	0.12	0.13	0.13
Ascorbic acid mg/100 mL					
	Harvest	21.40			
	control	19.37 n.s	19.39bc	20.07 n.s	18.04a
	pp	20.53	21.08a	20.40	16.93b
	pp + gas	19.40	20.47ab	19.67	-
	biodegradable film	19.38	18.98c	20.64	18.57a
	biodegradable film + gas	20.92	19.01c	20.58	18.13a
Total phenol /100 L					
	Harvest	138.12			
	control	173.93a	183.28ab	196.87a	217.91a
	pp	174.41a	188.85a	201.39a	223.02a
	pp + gas	153.41c	176.24b	173.56bc	-
	biodegradable film	152.26c	159.47c	178.84b	162.29b
	biodegradable film + gas	164.44b	163.92c	172.51c	159.02b
Total anthocyanins mg/100 g					
	Harvest	26.15			
	control	52.49a	59.00a	64.75a	81.08a
	pp	50.39ab	57.91a	61.03b	76.73b
	pp + gas	49.03b	50.28b	55.11c	-
	biodegradable film	45.80c	39.13d	47.24d	38.13c
	biodegradable film + gas	41.83d	45.61c	39.25e	36.16c
Antioxidants (% inhibition)					
	Harvest	46.01			
	control	53.46a	54.89a	55.02a	64.42a
	pp	53.96a	56.00a	56.40a	67.15a
	cp + gas	47.70c	51.41b	48.19c	-
	biodegradable film	45.10d	42.15c	51.42b	45.38b
	biodegradable film + gas	50.55b	48.25b	50.97b	42.22b

[a]For each variable (Citric acid, Malic acid, Ascorbic acid, Total phenol, Total anthocyanins, Antioxidants) means in column followed by different letters are significantly different at $P \leq 0.05$ according to the Duncan's test of the least significant difference.

ditions during all the storage. The ascorbic acid content of the berries decreased during ripening and was less unchanged by packaging conditions; the lowest ascorbic acid content (16.93 mg/100 mL) was found after 96 h of storage in fruits wrapped with the pp film.

According to the literature (WANG and LIN, 2000; BEEKWILDER *et al.*, 2005; VINCENTE *et al.*, 2006) the increase of total anthocyanins during raspberry ripening was observed for all fruits samples. The different packaging conditions strongly influenced the content of total anthocyanin after 24 h being higher than those in fresh berries (26.15 mg/100 g). Anthocyanins as red pigments are synthesized during the last stages of maturity when fruit become (KRÜGER *et al.*, 2011). High increases during the storage period were observed for the control and for fruits packed with pp film, while lower increases were observed in fruits packed with biodegrada-

ble film at every time. The accumulation of anthocyanins in raspberry fruits during postharvest was explained by KALT *et al.* (1999) by the decrease of organic acids which provide carbon skeletons for the synthesis of phenolics including anthocyanins red. The highest total anthocyanins values respectively of 81.08 mg/100 g (control) and 76.73 mg/100 g (pp) were observed after 96 h which correspond the lower total titratable acidity (Table 6).

The total phenolic compounds content significantly changed during the maturation process according to WANG and LIN (2000) that observed the total increase in red raspberry from the pink to the ripe stage. Particularly the highest values were observed after 96 h of storage for the control (217.91 g/100ml) and fruits wrapped with pp film (223.02 g/100 mL).

According to WANG *et al.* (2009) the total phenolic content of raspberry *cv.* Himbo Top showed a positive relationship with the antioxidant activity. Antioxidants values increased significantly during storage and particularly raspberry fruits which correspond the highest total phenol and total anthocyanins content showed at 96 h of storage the highest antioxidant activity (64.42 and 67.15 respectively for the control and the pp film). After 96 h of storage the lowest values were observed for fruits maintained in the biodegradable film and biodegradable film plus gas. The mechanism by which modified atmosphere storage prevents the increase in total antioxidant activity is not clear, but different atmosphere conditions might affect the release of bound phytochemicals that contribute to antioxidant activity.

4. CONCLUSIONS

Berry producers are seeking raspberry cultivars with a better potential for the fresh market. Large and firm berries with an attractive red color will be preferred. Since anthocyanins each have unique color characteristics, this may have contributed to apparent differences in anthocyanin content and to the darkening observed in the raspberry fruit. In this study the MAP technique with biodegradable film was useful to delay the ripening process of *cv.* Himbo Top raspberry through a delay in the changes in color, weight losses and acidity, and in turn an extension of shelf life could be achieved (96 h) as compared with pp film.

The results of this study indicate that nutritional quality of fruits remains stable throughout at the end of storage period.

More specifically, our data point to the stability of major phytochemical classes (phenols, flavonoids and anthocyanins) as well antioxidant capacity of raspberry fruits.

Higher antioxidant capacity values were, in most cases, maintained by fruits packed in biodegradable film (with and without gas). It may be concluded that, to improve the shelf life of raspberry fruits, the use of MAP is very important, but it is also important to choose the most suitable packaging material to maintain the best storage conditions and qualitative characteristics of the fruits.

The use of MAP as a supplement to proper temperature maintenance in the effort to delay ripening is consequentially more effective for storage sensitive fruits like raspberry, but is generally beneficial for all fruits (SANDHYA, 2010). This work demonstrates a well-performing method of reinvestigating the ripening process of aromatic fruit through chemical compound transformation. However, for a quantitative and conclusive discussion, the study must be improved with respect to statistics and matrix effects.

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PAPER

ITALIAN-TYPE SALAMI WITH PROPOLIS AS ANTIOXIDANT

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ABSTRACT

There is a world trend of replacing synthetic additives with natural ones. Thus, the purpose of this study was to evaluate the use of propolis, both free and microencapsulated, as a natural antioxidant in salami. For this, salamis were added with free propolis, propolis microencapsulated and other with sodium erythorbate (control). Weight loss, colour, pH, A_w, lipid oxidation, microbial spoilage and sensory acceptance were examined. Products containing propolis showed similar results to the control for colour, pH, weight loss and A_w. Propolis prevented the oxidation of salami during storage; however, it led to a lower sensorial acceptance.

- Keywords: antioxidant, bee glue, microencapsulation, quality, salami

INTRODUCTION

Salami is a product made with comminuted meat and fat that is embedded and subjected to fermentation and drying (CAMPAGNOL *et al.*, 2011). The lipids present in this product give it desirable sensory characteristics and nutritional and technological properties; however, they are easily oxidised, especially the linoleic (18:2, n-6) and oleic (18:1, n-9) acids (CHASCO *et al.*, 1993) included in this type of sausage. Oxidation degrades these compounds into secondary products, affecting the sensory attributes and nutritional value of the final product (OSAWA *et al.*, 2005).

To control this deterioration, in addition to the synthetic antioxidants currently used by the meat industry, it is possible and desirable to use natural antioxidants. Many natural compounds in bee products have significant antioxidant activity (NAGAI *et al.*, 2006), including propolis.

Propolis is a complex mixture of resinous material collected by honeybees from various plant sources (GUO *et al.*, 2011). Several studies have shown that propolis has biological properties such as antimicrobial, antifungal, antiviral and antioxidant activities (PENA, 2008). Attributed to the flavonoids and phenolic acids, most of the biological activities observed for propolis (FUNARI and FERRO, 2006).

Thus, propolis has potential to be used as a food additive because of its natural antioxidant and antimicrobial properties. However, due to its strong flavour and relative insolubility, the use of propolis in foods has been limited (NORI *et al.*, 2011). Microencapsulation can solve this limitation because of its ability to mitigate undesirable flavours; reduce volatility, hygroscopicity and reactivity; and increase solubility and stability in adverse conditions (FAVARO-TRINDADE *et al.*, 2008). A study by our research group demonstrated the feasibility of microencapsulating propolis by a complex coacervation method using isolated soy protein and pectin as a coating. The process yields an alcohol-free, stable powder with antioxidant activity and antimicrobial activity against *Staphylococcus aureus*, and it provides controlled release in food (NORI *et al.*, 2011).

Thus, the objective of this work was to use both free and microencapsulated propolis in Italian-type salami to evaluate their potential as a natural additive in this type of product.

MATERIALS AND METHODS

Salami sausage formulation and processing

The formulation followed the Brazilian legislation for Italian-type salami (BRASIL, 2000). Fresh boneless pork shoulder (600 g/kg), beef rib (200 g/kg) and pork backfat (200 g/kg) were obtained from a local supermarket. The following non-meat ingredients were added as a proportion of the total raw meat material: sodium nitrate (0.4 g/kg), sodium nitrite (0.1 g/kg), salt (25 g/kg), dextrose (7.5 g/kg), sodium tripolyphosphate (3.0 g/kg), monosodium glutamate (1.0 g/kg), white pepper (2.5 g/kg), garlic powder (2.0 g/kg), coriander (1.0 g/kg), nutmeg (0.5 g/kg), smoke aroma (0.3 g/kg) and starter culture (0.125 g/kg) Bactoferm F-1 (Chr.Hansen®, Valinhos, Sao Paulo, Brazil) containing *Staphylococcus xylosus* DD-34 and *Pediococcus pentosaceus* PCFF-1.

Salamis were divided into three treatments: treatment SE (control) with sodium erythorbate (0.5 g/kg), a synthetic antioxidant commercially used in salami production; treatment FP with free propolis (0.14 g/kg) prepared from the dehydration of ethanolic extract of propolis in a spray dryer and dissolved in 1 g/kg of propylene glycol; and treatment MCC with microencapsulated propolis with pectin and isolated soy protein (2.29 g/kg) prepared by complex coacervation according to methods described in NORI *et al.* (2011). All treatments with propolis were calculated to have the same amount of propolis solid in the final product. The propolis was prepared from green propolis, type 12, according to Brazilian classification (PARK *et al.*, 2002), and had its antioxidant activity and stability confirmed (NORI *et al.*, 2011).

The frozen beef and pork meat were cut and grounded separately using a grinder (Hobart, HB22-2 Troy, Ohio, USA) with stainless steel disks of 5 and 10 mm holes, respectively, and pork backfat was 8 mm hole. In the mixer (Beccaro, MB-25, São Paulo, Brazil), pork and beef were added first, followed by the pork backfat. After mixing of the raw meats, the non-meat ingredients were added, and the pre-hydrated starter culture and the propolis or sodium erythorbate were added last. The meat batter was stuffed into collagen casings 55 mm in diameter and 250 mm in length (Viscofan Brazil, São Paulo, Brazil).

The salami sausages were hung from metal rods and randomly placed in a fermentation chamber for 32 days with controlled temperature and relative humidity (RH) and protected from light. Fermentation was carried out at 23°-25°C, 88-90% RH for 24 h to reach a pH between 5.0-5.2. Then, the temperature and RH were reduced to 15°-17°C and 1-2% daily until reaching 75% respectively, where it remained until the 32nd day of the process when the salamis reached water activity less than 0.90. After the end of the process, the casings were removed manually and the final product was vacuum-packaged. The packages (Cryovac, Sao Paulo, Sao Paulo, Brazil) were composed of an ethylene vinyl acetate (EVA) multilayer (55 μm, O_2 trans. rate <30 $cm^3/m^2/24$ h/atm). The salami was stored away from light at ambient temperature (25°C) for 90 days.

This process was repeated three times, and each time, a sample of raw material (150 g) and three sausages from each treatment were randomly selected for analysis of quality and shelf life (1, 30, 60 and 90 days of storage).

Determination of lipid oxidation

Lipid oxidation was evaluated by measuring thiobarbituric acid reactive substances (TBARS) according to the method described by BRUNA *et al.* (2001). The results were expressed as mg of malonaldehyde per kg of a sample (mg MDA/kg). To obtain these results, the water content (% water) of the samples was quantified (PREGNOLATTO and PREGNOLATTO, 1985).

Measurement of pH, water activity (A_w) and instrumental colour

The pH was determined directly from samples (three measurement of each sample) using a potentiometer (Oakton pH 300 series 35618, Vemon Hills, Illinois, USA) with automatic temperature compensation and a glass penetration electrode (Digimed, Presidente Prudente, Sao Paulo, Brazil).

The water activity was measured at the temperature of 25.0°±0.3°C in salami slices approximately 3 mm thick which were placed in capsules suitable for analysis. The water activity was determined using the analyzer Aqualab CX2T (Decagon Devices Inc., Pullman, Washington, USA). This determination was performed on the raw sausage during the drying to identify the end of the process and during the storage for 90 days, with three measurements of each sample.

Colour was monitored (three measurements of each sample) using a Minolta colorimeter (Konica Minolta, Chroma Meter, 150 CR-400, Mahwah, New Jersey, USA). Measurements of L* and a* colour values (lightness and redness respectively) were carried out in 9 pieces of each treatment on the CIELab system. The colorimeter was standardized against a white tile (Y=93.7, x=0.3160 and y=0.3323), using D65 and 10° illuminant standard observer (CIE, 1978).

Weight loss

During the drying step, three salamis from each treatment were randomly selected from different locations in the drying chamber (front, right and left) were weighed daily until the end of the process. The Equations 1 and 2 were used to calculate the daily percentage of weight loss:

$$\%W_x = (mW_x \times \%W_{x-1})/mW_{x-1} \quad \text{(Eq. 1)}$$

$$\%WL_x = 100 - \%W_x \quad \text{(Eq. 2)}$$

Where $\%W_x$ is the total weight (%) remaining on day x, where $\%W_1$ is the weight at the first day of drying (100%); mW_x is the mean weight of 3 pieces of the same treatment on day x; $\%W_{x-1}$ is the total weight (%) remaining of the pieces on day x-1; mW_{x-1} is the average weight of 3 pieces of the same treatment on day x-1; $\%WL_x$ is the weight lost (%) of each treatment on day x in relation to the start of processing. Weight loss (WL%) was expressed as the percentage of the initial weight that was lost.

Microbiological analysis

Bacterial counts of coagulase-positive *Staphylococcus*, *Salmonella* spp. and thermotolerant coliform at 45°C were performed in the raw mixture and the salamis (1st day) in duplicate to evaluate the health and safety of the process as well as the effects of processing. Lactic acid bacteria were monitored to evaluate their resistance to processing and the propolis treatments employed.

For the presumptive analysis of *Salmonella* spp., the VIP® kit from Biocontrol for processed foods was used as indicated by the manufacturer. To count thermotolerant coliforms Petrifilm™ from 3M was used and coagulase-positive *Staphylococcus* was counted by inoculating samples onto the surface of sterile plates with Baird-Parker agar (LANCETTE and TANINI, 2001). Lactic acid bacteria were counted using MRS agar (DeMan, Rogosa, Sharpe Agar) and incubating the plates. The colonies were counted after 72 h of incubation at 37°C (GROSSO and FAVARO-TRINDADE, 2004).

Sensory analysis

An acceptance test was performed with 50 habitual salami consumers (selected according to willingness to participate) to determine whether the addition of propolis affects the sensory properties of salami. The sensory analysis was carried out in the Sensory Analysis Laboratory of Agro-industry, Food and Nutrition at São Paulo University in individual tasting booths under a fluorescent white light with 50 assessments per treatment in each period (1, 30, 60, and 90 days). The samples were presented in random order. The consumer acceptance test was conducted according to MEILGAARD *et al.* (1999) for the evaluation of taste, aroma, appearance and overall acceptance.

The slices of 2 mm thick were presented one at a time in disposable white plastic plates coded with random three-digit numbers. A nine-point structured hedonic scale was used, ranging from "disliked extremely" (1) to "liked extremely" (9).

Statistical analysis

TBARS, L*, a*, A_w and pH analyses were evaluated according to a completely randomised design (CRD) in a 3x4 factorial array according to the model specified in Equation 3:

$$Y_{ijk} = \mu + G_i + T_j + GT_{ij} + e_{ijk} \quad \text{(Eq. 3)}$$

Where, Y_{ijk} = value observed for the variable studied (TBARS, L*, a*, A_w and pH) in repetition k [k=1 (process 1), k=2 (process 2) and k=3 (process 3)] at storage day j [j=1 (1 day), j=2 (30 days), j=3 (60 days) and j=4 (90 days)] in comparison group i [i=1 (SE - control), i=2 (FP - free propolis) and i=3 (MCC - microencapsulated propolis)]; μ = a constant inherent in all observations (average); T_j = effect of the *j*th storage day; G_i = effect of the *i*th comparison group; GT_{ij} = effect of the double interaction of comparison group i with storage day j; e_{ijk} = experimental error associated with the variable studied in repetition *k* at storage time *j* and in comparison group *i*, assuming NID (0, σ_e^2).

Weight loss during the drying process of the salami tests was evaluated following the statistical model in Equation 4:

$$Y_{ijkl} = \mu + G_i + T_j + P_k + GT_{ij} + GP_{ik} + TP_{jk} + e_{ijkl} \quad \text{(Eq. 4)}$$

Where, Y_{ijkl} = value observed for the weight loss variable in repetition I [l=1 (process 1), l=2 (process 2) and l=3 (process 3)] of material processing k (k=1 to 3) at drying time j (j=1 day to j=32 days) and in comparison group i [being i=1 (SE - control), i=2 (FP - free propolis), i=3 (MCC - microencapsulated propolis)]; μ = a constant inherent in all observations (average); G_i = effect of the *i*th comparison group; T_j = effect of *j*th drying time; P_k = effect of *k*th processing where; GT_{ij} = effect of the double interaction of comparison group *i* with drying time j; GP_{ik} = effect of the double interaction of comparison group *i* with material processing *k*; TP_{jk} = effect of the double interaction of drying time j with material processing k; e_{ijkl} = experimental error associated with repetition *l* of material processing *k*, drying time *j* and comparison group *i*, assuming NID (0, σ_e^2).

For sensory analysis, which evaluated the attributes of appearance, aroma, flavour and overall quality, a mixed model was used according to the fixed effects used in the previous model, and the random effect of the consumer was included according to the recommendations of O´MAHONY (1986).

To compare groups regarding the main effect the multiple comparisons procedure of Student's *t*-test was used. In the case of significant interactions, the F test was used and, when necessary, regression analysis was also performed for stability studies. All tests were performed with the aid of the Statistical Analysis System© (SAS, 1995) using the PROC MIXED procedure.

RESULT AND DISCUSSION

It was possible to produce salami with either free (pure propolis powder obtained by atomizing) or microencapsulated propolis incorporated into it. However, the microencapsulated form was easily dispersed in the material, whereas the pure for clumped.

Lipid oxidation analyses

There was no significant difference between the TBARS values (approximately 0.47 mg MDA/kg) measured in the raw mixture and those of the salamis on the first storage day. Therefore, the presence of propolis (free and microencapsulated) and sodium erythorbate was effective and/or the conditions used in processing did not promote the oxidation process.

There was also no significant difference between the treatments with respect to TBARS values throughout the storage period under the conditions employed in this study (Fig. 1). This result allowed us to infer that, with respect to lipid oxidation, both free and microencapsulated propolis performed as well as sodium erythorbate. This result proves that the microencapsulated propolis was released from the microcapsule, because it acted as effectively as an antioxidant as the other additives used.

Fig. 1 shows a significant increase ($p<0.05$), although very small, in TBARS values with storage time that is independent of the treatment applied. The TBARS values, even after 90 days of storage, were considerably less than values close to 1 mg MDA/kg of fermented and dry meat sausages observed in another study (MARCO *et al.*, 2008). This result suggests that the conditions employed in the processing and storage together with the presence of erythorbate or free or microencapsulated propolis prevented lipid oxidation in the products, contributing to their preservation.

Microbiological analysis

The Italian-type salamis were within the standards of microbiological quality (BRASIL, 2001) for coliforms at 45°C, *Staphylococcus* co-

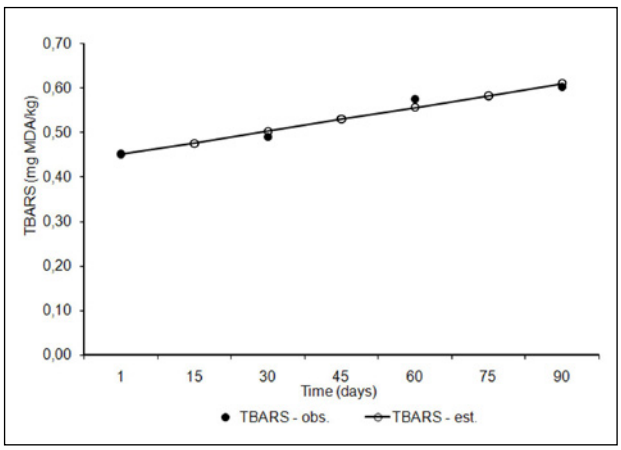


Fig. 1 - Results observed (obs.) and estimated (est.) for TBARS values (y = 0,4492 + 0,001783x) in Italian-type salami during 90 days of storage.

Table 1 - Microbiological evaluations of the raw mixture and prepared salamis (Day 1).

Microorganism	Sample	Treatments		
		SE	FP	MCC
Coliforms at 45°C (CFU/g)	Raw mixture	$1.7x10^2$	$1.7x10^2$	$3.0x10^1$
	Salami	<10	<10	<10
S. coagulase-positive (CFU/g)	Raw mixture	$<10^6$	$<10^6$	$<10^6$
	Salami	<10	<10	<10
Salmonella spp. (per 25 g)	Raw mixture	Absent	Absent	Absent
	Salami			
Lactic acid bacteria (CFU/g)	Raw mixture	$2.3x10^6$	$8.0x10^6$	$1.7x10^6$
	Salami	$1.3x10^7$	$2.4x10^7$	$2.2x10^7$

SE = control salami with sodium erythorbate; FP = salami with free propolis; MCC = salami with microencapsulated propolis.

agulase positive and *Salmonella* spp. (Table 1). Thus, it is observed that the sausages were produced properly in respect to good manufacturing practices for food and is suitable for use in sensory evaluation.

Considerably lower coliform counts were observed in the salami than in the raw mixture. This reduction can be attributed to the action of lactic acid bacteria, which can inhibit competing microorganisms by producing bacteriocins and by reducing the pH through the production of organic acids (HUGAS and MONFORT, 1997). Other factors are likely to have been responsible for the reduction of undesirable microbes, such as reduced A_w, the presence of nitrite and sodium nitrate and the presence of propolis that have antimicrobial properties.

The results observed for the lactic acid bacteria counts, approximately 10^7 CFU/g, are consistent with other studies whose counts were 10^6 for the raw mixture and 10^3 to 10^9 CFU/g in fermented sausages (DEL NOBILE *et al.*, 2009, ARO ARO *et al.*, 2010).

The sausages produced with propolis had the same lactic acid bacteria counts as those produced with erythorbate. This result suggests that the propolis used had no antimicrobial action against the starter culture, which is one more factor favouring its use as an additive in sausages.

Weight loss

Weight loss was significantly affected by the drying time ($p<0.05$) (Fig. 2) in a nonlinear manner. By the 8th drying day, the salamis had already lost about 20% of their weight, reaching a total of nearly 41% at the 32nd day. This drying behaviour differs from the linear weight loss observed by GARCIA *et al.* (2000) in Italian-type salami, even with a weight loss of 44%, higher than observed in this experiment. No differences were observed with respect to treatment ($p>0.05$).

Even with the non-linear weight loss of the salami, no crust formation was observed on its surface. This result shows indirectly that the amount of water removed from the product is dependent on the temperature and RH of the ripening chamber and the processing time (GARCIA *et al.*, 2000); thus, it appears that the procedures were conducted in a standardised manner. Other explanation is related to the increased acidification and is related to the lower pH found in the treatments, reducing the water retention capacity of the salami. Other study has reported weight loss of until 30% (GØTTERUP *et al.*, 2008), showing large variations between the processing of fermented and dry sausages, which may be due variables such as the formulation, the raw materials and the processing conditions.

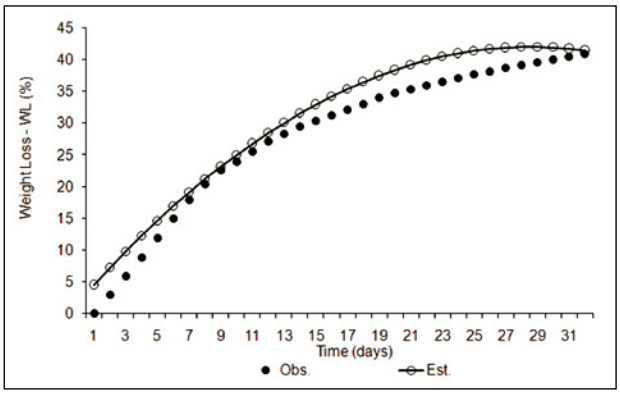


Fig. 2 - Observed (obs.) and estimated (est.) weight loss (%) results ($y = 1,7852 + 2,8163x - 0,04929x^2$) of Italian-type salami during 32 days of drying.

The above results indicate that the incorporation of propolis affected neither the drying process of Italian-type salami nor the materials used as coating agents, such as pectin and isolated soy protein.

pH

The pH measurements of the sausages (Fig. 3) showed a statistically significant interaction ($p<0.05$) between storage time and treatment.

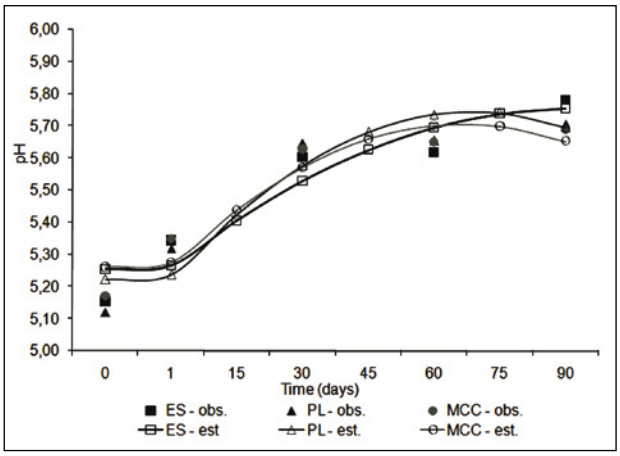


Fig. 3 - Observed (obs.) and estimated (est.) pH of Italian-type salami of different treatments during storage (SE: $y = 5{,}2541 + 0{,}01095x - 0{,}00006x^2$; FP: $y = 5{,}2219 + 0{,}01516x - 0{,}0001x^2$; MCC: $y = 5{,}2605 + 0{,}01335x - 0{,}00011x^2$).
SE = control salami with sodium erythorbate; FP = salami with free propolis; MCC = salami with microencapsulated propolis.

Time 0, in this case, indicates the salami after the end of the fermentation process, when the pH was reduced to between 5.10-5.20. This decrease is related to lactic acid production by lactic acid bacteria from the starter culture.

The pH increase during the 90 days of storage, also observed by LEE *et al.* (2009), may be related to the concentration of buffering proteins, the formation of ammonia and amines resulting from proteolysis by tissue enzymes or the decreasing dissociation of electrolytes in the middle (ARO ARO *et al.*, 2010).

Thus, it appears that the fermentation process was conducted properly and the desired pH of 5.0-5.2 was achieved at the end of fermentation. These results are consistent with commercial samples of Italian-type salami, which has a pH ranging from 4.86-5.78 (CACCIOPPOLI *et al.*, 2006).

The different treatments showed the same change in pH, as shown by the curves of Fig. 3, although there were points with slightly different results between treatments, as at the 75th and 90th days. However, small differences in pH between certain treatments in some areas are not significant from a technological standpoint.

Water activity

The water activity of the salami varied over time, showing a nonlinear reduction ($y = 0.86 - 0.00052x + 0.0000038x^2$) of A_w during the 90 days of storage.

The reduction of A_w from 0.86 on day 1 to 0.84 90 days later may be a result of free water binding to other compounds present in the material that were not identified. Similar behaviour was observed by LEE *et al.* (2009) in sausages stored for up to 120 days at room temperature of 25°C.

The observed reduction in A_w between the raw mixture (mean 0.97) and the prepared salamis at time point 1 (mean 0.86) is desirable, and it is due to the drying process, wherein the water essential for the growth of microorganisms is removed from the salami.

Salamis of all the treatments met the A_w standard established by the Technical Regulation of Identity and Quality for Italian-type salami, which is a maximum of 0.90 (BRASIL, 2000). The values obtained are also consistent with those of other study (HERRERO *et al.*, 2007). Thus, the incorporation of free or microencapsulated propolis left the A_w behaviour of this product unaffected.

Colour characterisation

For the Italian-type sausages studied, L* decreased linearly ($y = 40.90 - 0.01596x$) but modestly with storage time ($p<0.05$); however, there was no treatment effect. L* decreased from 40.86 to 39.38 during the storage period.

From the start of processing (raw butter) to the finished product (time 1), the salamis showed a reduction in the mean value of L* from 54.32 to 40.86. This decline seems related to the process of curing, drying and ripening. In particular, the drying process contributes to this decrease by concentrating the solids in the product by dehydration.

The L* value is highly variable in Italian-type salami sold in Brazil (CAVENAGHI and OLIVEIRA, 1999), and in typical Italian-type salami, L* values of 45.70 are observed (DEL NOBILE *et al.*, 2009). Thus, the L* values obtained herein are consistent with those obtained for similar products. This suggests that the incorporation of neither free nor microencapsulated propolis interfered with the lightness of the salami during its shelf life.

With respect to the a* parameter, only time contributed to the statistically significant linear increase ($y = 15.15 + 0.01772x$) in redness. In this case, a* increased from 15.23 to 16.77. Because it is an instrumental measure, this small difference of less than two units cannot be visually perceived by humans.

The increase in a* values may be related to the presence of viable lactic acid bacteria in the salami at the end of processing. *Staphylococcus xylosus*, which comprises the starter culture, shows nitrate reduction activity at the optimum temperature of 30°C, which is close to the storage temperature (25°C). This may have contributed to the formation of nitrosomyoglobin by residual nitrate reduction in the salami. Furthermore, the L* reduction observed may have helped to increase the intensity of the redness. The packaging used had an oxygen barrier and, together with storage away from light, was sufficient to prevent oxidation of the meat pigments, be-

cause the rate of reddening is more affected by oxygen than by light (MARCHESI *et al.*, 2006).

The a* values obtained also showed that there was no oxidation of nitrosomyoglobin of the salami during storage, unlike other studies that have indicated a significant correlation between the parameters of lipid oxidation (which increased with respect to time) and colour in vacuum packaged sausage (ZANARDI *et al.*, 2002).

The values obtained are lower than the 17.5-17.8 observed by GARCIA *et al.* (2000) and higher than the 7.99 observed by DEL NOBILE *et al.* (2009). This difference can be attributed to the raw meat used, which may have varying amounts of myoglobin, and oxidation of the red pigment.

Sensory analysis

The acceptance showed interaction between storage time and treatment with significant differences ($p<0.05$) in the appearance of the Italian-type salami of different treatments.

In general, the appearance of the sausage was accepted. The FP and MCC salamis received constant evaluations over the 90 days of storage in the range of "like regularly" (mean 7.0), whereas the SE salami showed a linear increase ($y = 6.4 + 0.007x$) in the same period from "like slightly" (mean 6.5) to "like regularly" (mean 7.2). On days 1 and 30, the salamis of treatments FP and MCC were the most accepted ($p<0.05$), whereas on the remaining days, no differences were observed ($p>0.05$) between the treatments applied.

In general, the FP and MCC salami had the most acceptable appearance over the 90 days of storage, and the SE salami was the least accepted. For the panellists, the MCC salami was characterised mainly by presenting a uniform appearance. Thus, in regard to the attribute of appearance, the addition of propolis did not impair the product's acceptance.

Regarding the attribute of aroma, the sausages studied differed only with respect to treatment ($p<0.05$), with no effect of storage time. The SE salamis showed a slightly higher acceptance ($p<0.05$; mean 7.3, in the range of "like regularly") than those from treatments FP and MCC (means of 6.6 and 6.8, respectively, in the range of "like slightly"). The more accepted aromas were described by the judges as characteristic, smooth and pleasant. Some panellists identified the odour of propolis in the FP and MCC salamis, they were unaware of the addition of this additive at the time of the evaluation, which may explain the lower acceptance of these.

However, the MCC salami was more accepted than the FP, which confirms that the encapsulation process was effective at attenuating the aroma of propolis, although incompletely so.

At no time did any consumer comment about the presence of a rancid aroma in the products, even after 90 days of storage. This confirms that the salamis remained stable with respect to lipid oxidation, confirming the results of TBARS.

The flavour of the SE salami increased linearly ($y = 6.8 + 0.0087x$) during the storage period of 90 days, that of the FP salami remained constant (mean 6.6), while the MCC salami's flavour declined between the 1st and 30th days but subsequently increased until the end of the 90 days of storage ($y = 6.7 - 0.026x + 0.00029x^2$). Until the 30th day, the treatments showed no difference between them ($p>0.05$), and subsequently, the SE salami was more accepted for its flavour ($p<0.05$) than the salami of the FP and MCC treatments, which did not differ ($p>0.05$).

The microcapsule incorporation was not effective for softening the flavour of the product, which was comparable to that with propolis incorporated in the free form (FP). Some consumers indicated that the FP and MCC salamis were less acceptable, with a strong residual uncharacteristic flavour that was unpleasant. However, they did not indicate that the flavour was directly from propolis. This suggests that the microcapsules released the compounds responsible for the characteristic propolis flavour during the storage, which was identified in the sensory analysis.

Only an effect of treatment was observed for the overall quality attribute. The control was significantly more accepted ($p<0.05$) than the treatments with propolis, which did not differ from one another. However, in general, the salamis were well accepted, with means that ranged between "like regularly" (mean 7.1 for SE) to "like slightly" (mean 6.7 for the FP and MCC treatments).

The lower acceptance assigned to treatments is possibly due to the aroma and taste of propolis, which persisted in the products. However, the difference in acceptance, although significant, was relatively small and should not be the only factor to consider in choosing or rejecting this additive, because propolis is a natural additive and has several therapeutic properties that may confer functional value to the product (PENA, 2008).

The SE salamis were the most accepted for the tested attributes, except for appearance, for which the MCC treatment was more accepted.

CONCLUSION

It is possible to prepare Italian-type salami using propolis as a replacement for the synthetic antioxidant sodium erythorbate. The products have similar technological characteristics to control products (SE treatment) and slightly lower sensory acceptance with respect to aroma acceptability. The uses of microencapsulated propolis are advantageous with regard to ease of incorporation of the additive to the raw mixture and because this process will be reduce

some strong sensorial characteristic of propolis. Thus, further research is needed with other microencapsulation techniques or even other microencapsulating agents to mitigate the strong aroma of propolis.

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PAPER

TABLE OLIVE CONSUMPTION BY SOCIOECONOMIC AND DEMOGRAPHIC GROUPS OF CONSUMERS IN TURKEY

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ABSTRACT

The objective of this study is to analyze the possible socioeconomic and demographic effects on consumers' table olive consumptions in Izmir. A questionnaire has been conducted with the participation of 384 consumes by using the face to face interview method in 2012. The binomial Logit was used to analyze data collected. According to the results of the analysis, consumer's gender and age , the size of the household, the location of olive production and also growing up in the city of Izmir have significant effects on the consumption of table olive. The findings will help table olive producers and marketing organizations to understand the critical factors that affect consumers' table olive consumption behavior and help them to make improvements accordingly.

- Keywords: consumption, consumer behavior, Mediterranean diet, table olive, Turkey -

INTRODUCTION

Known as Mediterranean diet, because of the spreading popularity owing to the healthy nutrition aspects of this diet (HELSING and TRICHOPOLOU, 1989), olive is consumed all over the world for health, personal hygiene and cleaning products as well as its usage for nutrition and nourishment features (LANZA and CORSETTI, 2009). Nowadays, people, who want to live a long and healthy life, have understood the importance of this diet and they have begun consuming more olives, which is one of the most important elements belonging to this diet. In fact, epidemiological data shows that the Mediterranean diet has significant protective effects against cancer and coronary heart diseases (KEYS, 1995; TRICHOPOULOU and LAGIOU, 1997).

As being the symbol of the peace on earth, olive is one of the most important trading prospects (commodities) of the Mediterranean countries as well as it represents a different aspect of Turkish culture and plays important role in Turkish Economy. The share of the table olive production of Turkey in the world is about 17%. Turkey is the number one country in the world for producing black olives. Moreover, 11% of the world table olive consumption was provided by Turkey (ANONYMOUS, 2013).

Olive fruits cannot be consumed as they are harvested from trees. In general, every processing method aims to remove the natural bitterness of this fruit, caused by the glucoside oleuropein (AKTAN and KALKAN, 1999). After removing this natural bitterness, the table olive is produced in two commercial types: green and black types. An amount of 84.2% of the total olive production has been processed as black and 15.8% as green (ANONYMOUS, 2013).

Per capita olive consumption is 0.35 kg in Argentina, 1.88 kg in Greece, 3.13 kg in Egypt, 3.69 kg in Turkey, 3.88 kg in Spain and 6.11 kg in Syria (ANONYMOUS, 2011). The reason for why Turkey takes place in the fourth place in the world olive consumption is because olives constitute a major part of traditional Turkish breakfast combined with bread, cheese, and of course black tea.

Besides its high nutritive values, fiber content, good taste, and high protein amount, olives contain also the mandatory amino-acids (especially leucine, aspartic acid and glutamic acid), unsaturated acids, vitamins and main nutrients, which are the main reasons of its consumption and its products (SIMOPOULOS, 2001; ERTAS, 2011). And also the consumers pay attention to core growth, color, salt portion and package of olives.

Lately, about the olive oil, which is one of the most significant components of the Mediterranean diet, there have been many researches, such as consumer preferences (AKBAY, 2007; DELGADO and GUINARD, 2011; DEKHILI *et al.*, 2011; GUERRERO *et al.*, 2012), sensorial assessment and consumer perception (SATOSA *et al.*, 2010; YILMAZ and AYDENIZ, 2012), importance of origin cues (DEKHILI *et al.*, 2011) and consumer behaviors (TIRYAKI, 2008; Duquenne and Vlontzos, 2012). However, the analysis of the factors affecting the table olive consumptions and decisions of consumers has not been found in the literature. Therefore, the main objective of this paper is to provide a better understanding of consumers' socioeconomic and demographic characteristics affecting the table olive consumption.

In this study, Binomial Logit Model is estimated using data collected from the consumers in Urban area of Izmir. Section two briefly describes the material and method and is followed by results and discussion. Conclusion and implication for future research are then presented.

MATERIAL AND METHOD

The data used in this study were collected from the consumers in Urban area of Izmir, Turkey. Sample size was calculated using Proportional Sampling Method which makes it possible for researcher to achieve greater representativeness in the sample of the population. According to this method, samples was selecting randomly from the subgroup in proportion to the actual size of the group in the total population (NEWBOLD, 1995; MIRAN, 2013):

$$n = \frac{N * p * (1-p)}{(N-1) * \sigma_p^2 + p * (1-p)} = 384$$

where, n is sample size, N is population size, σ_p^2 is variance (in 95% confidence interval and 5% means deviation) and p is the estimating rate (for this study, it is assumed to be 0.5 for reaching maximum sample size). The questionnaire was completed with a total of 384 randomly selected consumers in 2012. Interviewers collected the data in a home visit conducting face-to-face interviews with the consumers in order to encourage a high level of cooperation and complete reporting.

In this study, binomial logit analysis was implemented to identify consumers' socioeconomic and demographic characteristics effective in table olive consumption. The model is assumed that a consumer has faced with making choices between two alternatives: consuming or not consuming table olive.

If it is assumed that a member of consumer preference alternatively from the two options, then the observations of the choices reveal the consumer's preferences:

$$U_{ij} = \overline{U} + \varepsilon_{ij}$$

If we observe that a consumer (designated *i*) chooses table olive consumption, this implies

that: $U_{i1} > U_{i0}$, where U_{i1} and U_{i0} are the utilities that i associates with table olive consumption and non-consumption, respectively. The utility U_{ij} that the alternative j (j = 1: consume; j = 0: not consume) gives individual i, is composed of two parts: a systematic term, which depends on an attributes vector X, and another random one ε_{ij}. But utility U_{ij} is not observable. What we observe is decision Y_i, which is worth 1, if individual i chooses table olive consumption and 0 otherwise. If a rational individual chooses the alternative that gives her/him the greatest utility, then we would have:

Probability [Yi =1] Probability [$U_{i1} > U_{i0}$]
Probability [Yi =0] Probability [$U_{i0} > U_{i1}$]

In this case the probability that the consumer i chooses alternative 1 is (MCFADDEN, 1974).

In a logit model, it is postulated that its probability distribution conditional on a vector of covariates Xi is given by:

$$\pi(x) = P(Y = 1 \mid X) = \frac{e^{\alpha + X\beta}}{1 + e^{\alpha + X\beta}}$$

This would be the reduced form for the binomial logit model, where the row vector of explanatory variables for the i^{th} individual contains the independent or explanatory variables considered in the previous section and where we assume that the non-observed ε's follow a distribution of logistic probability (GUJARATI, 1995).

RESULTS AND DISCUSSIONS

Descriptive statistics regarding the consumers participated in the questionnaire are presented in Table 1. Fifty percent of the consumers participated in the questionnaire are male. The average age of consumers is 36.6 and 57% of the consumers are between 26 and 45 years old. 54% of the consumers are college and university graduates and 77% of consumers are married. In the research area, average household size is 3.46. The average olive consumption amount of the consumers has been calculated as 1.74 kg per month. The 82% of the consumers were born in Izmir and grew up with the culture of the location and 89% of them question the origin of the olive. According to results, 21% of the participants are consuming table olive.

Binomial Logit Model estimates the probability of whether or not the consumer consumes table olives. The results of maximum likelihood estimation of this model are presented in Table 2. Overall performance of model is statistically significant. Log likelihood is the basis for tests of a logistic model. Likelihood Ratio Test that tests whether all the coefficients in the Binomial Logit Model equal zero or joint significance of all coefficient estimates is highly significant at the %1 level as indicated by the chi-square value of 121.69. Eight of 13 variables in the model are statistically significant at 10% level.

For the table olive consumption: gender, age and education level of consumers, size of household, the origin of olive and the culture of Izmir

Table 1 - Descriptive statistics.

Variable name		Mean	Std. Dev.
Gender	Gender of consumer (Woman:0, Man:1)	0.50	0.50
Age	Age of the consumer (year)	36.61	12.31
Age1*	1:If less than 26 years old; 0:Otherwise	0.22	0.41
Age2	1:If between 26 and 45 years old; 0:Otherwise	0.57	0.50
Age3	1:If older than 46 years old; 0:Otherwise	0.21	0.41
Edu1*	Elementary school:1; Otherwise:0	0.14	0.35
Edu2	Secondary school:1; Otherwise:0	0.14	0.35
Edu3	High school:1; Otherwise:0	0.17	0.38
Edu4	College and University:1, Otherwise :0	0.54	0.50
Marital	Marital status (Unmarried:0, Married:1)	0.77	0.42
Hsize	Number of members in the household	3.46	1.35
Inc	Household income (TL/month)**	2088.78	1216.93
Inc1*	1:If less than 1200 TL:1; 0:Otherwise	0.25	0.43
Inc2	1:If between 1250 TL and 2500 TL:1; 0:Otherwise	0.55	0.50
Inc3	1:If older than 2550 TL:1; 0:Otherwise	0.20	0.40
Origin	1: If consumer is asking country of origin	0.89	1.18
for table olives; 0:Otherwise			
Izmir	1: If consumers are from Izmir; 0:Otherwise	0.82	0.38
Table olive	1: If prefer to consume table olives; 0:Otherwise	0.21	0.41
Exp	Household expenditure for table olive (TL/month)**	18.34	14.85

*Reference category which indicates base variable omitted from models to avoid multicollinearity
**1$= 1.79 TL

Table 2 - The result of binomial logit model.

	Coef.	Std. Err.		Odds Ratio	Std. Err.
Gender	0.9299	0.3465	***	2.5343	0.8781
Age2	1.0100	0.5939	*	2.7456	1.6306
Age3	0.3617	0.6652		1.4358	0.9552
Edu2	0.3890	0.6076		1.4755	0.8966
Edu3	1.0842	0.6098	*	2.9571	1.8032
Edu4	1.1484	0.5323	**	3.1532	1.6785
Hsize	0.2708	0.1279	**	1.3111	0.1677
Marital	-0.1204	0.5364		0.8865	0.4756
Inc2	0.3427	0.4271		1.4088	0.6016
Inc3	0.8007	0.5225		2.2271	1.1637
Origin	0.9962	0.1374	***	2.7080	0.3722
Izmir	1.5739	0.6108	***	4.8256	2.9476
constant	-7.3703	1.1030	***		
Log likelihood	-138.3014				
LR $X^2(12)$	121.69***				

***, ** and * indicate statistical significance at the 1%, 5% and 10% levels, respectively.

Province are the major factors. According to the results of the analysis, gender of consumer has a positive effect on the olive consumption decisions. Odds Ratio results show that the probability of men, comparing to women, belonging to the group of olive consume is 2.53 times higher than belonging not to this group.

There has been found a positive relation between age of consumer and table olive consumption. The consumers between ages 26 and 45 consume more olives than the consumers under the age of 26. As the Odds Ratio results have proved that the probability of belonging to the olive consuming group for the consumers between 26 and 45 is 1.63 times higher than the group, in which there is no olive consumption.

It is also proved that there has been a positive relationship between education and table olive consumption. The higher the education level becomes, the more table olive have been consumed. Consumers graduated from high schools or university consumes more table olives than the others. The probability of the high school graduates (Edu3) against the ones, who do not consume olive, is 2.96 times more likely being in the group of table olive consumption. And the same relation for the university or college graduates (Edu4) has been calculated as 3.15 times higher. The consumers, who only finished the elementary schools, should be informed more about the benefits of the olive to the health; then the amount of the consumption could be raised to a higher level.

There has been also a positive relationship between the number of the individuals in household and table olive consumption. As the members of the household increase, the consumption of olive is increasing relatively as well. The probability of this equation is calculated as 1.31 times more likely than the other group of households.

Olive qualities may differ by its size, size of the core, oil rate and many others according to its species and origins. The consumers, who accustomed to the taste of one olive type, want to continue their habits and that is why they question the origins of the olive. The results have shown that there has been also a positive oriented relation between olive consumption and the questioning the origin of it. With another expression, the consumers, who pay attention to the origin of olives, consume more olives than the others. And the probability of this situation is 2.71 times more than the ones, who do not ask about the olives origins. This result overlaps with the previous researches. YILMAZ and AYDENIZ (2012) have found the positive relationship between taste and consumption of olives.

Furthermore, the consumers from the Izmir province and grew up with the nutrition habits of this area consume more table olives than the others. The probability of this likelihood is 4.83 times higher than the others. This explains us the differences between Izmir and the other regions.

According to the results of the binomial logit analysis, it is concluded that household income is statistically insignificant. This is not surprising because olive is one of the most indispensable and vital components of the breakfast even among the families with low income in Turkey.

CONCLUSIONS AND RECOMMENDATIONS

The findings of this study have shown the relations and effects of the socioeconomic and demographic groups on table olive consumptions of the consumers. As mentioned before; gender, age and education of consumers, size of the household, origin questioning and the culture and nutrition habits of the region have significant influences on the olive consumption of the consumers. Within the area of the research, it is also found out that males consume more olive

than women. For a higher amount of olive consumption among women, they should be gained the consciousness for the healing and other positive effects of the olive (against cancer and other coronary heart diseases) on the human health system. Thanks to this awareness of olive consumption, mothers will also lead and influence their children positively towards to the olive consumption. Thus, there will be a wide and conscious wave on the olive consumption not only for present, but also for the future generations. As the one of the main components of breakfast culture besides cheese and bread, the olive consumption rate increase depending on the size and members of the household. Even among the families with low income, olive is one of the most indispensable and vital components of the breakfast. As mentioned above, the consumer questioning the origin of olive consume more olives than the consumers, who do not care about its origins. This means that the consumers pay attention to their accustomed taste and flavor. Under the lights of this result, the producers and marketing organizations could give their priorities to the consumer habits and behaviors. The producers and marketing organizations can also utilize through the useful information about the olive on the packages or commercials of the olive products.

In Turkey, several commercials and advertisements are being broadcast for the increasing of hazelnut consumption, reducing on the cigarette addiction and fighting against the obesity under the name of 'Public Spot', which aims increasing the consciousness of the public about several important incidents or developments through the press publishing organs (ANONYMOUS, 2012). The advantages and benefits of the olive consumption and its significance for the Mediterranean diet could and should be added and published through these public spot advertisements. The olive containing the antioxidant materials, could reduce risk of the coroner-heart diseases and cancer, which are probably the most serious and dangerous diseases of modern time. Thus, health expenses related to these diseases would be spontaneously reduced and prevented as well. Additionally, publishing of these studies could have a great influence on the literature; thus the source materials about this subject could be created for the consumers, producers and related institutions and enterprises.

These socio-demographic factors should be taken into considerations by table olive producers and marketing organizations in the formulation of marketing strategies designed to promote consumption. The production depending on the needs and preferences of the consumers will also raise the marketing participation and share of the producers and marketing organizations. As long as the table olive consumption grows, the value of this fruit will also grow in the eyes of the public, and thus the marketers and producers will specialize in this field, so they could increase their profits. And hereby, this profit raise will contribute also the development of the country especially in rural areas, in which the agriculture has a great importance.

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PAPER

MICROBIOLOGICAL QUALITY OF LEAFY GREEN VEGETABLES SOLD IN THE LOCAL MARKET OF SAUDI ARABIA

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ABSTRACT

Fresh leafy green vegetables (LGV) are frequently linked with outbreaks of foodborne illness in humans worldwide. The objectives of this study were to investigate the prevalence of some common foodborne pathogens and indicator bacteria that are likely to occur in LGV. In this study 105 samples of LGV, namely, lettuce, coriander, leek, dill, rocket leaves, parsley, and green onions were investigated. High total mesophilic count (> 8 $\log_{10}$ CFU/g) was detected in several types of the analyzed LGV. The counts for *Enterobacteriaceae* ranged from 5.72 to 7.06 $\log_{10}$ CFU/g in lettuce and leek, respectively. High total coliform counts were also detected in the tested vegetables, where counts ranged from 5.86 to 6.59 $\log_{10}$ CFU/g. *Pantoea agglomerans* was recovered most frequently from the LGV (13% of the samples). *E. coli* was identified in rocket leaves and green onions. The resistance of the identified bacterial isolates to a set of 20 different antimicrobial agents was tested. Most of the bacterial isolates were sensitive except for *Stenotrophomonas maltophilia.* The presence of high total count, *Enterobacteriaceae* and coliform counts and the presence of *E. coli* entail implementing effective control measures to minimize risk factors that may be accrued from fresh LGV consumption.

- Keywords: microbial quality, leafy green vegetables, *Enterobacteriaceae*, total coliform -

INTRODUCTION

Fresh leafy green vegetables (LGV) are considered an important part of a healthy and nutritious diet. It was generally recognized that plant-based foods are free from enteric bacteria that may pose adverse health effects on human, and mostly foodborne ailments are associated with meat and poultry consumption (ALBRECHT *et al.*, 1995). However, LGV are frequently linked with outbreaks of foodborne illnesses in humans worldwide, particularly because these vegetables or their ready-to-eat (RTE) salads are frequently consumed raw or with minimal or no further treatment (ILIC *et al.*, 2012). The United States Centers for Disease Control and Prevention (CDC) reported that the number of outbreaks associated with fresh produce consumption has increased prominently during the past decade (1995-2005) (JOHNSTON *et al.*, 2005). Usually, several factors contribute to microbial contamination of fresh produce. These factors could be attributed to certain pre- and post- harvest practices including but not limited to the use of contaminated irrigation water, soil and seed quality, surrounding wildlife, inappropriate fertilizers, especially untreated animal manure, and harvesting and packing workers with poor commitment to good hygienic practices (FAN *et al.*, 2009).

People at high risk of contracting foodborne illnesses such as children, elderly, and immunocompromised people are more likely to be subject to developing infections from pathogens that may be harbored into contaminated LGV and their RTE salads. In the United States of America (USA) and Europe, pathogens such as *Salmonella* spp. and *Escherichia coli* O157:H7 are among the most common pathogens that occur in LGV, especially, lettuce, spinach and their RTE salads (GOLBERG *et al.*, 2011; TABAN and HALKMAN, 2011; TOMAS-CALLEJAS *et al.*, 2011). Raw vegetables have been reported as a source of enterotoxigenic *E. coli*, a causative agent of traveler's diarrhea (BEUCHAT, 1996). Lettuce and spinach contaminated with *E. coli* O157:H7 were involved in outbreaks of severe gastroenteritis in different states in the USA (FAN *et al.*, 2009). It was demonstrated that this organism could be adaptable to stressful environmental and nutrient scarcity conditions available in LGV (DELAQUIS *et al.*, 2007).

In a study by JOHNSTON *et al.* (2005), the total plate, total colifrom and *E. coli* counts were in the range of (4.5-6.2), (1.0-3.4) and (1.0-1.5) $\log_{10}$ CFU/g, respectively, for LGV and herbs collected from the southern USA. In a surveillance study of microbial quality of fresh produce in Singapore, no *E. coli* O 157:H7 or *Salmonella* spp. were detected, nonetheless, high level of coliforms was detected in bean sprouts and fresh-cut salad, with 50% of samples containing more than 5 $\log_{10}$ CFU/g (SEOW *et al.*, 2012). High prevalence rate (5.7%) of *Salmonella* spp. was reported in fifteen different types of fresh vegetables in Mexico. The most predominant isolate was *S. Typhimurium*. The same study reported isolating *S. Typhi* from parsley (QUIROZ-SANTIAGO *et al.*, 2009).

To our knowledge, no previous reports have been undertaken to explore the microbiological quality of fresh produce in Saudi Arabia. Therefore, the objectives of the current study were to 1) investigate the prevalence and the concentration of some common foodborne pathogens that are likely to occur in LGV along with indicator intestinal microflora, 2) identify differences in microbiological quality of different types of LGV, and 3) explore the antimicrobial susceptibility of the bacterial species isolated from LGV.

MATERIALS AND METHODS

Sample preparation and bacterial counts

In this study, the presence of some indicator microorganisms (*Enterobacteriaceae*, and total coliform), and other selected foodborne pathogens such as *E. coli* O157:H7, *Salmonella* spp.

and *Vibrio* spp. in 105 samples of different types of LGV (15 samples of each type) was evaluated. Different types of LGV, namely, lettuce, green onions, dill, coriander, leek, parsley, and rocket leaves were purchased from different major suppliers of vegetables in the local market of Hail region-Saudi Arabia between October/2011 and January/2012. The samples were purchased from 8 different gross suppliers to ensure the diversity and the representativeness of the samples for all the vegetables consumed in the region. The samples were chosen independently and randomly from the various locations. The samples were kept in an ice box after purchasing and analyzed the same day or the next day under aseptic conditions. A fifty gram sample of each of the LGV was placed in a sterile plastic bags and 10-fold serially diluted in 0.1% peptone water after being vigorously mixed for 2 min. Thereafter, appropriate dilutions were plated on tryptic soy agar (TSA) (Difco, Becton Dickinson, Spark, MD) for the enumeration of total mesophilic bacteria. The TSA plates were incubated at 37°C for 48 h. *Enterobacteriaceae* count was determined on violet red bile glucose (VRBG) agar (Oxoid, Hampshire, England); total coliform count was obtained by plating on violet red bile lactose (VRBL) agar (Oxoid). The VRBG and VRBL plates were incubated at 37°C for 24 h. A possible presence of *E. coli* O157:H7, *Salmonella* spp. and *Vibrio* spp. was examined using the overlay method (LEE and KANG, 2001). This method includes plating aliquots of the samples on TSA plates and incubation at 37°C for 2 h to allow injured cells of

pathogens to repair and resuscitate. Then about 7 ml of each of tellurite cefixime sorbitol MacConkey Agar (TC-SMAC) (Difco), xylose lysine deoxycholate iron agar (XLD) (Difco) and TCBS *cholera* medium (TCBS) (HIMEDIA Laboratories, Ltd, Mumbai, India) were overlaid onto the TSA, to test for *E. coli* O157:H7, *Salmonella* spp. and *Vibrio* spp, respectively, and incubated for additional 22 h at 37°C.

Identification of presumptive colonies

Typical colonies of *Enterobacteriaceae* (red or pink colonies on VRBG agar), coliform (red or pink colonies on VRBL agar), *E. coli* O157:H7 (colorless colonies on TC-SMAC agar), *Salmonella* spp. (black colonies on XLD agar), and *Vibrio* spp. (yellow or green colonies on TCBS agar). Suspected presumptive colonies from different media were streaked on TSA and were subject to biochemical identification using the PHOENIX Automated Microbiology System (PHX system; BD, Sparks, MD). Each of the selected suspected colonies was suspended into 1 ml of PHORNIX system ID broth. Twenty five microliters were transferred to the PHOENIX automated system instrument. The results were obtained within 12 h after incubation. The isolated suspected isolates were identified at the species level.

Susceptibility to antimicrobial agents

The susceptibility of the isolated bacterial species to different antimicrobials was also tested. Twenty different antimicrobials were tested, namely, gentamicin, amikacin, tobramycin, imipenem, meropenem, cefpodoxime, cefuroxime, ceftazidime, cefotaxime,nitrofurantoin, ciprofloxacin, levofloxacin, moxifloxacincefepime, aztreonam, ampicillin, piperacillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, and trimethoprim-sulfamethoxazole were tested against each of the isolated species as described by FUNKE and FUNKE-KISSLIN (2004). The results of antimicrobial agents' susceptibility of the bacterial isolates were recorded as percentage of resistant isolates for each species (the number of resistant isolates for a particular antimicrobial agent relative to the total number of resistant and sensitive isolates of the same bacterial specie).

Statistical analysis

The mean values obtained from the microbiological evaluation of LGV were analyzed by one-way analysis of variance (ANOVA) and subjected to Turkey test to determine the significant differences ($P< 0.05$) among the means using Origin 8.0 software (Origin Lab Corporation, Northampton, MA, USA).The results were reported in terms of colony forming units ($\log_{10}$ CFU/g).

RESULTS AND CONCLUSIONS

In the current study, the microbial quality of LGV produced in some of the local markets of Saudi Arabia was investigated. The counts for total mesophiles, *Enterobacteriaceae* and total coliform were determined. Also, the possibility for the presence of contaminating *E. coli* O 157: H7, *Salmonella* spp. and *Vibrio* spp. was also investigated. Remarkably, high total counts of mesophilic bacteria were detected in the seven LGV examined in the current study (Fig. 1). Counts were in the range of 7.8-8.4 $\log_{10}$ CFU/g of the vegetables. Although there was almost 0.6 $\log_{10}$ CFU/g difference in the mesophilic counts of the LGV, there were no significant differences ($P> 0.05$) in the counts among all of the seven LGV explored in the study. The highest mesophilic count was detected in the rocket leaves (8.4 $\log_{10}$ units) and the lowest was in lettuce.

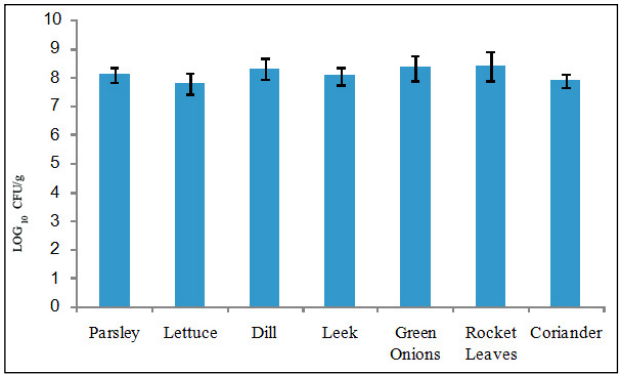


Fig. 1- Counts of total mesophilic bacteria ($\log_{10}$ CFU/g) in seven different types of leafy green vegetables. (N=105).

In comparison, the mean mesophilic bacterial count of LGV obtained in this study was higher than that reported in a recent study conducted under the Food and Drug Administration (FDA), where 100 bagged lettuce and spinach mixes were sampled. The mean total plate count was around 7.0 $\log_{10}$ CFU/g, with a range of < 4.0 to 8.3 $\log_{10}$ CFU/g (VALENTIN-BON *et al.*, 2008). Similar results for the total plate count of 140 lettuce samples in Spain were reported, where counts in the range of 3.01 to 7.81 $\log_{10}$ CFU/g were recorded (SORIANO *et al.*, 2000). In another study that included exploring the microbial quality of RTE leafy salads in Brazil, more than half of the samples (133 samples) had total aerobic counts of about 6.0 $\log_{10}$ CFU/g or higher (FRÖDER *et al.*, 2007).

High counts of *Enterobacteriaceae* were detected in the LGV (Fig. 2). Leek exhibited the highest count of *Enterobacteriaceae* (7.06 $\log_{10}$ CFU/g) and lettuce had significantly ($P < 0.05$) lower count compared to the other vegetables. Total coliform counts showed the same trend as for *Enterobacteriaceae*, where the counts ranged

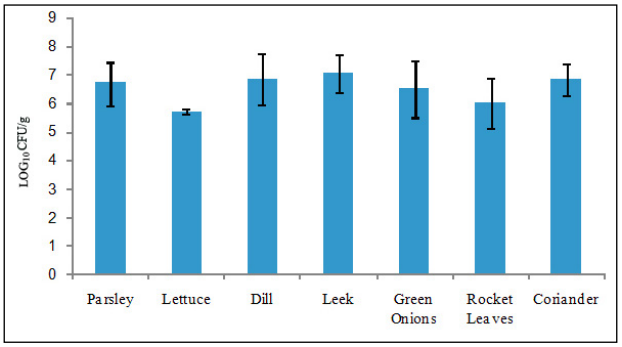


Fig. 2- Counts of *Enterobacteriaceae* (log_{10} CFU/g) in seven different types of leafy green vegetables. (N=105).

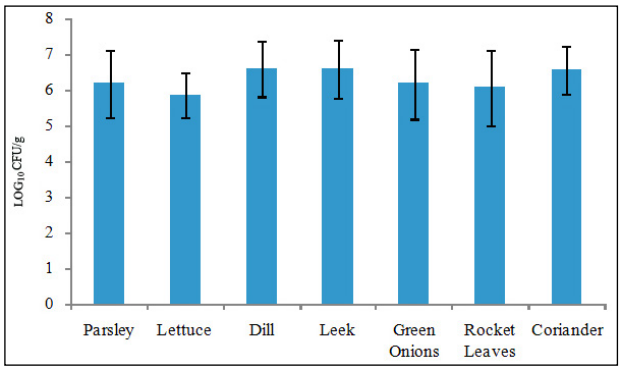


Fig. 3- Counts of total coliform (log_{10} CFU/g) in seven different types of leafy green vegetables. (N=105).

from 5.86 log_{10} CFU/g in lettuce to about 6.60 log_{10} CFU/g in leek and coriander (Fig. 3). Nonetheless, the differences in the coliform count among the seven LGV were not significant (P >0.05). In Brazil, the populations of total coliform and *Enterobacteriaceae* that exceeded 10^6 CFU/g in six different types of leafy vegetables and vegetable salads were 21 and 22%, respectively (FRÖDER *et al.*, 2007). Another study demonstrated that total coliform counts in cauliflower, broccoli, lettuce and tomatoes ranged from 4.89 to 6.30 log log_{10} CFU/g (ALBRECHT *et al.*, 1995), which is generally lower than the values reported for the total coliform in the present study. In the current study, all the samples were collected in the winter time (October-January) where temperatures are relatively low. However, it is anticipated that the bacterial counts of all tested bacterial groups to be higher if the samples were to be collected in the summer time.

Table 1 shows 23 different bacterial species that were isolated from seven different types of LGV. The majority of the isolates belong to the *Enterobacteriaceae* family (14 isolates). Whereas, the rest were *Aeromonas* spp., *Pseudomomas* spp. *Acinetobacter baumanni*, *Shewanella putrefaciens*, and *Stenotrophomona smaltophilia*. The most frequently isolated specie was *Pan-*

Table 1 - Frequency of different members of the *Enterobacteriaceae* and other bacterial species isolated from selected leafy green vegetables sold in the local market of Saudi Arabia. (N=105).

Isolated microorganisms	Parsley	Rocket leaves	Lettuce	Dill	Leek	Green onions	Coriander	Total
Acinetobacter baumannii	2	0	0	0	0	0	0	2
Aeromonas caviae	0	1	2	1	2	1	0	7
Aeromona sveronii	0	1	0	1	0	0	0	2
Citrobacte braakii	0	0	0	0	0	1	0	1
Citrobacter freundii	1	0	0	2	1	2	2	8
Citrobacte youngae	0	0	0	0	0	1	0	1
Escherichia coli	0	1	0	0	0	1	0	2
Enterobacter cloacae	1	1	0	1	4	2	0	9
Enterobacter gergoviae	0	0	1	0	0	0	0	1
Klebsiella pneumonia spp. *ozaenae*	0	1	0	0	0	0	0	1
Klebsiella oxytoca	0	1	1	0	0	2	2	6
Klebsiella pneumoniae spp. *pneumoniae*	0	0	0	0	1	3	0	4
Leclercia adecarboxylata	3	1	0	2	2	0	0	8
Pantoea agglomerans	3	0	2	1	1	2	5	14
Pseudomonas fluorescens	1	1	1	0	0	0	0	3
Pseudomonas pseudoacaligenes	1	0	0	0	0	0	0	1
Pseudomonas putida	0	0	1	1	0	0	1	3
Pseudomonas species	0	1	0	0	0	1	1	3
Serratia odorifera	0	0	1	0	0	0	0	1
Serratia marcescens	0	0	1	0	0	0	0	1
Shewanella putrefaciens	0	0	0	1	0	1	0	2
Stenotrophomonas maltophilia	0	1	0	0	0	0	0	1
Tatumella ptyseos	0	0	1	0	0	1	1	3

toea agglomerans. The frequency of *P. agglomerans* isolation was14 out of 105 samples. *Enterobacter cloacae* and *Leclercia adecarboxylata* were isolated at high frequency rates as well (9 and 8 times, respectively). *Citrobacter freundii* and *Aeromonas caviae* had also been isolated at a high frequency rates (8 and 7 times, respectively). Of a particular significance is *Acinetobacter baumannii,* an opportunistic pathogen that may cause pneumonia and infections of the urinary tract (MUSSI *et al.*, 2010). *E. coli* was isolated from rocket leaves and green onions. *E. coli* is often used as an indicator for fecal contamination, since this organism mostly emanate from intestinal tract of human and animals. The presence of *E. coli* could be attributed to the problem of utilizing untreated manure as soil fertilizers in the field or due to the use of contaminated irrigation water (AYCICEK *et al.*, 2006). Yet, this organism can be isolated also from non-fecal sources. In a study conducted to examine the microbial quality of baby spinach and romainie lettuce in the USA, no *E. coli* was detected, though the tested samples contained relatively high coliform counts (KASE *et al.*, 2012). However, in comparison, *E. coli* was detected in up to 9.7% of the tested samples of fresh produce, with the highest prevalence of the organism in organically grown compared to conventionally grown produce in Minnesota-USA. The level of *E. coli* contamination in organic produce reached 22.4% of the lettuce samples (MUKHERJEE *et al.*, 2004). Non-fecal coliforms such as *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp. have been isolated from several samples in the current study. However, their presence is not of public health concern as they exist commonly in water, soil and vegetation, and have long been recognized as common in fresh produce such as lettuces and fresh-cut salads (JOHANNESSEN *et al.*, 2002). Nonetheless, *Klebsiella pneumoniae* could cause pneumonia and urinary tract infections in people with weak immune system. A study conducted in Mexico that surveyed 130 salad samples made from vegetables irrigated with untreated sewage water indicated that about 85% of the samples contained *E. coli* and 7% of the samples harboured diarrheagenic *E. coli* pathotypes (CASTRO-ROSAS *et al.*, 2012).

All of the suspected isolated colonies from the 105 samples of the LGV were subject to further biochemical identification. Yet, none of the LGV samples was found to contain *Salmonella* spp., *E. coli* O157:H7, or *Vibrio* spp. This is in fact agrees with the results of the FDA survey which indicated that 99% of the local fresh produce sampled in the USA to be free of *Shigella*, *Salmonella*, and *E. coli* O157 (FDA, 2003). In another study undertaken to explore the microbial quality of fresh produce in Norway, *E. coli* O157:H7 and *Salmonella* spp. were not detected in about 900 samples of fresh produce (JOHANNESSEN *et al.*, 2002). The absence of such pathogens from fresh produce is not unusual, possibly because some natural microflora on LGV may pose competitive or inhibitory activity against some pathogens that may contaminate LGV such as *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Salmonella*, and *Listeria monocytogenes* (SCHUENZEL and HARRISON, 2002).

High bacterial counts of *Enterobacteriaceae* and coliform reported in the current study suggest that effective control measures should be applied to improve the microbiological quality of LGV produced and distributed in the local markets of Saudi Arabia. The most commonly used method to control microbial contamination of LGV is to apply chlorine-based washing solutions and less likely chlorine dioxide, electrolyzed oxidizing water, ozone, UV-light, hydrogen peroxide, peroxiacetic acid and organic acids (ILIC *et al.,* 2012). Slightly acidic electrolyzed oxidizing water treatment exhibited a considerable antimicrobial effect in reducing total mesophilic count, *E. coli* and *Salmonella* spp. in fresh produce and sprouts (ISSA-ZACHARIA *et al.*, 2011).

The resistance profile of *Enterobacteriaceae* members and other bacterial isolates to different antimicrobial agents is presented in Table 2. Twenty different antimicrobial treatments were examined against the 23 bacterial isolates. The percentages of resistant isolates that belong to the same bacterial specie were calculated (number of resistant isolates of the same specie relative to the total number of sensitive and resistant isolates of the same bacterial specie). Cefepim, piperacillin-tazobactam, levofloxacineceftazidime, and trimethoprim-sulfamethoxazole showed a strong antimicrobial activity against almost all of the bacterial isolates. *Stenotrophomonas maltophilia* exhibited a remarkable degree of resistance against the majority of the antimicrobial treatments used except for cefotaxime, ciprofloxacin, cefepim, piperacillin-tazobactam, levofloxacine and colistin. *E. coli*, on the other hand, was sensitive to all antimicrobial agents used.

This study was an attempt to shed light on the safety of the fresh LGV produced and sold in the local market of Saudi Arabia. Although no particular foodborne pathogen was detected, astonishingly, high total mesophiles, *Enterobacteriaceae*, and colifrom counts were detected in the LGV. This implies poor hygienic conditions of fresh produce. These results suggest implementing more strict hygienic practices during production, harvesting, packaging and transportation of fresh produce. Additionally, these results may also necessitate applying more sound agricultural practices and avoid using untreated animal manure or contaminated water for irrigation. At household level, refrigeration of the purchased vegetables, proper washing and using diluted chlorinated solution

Table 2 - Percentages of antibiotic resistance of members of *Enterobacteriaceae* and other bacterial species isolated from leafy green vegetables sold in the local market of Saudi Arabia.

Isolated organisms	*Type of antimicrobials*[*]												
	Ertapenem	Meropenem	Cephalothin	Cefuroroxime	Cefoxitin	Ceftazidime	Cefotaxime	Aztreonam	Amipicillin	Amoxicillin-clavulanate	Trimethoprim-sulfamethoxazole	Nitrofurantoin	Ciprofloxacin
Acinetobacter baumannii	100	0	100	100	100	0	0	100	100	100	0	100	0
Aeromonas veronii	100	0	100	0	50	0	0	0	100	0	0	0	0
Aeromonas caviae	100	0	100	100	100	0	0	0	100	0	0	0	0
Citrobacter braakii	0	0	100	0	100	0	0	0	100	100	0	0	0
Citrobacter freundii	0	0	100	100	100	14	14	0	85	85	0	0	0
Citrobacter youngae	0	0	100	0	100	0	0	0	100	0	0	0	0
Enterobacter cloacae	0	0	100	100	100	0	0	0	100	100	0	0	0
Enterobacter gergoviae	0	0	100	0	100	0	0	0	100	100	0	0	0
Escherichia coli	0	0	0	0	0	0	0	0	0	0	0	0	0
Klebsiella oxytoca	0	0	16	16	0	0	0	16	83	0	0	0	0
Klebsiella pneumoniae spp *ozaenae*	0	0	100	0	100	0	0	0	100	0	0	0	0
Klebsiella pneumoniae spp *pneumonia*	25	0	50	0	50	0	0	0	100	25	0	25	0
Leclercia adecarboxylata	0	12	12	0	0	0	0	0	12	0	0	0	0
Pantoea agglomerans	0	7	57	7	0	0	0	7	0	7	0	0	0
Pseudomonas fluorescens	0	0	100	100	100	0	0	0	100	100	0	66	33
Pseudomonas putida	0	0	100	100	100	0	33	33	100	100	33	100	0
Pseudomonas pseudoacaligenes	0	0	0	0	0	0	0	0	0	100	0	100	0
Pseudomonas species	0	0	0	0	0	0	0	50	0	100	0	100	0
Serratia marcescens	0	0	100	100	100	0	0	0	100	100	0	100	100
Serrata odorifera	0	0	100	100	0	0	0	0	100	100	0	100	0
Shewanella putrefaciens	0	0	0	0	0	0	0	0	0	100	0	100	0
Stenotrophomonas maltophilia	100	100	100	100	100	0	100	0	100	100	0	100	0
Tatumella ptyseos	0	0	0	0	0	0	0	0	0	0	0	0	0

*All isolated organisms were sensitive to cefepim, piperacillin-tazobactam, and levofloxacine.
All isolated organisms (except *Stenotrophomonas maltophilia* isolates) were sensitive to amikacin, gentamicin, and imipenem.
All *Enterobacteriaceae* isolates (except *Serratia marcescens*) were sensitive to colistin.

for treating LGV before consumption are prudent preventive measures that could considerably reduce the risk of contracting pathogens that may lodge on LGV.

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PAPER

ANTIOXIDANT ACTIVITY AND BIOCHEMICAL VARIATION AMONG JUICES OF DIFFERENT GENOTYPES OF SUGARCANE

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ABSTRACT

Sugarcane (*Saccharum officinarum* L.) juice is widely consumed by the people of the tropics and subtropics. It has been used to cure jaundice and liver-related disorders in medicine. Its possible mechanism of action was examined in terms of antioxidant activity and phenolic contents. The antioxidant activity was assayed by using the scavenging of a stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical at 517 nm. In addition, the phenolic content, reducing sugar, non-reducing sugar and Brix contents were measured to evaluate the biochemical variation. The juice from thirteen important genotypes of sugarcane was included in the study. Among these genotypes S-2003-CP-43-33, CP-77-400, SPF-213 and HSF-240 showed good antioxidant activities (IC_{50} values varied from 17.04±0.6 to 21.90±0.45 μg/mL). Cluster analysis showed wide variation between the genotypes based on biochemical assays. HSF-240 and CP-43-33 showed 50 gene-linkage distances in clustering pattern.

- Keywords: sugarcane (*Saccharum officinarum* L.) juice, antioxidant activity, sugars, Brix, phenolics, clutster analysis -

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is considered to be the most important cash crop not only in Pakistan but also in different parts of the world. It is cultivated on large scale in tropical and subtropical regions primarily for its ability to store high concentrations of sucrose or sugar in the internodes of the stem and raw material for industrial products such as furfural, dextrans and alcohol (MARTIN *et al.*, 1982). Some natural pharmaceutical compounds are also derived from sugarcane (MENEDEZ *et al.*, 1994). Additionally agricultural and industrial by-products of the sugar production process are extensively employed for animal nutrition, food processing, paper manufacturing and fuel (PATRAU, 1989). It is a primary raw material for the sugar industry as 70% of the world's sugar comes from sugarcane. Antioxidants recognized their ability to scavenge the free radicals and protect living beings from oxidative damage (SIES, 1996). Plants as well as animals are continuously exposed to free radicals. Free radicals are highly unstable and reactive molecules and present a formidable challenge to all living systems (HALLIWELL *et al.*, 1997). If left unchecked, they can cause oxidative injury by initiating chain reactions that disrupt membranes, denature proteins, fragment DNA and consequently in the end participate in cell death, ageing and cancer (AMES *et al.*, 1995). Sugarcane juice has broad biological effects in raising innate immunity to infections (LO *et al.*, 2005). According to OLIVEIRA and HORII (1998) the sugarcane juice obtained by grinding sugarcane stalk contains approximately 78 to 86% water, 10 to 20% sucrose, 1 to 2% reducing sugar, 0.3 to 0.5% ash and 0.5 to 1.0% of nitrogen compounds (OLIVEIRA and HORII, 1998). The pH of the sugarcane juice varies between 5.2 and 6.8 (CORAZZA *et al.*, 2001). The Brix variation in sugarcane juice is 14° to 22°, depending upon the extraction process. The sugarcane juice contains flavonoids such as apigenin, luteolin and tricin derivatives and among phenolics, hydroxycinnamic, caffeic and sinapic acid, representing a total content of around 160 mg/L (JOAQUIM *et al.*, 2006). The DPPH radical has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts and foods (PORTO *et al.*, 2000; SEOW *et al.*, 2012). In view of the potential role of sugarcane as a dietary source of flavonoids as well as its possible use in functional food, the study was aimed to evaluate the antioxidant activity, phenolic and sugar contents among thirteen genotypes of sugarcane. It is the first report on the antioxidant and biochemical analysis of the above sugarcane genotypes. The study is particularly important in view of the fact that sugarcane juice is widely consumed in summer, as a drink in Pakistan.

MATERIAL AND METHODS

Studies were conducted on thirteen genotypes of sugarcane which were collected from different growing areas of Pakistan. Genotypes selected for this experiment were HSF-240, SPF-213, CP-77-400, CP-43-33, HSF-242, NSG-60, NSG-45, CPF-198, Lho-83-153, NSG-555, CSSG-668, S-2003-US-718 and S-2002-US-133. All the genotypes were grown under glass house conditions under normal soil conditions in Faculty of Agriculture, University of the Poonch Rawalakot, A.K. Pakistan.

Sugarcane juice was extracted from the genotypes and their Brix content was analyzed through a digital refractrometer (AST, 1230, Japan).

Reducing sugar was estimated by using Benedict's method (A.O.A.C. 1990). Two gram of anhydrous sodium carbonate was added to 5 mL of Benedict solution, warmed it and finally titrated against the sugarcane juice drop by drop through burette until color is changed. The volume of sample solution was recorded in triplicate.

Non-reducing sugar was determined by using Benedict's method (A.O.A.C. 1990). The sugar solution was hydrolyzed with 5 M HCl for 30 min at 100°C and then titrated against the Benedict's solution as mentioned above.

The total phenolic contents were determined by adding 0.5 mL of the sugarcane juice to 2.5 mL, 10% Folin-Ciocalteau's reagent (v/v) and 2 mL of 7.5% sodium carbonate. The reaction mixture was incubated at 45°C for 40 min, and the absorbance was measured at 765 nm in the spectrophotometer (Shimadzu, Japan). Gallic acid used as a standard phenol (SINGLETON *et al.*, 1999). The mean of three readings were used and the total phenol contents were expressed as microgram of gallic acid equivalents/g extract.

Scavenging of the stable radical, DPPH was assayed *in-vitro* (HATANO *et al.*, 1988). Briefly 0.25 mM solution of DPPH radical (0.5 mL) was added to the sample solution in ethanol (1 mL) at different concentrations (25-100 μg/mL) of sugarcane juice. The mixture was shaken vigorously and left to stand for 30 minutes in the dark, and the absorbance was measured at 517 nm. The capacity to scavenge the DPPH radical was calculated using the following equation:

$$(\%)\ \text{scavenging} = [(Ao - A_1)/Ao)] \times 100$$

Where, Ao is the absorbance of the control reaction and A_1 is the absorbance of the sample itself. Ascorbic acid was used as a standard compound in the assay. The IC_{50} values (extract concentration that cause 50% scavenging) were determined from the graph of scavenging percentage against the extract concentration. All determinations were carried out in triplicate.

The results were expressed as means ± stand-

Table 1 - Cluster analysis of thirteen genotypes of sugarcane on the basis of biochemicals.

Clusters	Genotypes	Sub-Clusters	Genotypes
I	HSF-240, NSG-45, CPF-198,NSG-555 and Lho 83-153	IA	HSF-240
		IB	NSG-45, CPF-198, NSG-555 and Lho 83-153
II	HSF-242 and S-2002-US-133		
III	NSG-60, S-2003-US-718 and CSSG-668	IIIA	NSG-60 and S-2003-US-718
		IIIB	CSSG-668
IV	SPF-213, CP-77-400 and CP-43-33	IVA	SPF-213 and CP-77-400
		IVB	CP-43-33

ard deviation. The data was analyzed by one way ANOVA. $P < 0.05$ was considered significant in all cases. The software Package Statistica was used for the analysis of data.

RESULTS AND DISCUSSION

The Brix represents the amount of sucrose and is usually extended to the soluble sugars of the sugarcane juice (SINGH *et al.*, 2001). The concentration of sugars in sugarcane is highly variables seasonally; average contents range is from 15 to 20% (SMITH and INMAN, 2005). NOGUEIRA and VENTURINI (2005) state a variation in sugarcane juice which ranges between 14° to 22°. The value of Brix obtained for the raw sugarcane juice was 20° that can be considered normal during the present year. In our studies soluble sugars were found to be high in HSF-242 and CSSG-668, which were 15.6° and 12.8° respectively. Some genotypes showed very low Brix values such as CP-77-400 and NSG-60 (4.5° and 7.4° g/L respectively) (Fig. 1).

The amounts of reducing sugar in sugarcane juice showed a high variation among the genotypes. The highest reducing sugar content was found in S-2003-US-133 and Lho 83-153 (10.8 and 7.3 mg/mL). Whereas, the NSSG-55 and CSSG-668 showed the lowest values of reducing sugar which were 4 mg/mL (Fig. 2). SHARMA and CHATTERJI (1971) and KNAPP *et al.* (1965) observed 50% more reducing sugars in susceptible varieties to corn earworm. Since, sugar is considered as one of the important nutrients in plants and sugar contents reproduce the metabolic state of the sugarcane shoot, the differences in the relative amounts of sugars among different genotypes with differential susceptibilities to ESB point out that these compounds might act as phagostimulants to *C. infescatellus* feeding on sugarcane. *C. infuscatellus* is an insect stem borer that infests sugarcane. Similar relationship has been reported by RAO and RAO (1961) for *C. auricilius*.

The infestation of sugarcane stem borers was found to be inversely proportional to the quantity and quality of sugarcane. Maximum cane weight of 13.5 ± 0.21 and 12.1± 0.05 kg were recorded in CPF-237 and Thatta-10 varieties from Pakistan at 0% infestation level, while minimum at 20% infestation (9.3±0.12 and 8.5 ±0.12 kg), respectively. The highest sugar recovery (11.0 ±0.08 and 10.2±0.11%) was observed at 0% in-

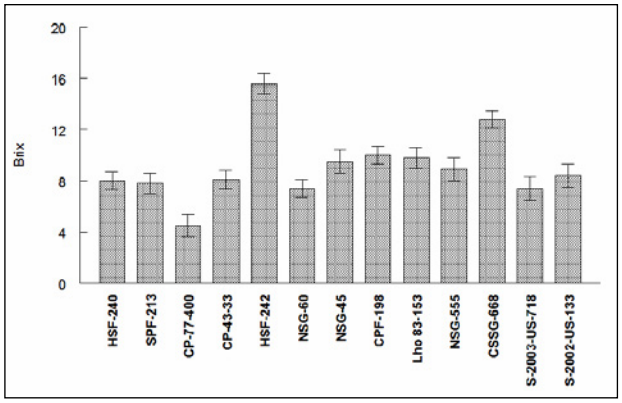


Fig. 1 - Variation of Brix among different genotypes of sugarcane. Values are mean ± SD (n=3). The mean values are significantly different ($P < 0.05$) by one way ANOVA.

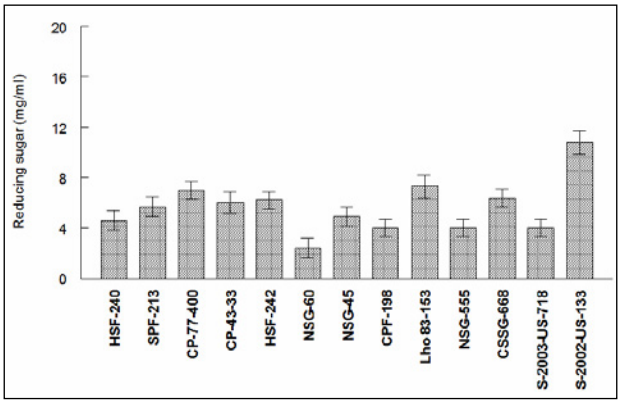


Fig. 2 - Reducing sugar contents among different genotypes of sugarcane. Values are mean ± SD (n=3). The mean values are significantly different ($P < 0.05$) by one way ANOVA.

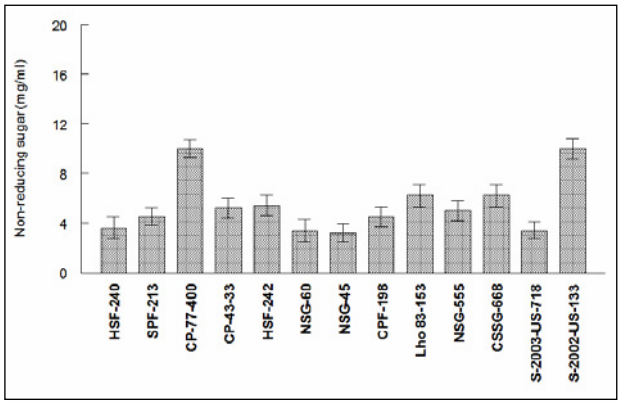


Fig. 3 - Non-reducing sugar contents among different genotypes of sugarcane. Values are mean ± SD (n=3). The mean values are significantly different (P < 0.05) by one way ANOVA.

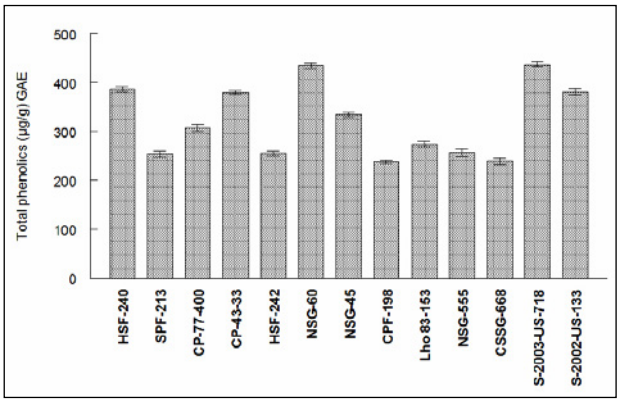


Fig. 4 - Total pheolics content among different genotypes of sugarcane. Values are mean ± SD (n=3). The mean values are significantly different (P < 0.05) by one way ANOVA.

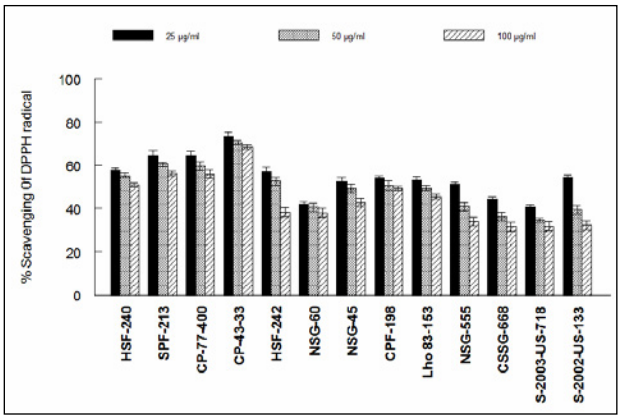


Fig. 5 - Antioxidant activity of sugarcane juice. Values are mean ± SD (n=3). The mean values are significantly different (P < 0.05) by one way ANOVA.

festation while, the lowest (9.3±0.12 and 8.5 ±0.12%) at 20% infestation in both the varieties (RAZZA *et al.,* 2012).

The non reducing sugar content was found between 3.2 to 3.4 mg/mL. The highest amount of non-reducing sugar was detected in CP-77-400 and S-2002-US-133 (10 mg/mL). While, the lowest amount was detected in NSG-45 and NSG-S-2003-US-718 (3.2 and 3.4 mg/mL) (Fig. 3).

The total phenolic contents of the sugar manufacturing products was determined according to the Folin-Ciocalteu,s method, which proved to be a suitable, simple, and rapid method for the estimation of total phenolic contents of various samples (SINGLETON *et al.,* 1999). The total phenolic contents of sugarcane genotypes ranged from 237.5 to 437.3 µg/g GAE. S-2003-US-718, demonstrated the high phenolic content (437.3 µg/g) as compared to CPF-198 (237.5 µg/g) (Fig. 4). It is noteworthy that high phenolic contents were observed for sugarcane because, they are highly colored materials and phenolic compounds are strongly involved in the formation of color (GODSHALL *et al.,* 2002). However, the values obtained with the Folin-Ciocalteu·s assay may be over estimated due to the existence of sucrose, glucose, fructose, and Millard's reaction compounds in the sugar products, which may interfere with the test by enhancing the development of the blue color (PAYET *et al.,* 2005, SINGLETON *et al.,* 1999).

Free radicals involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies such as cancer and cardiovascular diseases (HALLIWELL, 1992). 2, 2-diphenyl-1-picrylhydrazyl is considered to be a model of a stable lipophilic radical. A chain reaction of lipophilic radicals is initiated by lipid autoxidation. Antioxidants respond with DPPH•, reducing the number of DPPH free radicals to the number of their available hydroxyl groups. Therefore, the absorption at 517 nm is proportional to the amount of residual DPPH•. It is visually noticeable as a discoloration from purple to yellow. The results obtained in this study showed the high antioxidant activity of sugarcane genotypes and suggest their use in diseases arising from the attack of free radicals. The results indicated high antioxidant activity of sugarcane genotypes and suggested their use in diseases arising from the attack of free radicals. Such antioxidant activity of sugarcane has also been supported by the literature (JOAQUIM *et al.,* 2006; FABIANA *et al.,* 2008; ABBAS *et al.,* 2013). Among different genotypes CP-43-33, SPF-213 and CP-77-400 significantly displayed high DPPH radical scavenging activities (Fig. 5), whereas, S-2003-US-718, NSG-60 and CSSG-668 showed weak antioxidant activities (Fig. 5). It was visually noticeable as a discoloration from purple to yellow occurred and displayed antioxidant activity higher than 50% even at a low concentration of 25 µg/mL and was found to be comparable to that of ascorbic acid which is a well known antioxidant. However, there was a small but significant decrease in antioxidant activity with increasing concentration of juice (Fig. 5). This might be due to the pro-oxidant action of phenolics and flavonoids at higher concentrations. Compounds with antioxidant ac-

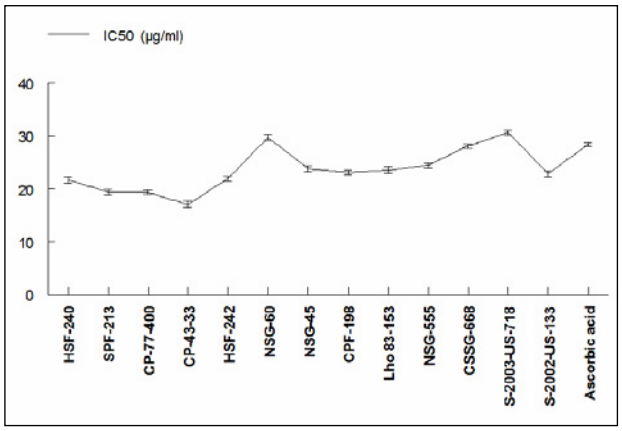


Fig. 6 - Calculated IC_{50} values (extract concentration that cause 50% scavenging) for juices of different sugarcane obtained by DPPH radical scavenging.

tivity may exhibit pro-oxidant behavior under certain conditions especially in *in-vitro* experiments. Pro-oxidant action can accelerate damage to molecules such as DNA, carbohydrates, lipids and proteins (AUROMA *et al.,* 1997; SABIR *et al.,* 2012).

The IC_{50} value (extract concentration that causes 50% scavenging) is inversely proportional to the scavenging activity of the extract. The IC_{50} values of different genotypes of sugarcane varied from 17.04±0.6 to 30.63±0.45 µg/mL showing the high radical scavenging ability of the genotypes compared. These values are comparable to the reference standard ascorbic acid (IC_{50} = 28.4 µg/mL). Among different genotypes, CP-43-33 showed the highest antioxidant activity (IC_{50}, 17.04±0.6) whereas, S-2003-US-718 relatively showed weak antioxidant activity (IC_{50}, 30.63±0.45 µg/mL). The genotypes such as NSG-45, CPF-198, and Lho-83-153 showed similar IC_{50} values (Fig. 6).

In cluster analysis, four clusters and six sub clusters were formed based on their biochemical expression. HSF-240, NSG-45, CPF-198, NSG-555 and Lho 83-153 fall in a same (cluster-I) as, these genotypes also had similarities in DPPH and Brix assays, but cluster I further split in to two sub clusters IA and IB. Both sub clusters were differentiated on the bases of phenolic content, non-reducing and reducing sugars (Fig. 7). Cluster II which comprise HSF-242 and S-2002-US-133 was not further divided into sub clusters because, these genotypes have same expression in all biochemical analysis. On the basis of phenolic contents and DPPH activity NSG-60, S-2003-US-718 and CSSG-668 fall in Cluster III and further sub clustered into IIIA and IIIB based on non-reducing, reducing sugars and Brix values. From cluster analysis, we concluded that cluster I and cluster III were present at a large distance and having maximum dissimilarities, which provide good idea for plant breeders to breed suitable varieties. TAHIR *et al.* (2013) showed genetic divergence among twenty five genotypes of sugarcane growing in different areas of Pakistan. Random Amplified Polymorphic DNA (RAPD) showed genetic diversity among twelve sugarcane genotypes (HSF-240, CP-77-400, HSF-242, CP-43-33 etc.) (MUMTAZ *et al.,* 2011). Therefore, the observed biochemical variation in this study may be due the genetic variation among different genotypes of sugarcane.

CONCLUSION

On the basis of the above results it is concluded that juices of sugarcane genotypes collected from different areas of Pakistan are the rich source of sugars. Among these genotypes, S-2003-CP-43-33, CP-77-400, SPF-213 and HSF-240 are potential candidates for antioxidant activities. They also underline importance

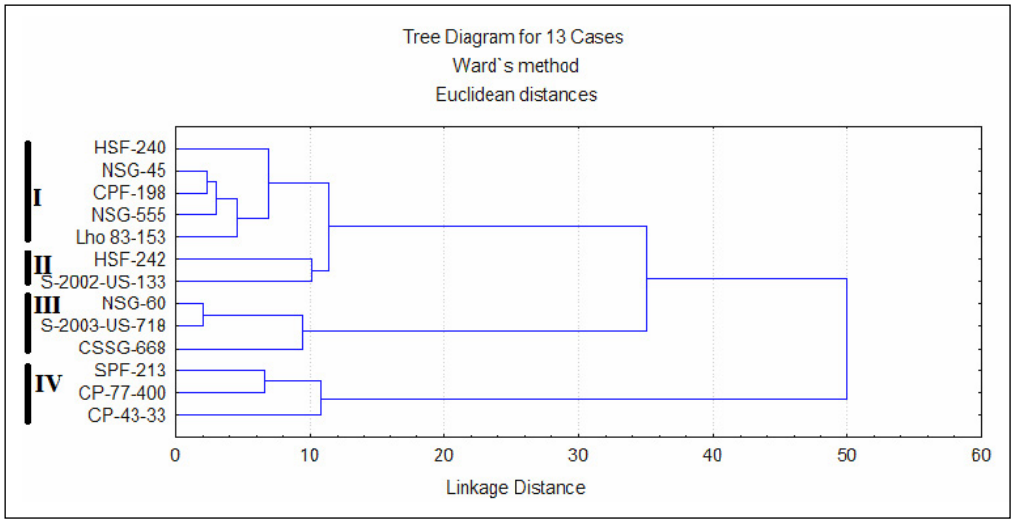


Fig. 7 - Biochemical dendogramme of thirteen genotypes of sugarcane.

as food supplements and the very promising source of indigenous drugs. In general, the variation among phytochemical contents and antioxidant activity indicates that the genetic variability can be used to develop cultivars with enhanced health benefits.

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PAPER

THE INFLUENCE OF TEMPERATURE AND STORAGE TIME ON CANTALOUPE MELONS PHYSICOCHEMICAL QUALITY

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ABSTRACT

Cantaloupe melons (*Cucumis melo* L. subsp. *melo* var. *cantalupensins* Naudin) *cv* 'Chianti' were evaluated for quality traits during 14 days of storage at three different temperatures i.e., 2°, 10° and 18°C with 85-95% relative humidity. The weight loss of fruits slightly increased during the stored period studied, as well as insignificant differences between the temperatures. Whereas significant texture was lost more rapidly in the samples stored at 18° and 10°C than those stored at 2°C. TSS (total soluble solids) were also affected by storage time and temperature. The TSS content of fruit at 2°C increased and then remained constant over storage. At higher temperatures and at every stage of storage time TSS increased as storage time increased. The predominant carotenoid in all samples was β-carotene. The carotenoids were increased and then decreased with the time; however, the decrease processes were delayed by low temperature. The alpha form was the predominant tocopherol fraction. The level of tocopherol isomers significantly (α-tocopherol) and gradualy (γ- and δ- tocopherol) increased during the 7 days, but after 1st week of storing for all isomers a significant decrease was measured. High temperature storage at 18°C in comparison to 10°C and 2°C promoted γ- and δ- tocopherol level.

- Keywords: Cantaloupe melons, storage, weight loss, texture, soluble solids, carotenoids, tocopherol -

INTRODUCTION

Cantaloupe melons (*Cucumis melo* L. subsp. *melo* var. *cantalupensins* Naudin) is one of the most important horticultural crops in the world of agriculture (NUÑEZ-PALENIUS *et al.*, 2008). According to BAN *et al.* (2011) the global consumption of these fruits are greater than that of any other species in the Cucurbitace family. Cantaloupes are a popular delicacy and exclusively consumed as fresh or minimally processed products such as fresh cut pieces of melon with rind or as cubes without rind.

Some researchers suggest that extract of cantaloupe melon may serve as a potential source of natural phytochemicals for food and nutraceutical application (LESTER, 2006). Over the last decade, there has been increased interest in plant foods that are rich in health-protecting source of minerals, vitamins and phytochemicals (VEBERIČ *et al.*, 2009). Among these, carotenoids and tocopherols constitute important groups in human diet (LANDRUM and BONE, 2001). The protection provided by fruits and vegetables against a wide range of several degenerative diseases, including cancer, cardiovascular diseases and other chronic diseases, has been attributed to the antioxidants they contain (SANGEETHA and BASKARAN, 2010). The reasons for this relationship appear to be multi-faceted and include components in plants that are used as a free radical scavengers, singlet and triplet oxygen quenchers, detoxification agents, or as plant defense response components (PERKINS-VEAZIE *et al.*, 2003).

One of the most important factors in the production and marketing of cantaloupes is the postharvest quality. Cantaloupe fruits in general have limited storage time and become soft and shriveled after about two weeks because the open, netted epidermis favors high transpiration rates (FALLIK *et al.*, 2005). The recommended storage temperature for whole cantaloupe is varied between 2 (FLORES *et al.*, 2007) to 18°C (RADULOVIČ *et al.*, 2007).

The development of new cultivars of cantaloupe melons with high added value and which are well adapted to the Slovenian growing conditions has stimulated the interest in the commercial cantaloupes production. During the 2012 season, study was conducted to evaluate the effectiveness of holding fresh cantaloupe fruits in different storage temperatures of 2°, 10° and 18°C to extend their post harvest life and maintain fruit quality parameters. Further, research focused on the degradation of carotenoids of cantaloupe fruits, which data are missing in the literature

MATERIALS AND METHODS

Processing of samples for analyses

Cantaloupe melon *cv* 'Chianti' (Semenarna d.o.o., Ljubljana) was grown at the Experimental Station of the Biotechnical Faculty in Ljubljana. Standard agricultural practices were adopted uniformly according to crop requirements. Thirty of the tagged fruits per plot were harvested on the same day when they reached the horticultural maturity (i.e. at the 3/4 to full-slip stage). After harvest fruits were held overnight at about 10°-15°C and transferred the following day to the laboratory. Fruits were randomly divided into three groups, each group containing 30 fruits in four replicates. Fruits were then placed in the chambers for 14 days at 85-95% relative humidity to simulate a commercial storehouse situation. The first group was stored at 2°C, the second one at 10°C and the third one at the 18°C. The fruits were analysed three times during storage from August 28th to September 10th 2012.

Determination of weight loss

Weight loss was evaluated by the difference in fruit weight on the first day of the experiment and each sampling. The weight of fruits was recorded using a Mettler balance model Toledo PB 602.

Determination of texture

Texture (firmness) was measured in the pulp, by removing 1 cm^2 of the fruit skin, on opposite sides using a Chatillion penetrometer (model DFG 50), equipped with an 11 mm diameter round stainless steel probe with flat end (John Chatillion & Sons, U.S.A.). The crosshead speed during the firmness testing was 10 mm/min. Two measurements of the force needed to penetrate the mesocarp tissue were taken for each fruit, and an average was calculated as a puncture force (kg cm^{-2}) recorded during penetration.

Chemical analyses

Total soluble solids concentration (Brix was noted in percentage) of the expressed juice was determined using a hand-held Atago PR1 refractometer (brix range 0-20% at 20°C).

When weight, texture and soluble solids measurements had been performed, sample of fruits flesh were chopped, frozen in liquid nitrogen and stored at -20°C. For detection of dry weight (DW), 2 g of the frozen sample was freeze-dried for 22 h in a Gamma 2-20 lyophilizer (Christ, Germany). Water content (%) was calculated from the difference between the masses before and after the lyophilization.

Carotenoid components and isomers' composition of tocopherols were determined using an improved method described in KACJAN-MARŠIČ *et al.* (2010). Carotenoids and isomers' composition of tocopherols were extracted from the dry fruit powder with ice-cold acetone. All extraction procedures were performed in dim light.

Acetone extracts were subjected to HPLC gradient analysis (column Spherisorb S5 ODS-2 250 x 4.6 mm with precolumn S5 ODS-2 50 x 4.6 mm). Tocopherols were separated using methanol as solvent. Tocopherols were detected directly by fluorometry (excitation 295, emission 325). Carotenoids were separated using the following solvents: solvent A, acetonitrile/methanol/water (100/10/5, v/v/v); solvent B, acetone/ethyl acetate (2/1, v/v), at a flow rate of 1 mL min^{-1}, linear gradient from 10% solvent B to 70% solvent B in 18 min was applied, run time 30 min, photometric detection at 440 nm.

Statistical evaluation

All measurements were performed in triplicates (n = 3). Statistical analysis was performed using the Statgraphics programme, version 4.0. Results were compared by Tukey HSD test

RESULTS AND DISCUSSION

Weight loss

The average weight of randomly chosen cantaloupe fruits measured after harvesting was 1,142 g. It was found that weight loss is quite uniform irrespective of storage temperatures. Effects of storage conditions on weight loss of stored fruits are listed in Fig. 1. Maximum weight loss occurred at 18°C (7.6%), while lowest loss was recorded at 10°C (0.9%), after the first week of storage, and 13.6% (at 18°C) and 3.5% (at 2°C) for fruits stored for two weeks. However, the difference was not statistically significant.

The weight loss experienced for other fruits and vegetables, during recommended storage conditions and storage period varies between 2 and 10%, due to moisture losses (ELKASHIF *et al.*, 1989). Minimal economical loss (weight loss) for cantaloupe melon during storage is due to the rind thickness and wax layer on the surface of the fruit, and for the cantaloupe melons are

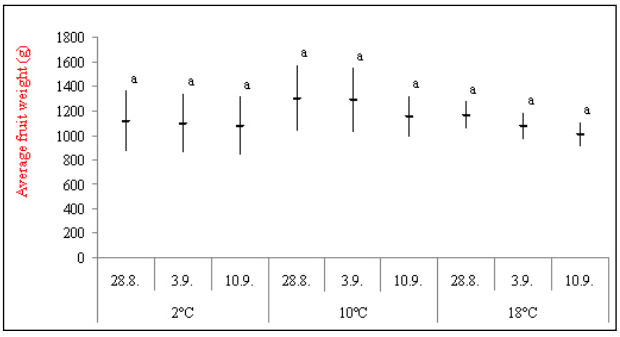


Fig. 1 - Changes in the weight loss (g $fruit^{-1}$) during 14 days storage at 2°, 10° and 18°C (vertical bars represent standard errors of the means).

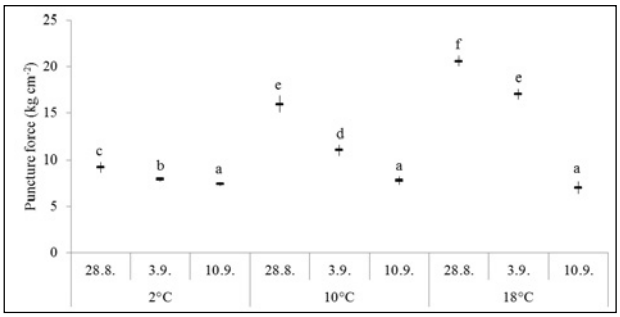


Fig. 2 - Changes in the puncture force (kg cm^{-2}) during 14 days storage at 2°, 10° and 18°C (vertical bars represent standard errors of the means).

reported to show no climacteric activity (KARAKURT and HUBER, 2002).

Texture

The textural quality of fruits is influenced by flesh firmness. The development of pericarp firmness, i.e. the softening of the fruits, was significantly affected by storage time and temperature (Fig. 2). Firmness decreased notably at all temperatures during the storage period. It was observed from the present research intervention that firmness decreased as a function of storage time (POŽRL *et al.*, 2010). However, firmness decreased slowly in fruits stored at 2°C. At the higher temperatures, the decrease in firmness was more noticeable. This was in agreement with the slow maturation expected at low temperatures (LAINEZ and KRARUP, 2008; GARCÍA *et al.*, 2005). A close relationship between the softening of the fruits, higher temperature and extension of storage time was described in previous studies (BEAULIEU and LEA, 2007). It was suggested by ŽNIDARČIČ *et al.* (2010) that a postharvest change in texture can occur due to the loss of moisture through transpiration, as well as enzymatic changes. In addition, hemicelluloses and pectin become more soluble, which results in disruption and loosening of the cell wall structure and composition (PAUL *et al.*, 1999).

Total soluble solids (TSS)

TSS content is related to the balance of sugars and acids and it has a major impact on the flavor of the vegetable (GUILEN-RIOS *et al.*, 2007). Fig. 3 show that the storage period has a significant effect on TSS. It is clear from the obtained data that TSS has a significant rise through fruit development regardless of temperature and the maximum is observed in the end of the storage period. The gradual increase of TSS up to 21 days of storage might be due to conversion of insoluble to soluble forms of sugars and the least utilization of organic acid.

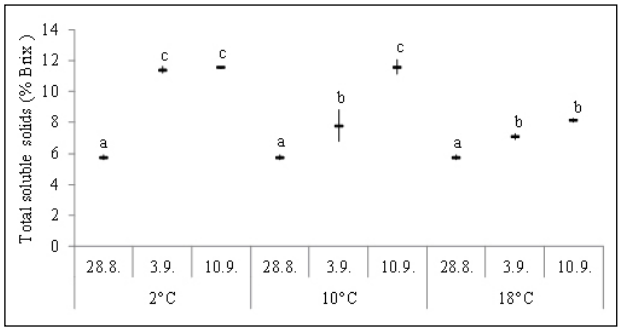


Fig. 3 - Changes in the total soluble solids (% Brix) 14 days storage at 2°, 10° and 18°C (vertical bars represent standard errors of the means).

According to RATHORE *et al.* (2007), the increment of TSS might be due to the alteration in cell wall structure and breakdown of complex carbohydrates into simple sugars during storage.

Carotenoids

Carotenoid levels appears to vary by species, variety, cultivar, biochemical attributes, irrigation, degree of maturity at harvest, growing site, climate, soil fertilization (van den BERG *et al.*, 2000). Some authors have observed that operation carried out during the post-harvest storage is also very important (KIDMOSEA *et al.*, 2006). For example, some authors (MENCARELLI and SALVEIT, 1988) mention that fruits biosynthesize carotenoids during ripening through-out the storage time. In our study, the major carotenoid found in the melon fruits was β-carotene, making up on average 90% of the total carotenoids. β-carotene have been widely reported as being one of the major carotenoids found in vegetables (CALVO, 2005).

Changes in carotenoid components of fruits are presented in Table 1. No significant differences in β-carotene content were found between fruits stored at 2° and 10°C. On the contrary, β-carotene content showed significant increase in fruits stored at 18°C. A steady loss in β-carotene was observed during storage. Initial β-carotene content of fruits was 90.41±28.19 mg/100 g, respectively, on dry weight basis. The lowest value was registered in fruits within three weeks of storage (51.25±3.88 mg/100 g).

In general, lutein content progressively increased with increasing temperatures. The initial level of lutein content was about 0.82±0.24 mg/100 g dry weight. During the storage the lutein content was increasing slightly in the first seven days and afterward the content of lutein started to decrease. As far as we know, there are no published works quantifying lutein content throughout storage of melon fruits.

The lycopene content was found to increase from a low value (2.74±0.16 mg/100 g) at 2°C g to a maximum (6.01±1.34 mg/100 g) at 18°C. Lycopene concentrations were also affected by storage time. Maximum lycopene accumulation (3.71±1.18 to 4.81±1.93 mg/100 g) occurred in fruits that were 0-7 days in age, while the lowest lycopene concentration (1.91±0.52 mg/100 g) was in fruits at the end of storage.

Some authors (LEE and CHEN, 2002) are generally in agreement that lycopene remains relatively stable during typical food processing procedures, except at extreme conditions. On the other hand, TONUCCI *et al.* (1995) reported that lycopene content of vegetables increased by thermal process. For example, SHI *et al.* (1999) explained that this change might be caused by an increased release of lycopene from the cell, skin and insoluble fibre of tomato.

Tocopherols isomers

Tocopherols, collectively known as vitamin E, are one of the most important components of cellular antioxidant systems. Although tocopherols

Table 1 - Changes in the carotenoid components (mg/100 g) during 14 days storage at 2°, 10° and 18°C.

	β-carotene	Lutein	Lycopene	Total carotenoids
Temperature (°C)				
2°C	69.06±7.05	0.56±0.03	2.74±0.16	72.35±7.23
10°C	69.58±20.02	1.02±0.49	3.42±1.67	74.02±22.18
18°C	145.31±15.51	1.31±0.38	6.01±1.34	152.64±32.33
Days				
0	90.41±28.19	0.82±0.24	3.71±1.18	94.92±20.27
7	93.76±15.79	1.31±0.71	4.81±1.93	99.87±26.14
21	51.25±3.88	0.48±0.02	1.91±0.52	53.63±4.25
Significance				
Temperature (T)	*	*	*	*
Days (D)	*	*	*	*
T x D	NS	NS	NS	NS

NS, non-significant; asterisk indicates significance at $P<0.01$.

Table 2 - Changes in the isomers' composition of tocopherols (mg/100 g) during 14 days storage at 2°, 10° and 18°C.

	α-tocopherol	γ-tocopherol	δ-tocopherol	Total tocopherols
Temperature (°C)				
2°C	4.32±0.97	1.23±0.07	0.08±0.04	5.63±0.89
10°C	4.92±0.95	1.24±0.68	0.27±0.17	6.43±0.96
18°C	7.06±1.48	2.61±0.44	0.48±0.38	10.15±1.52
Days				
0	4.82±0.87	1.42±0.61	0.17±0.08	6.41±0.87
7	8.86±0.64	1.98±0.62	0.41±0.12	11.25±2.07
21	3.44±1.59	0.89±0.33	0.06±0.02	4.39±1.92
Significance				
Temperature (T)	*	*	NS	*
Days (D)	*	*	*	*
T x D	NS	NS	NS	NS

NS, non-significant; asterisk indicates significance at $P<0.01$.

appear to be universal constituents of all higher plants (THRELFALL and WHISTANCE, 1971), to our knowledge, there have been no reports of the qualitative composition of tocopherols in cantaloupe fruits. In our study, three isomers of tocopherols were detected (α-, γ- and δ-tocopherol), and the results are shown in Table 2.

Among the tocopherols we determined after harvest (at 0 day) α-tocopherol was predominant at the level of 4.82±0.87 mg/100 g. The contents of γ- and δ-tocopherol were 1.42±0.61 mg/100 g and 0.17±0.08 mg/100 g, respectively.

During 21 days of post harvest the higher the storage temperature, the higher the content of tocopherols. Only exception is δ-tocopherol which concentration showed no substantial variations due to temperature conditions.

As shown in Table 2 content of tocopherols grew and peaked after 1st week of storage. Decrease of tocopherols after seven days was almost linear in all cases although the rate of decrease was slightly higher in α- and δ-tocopherol. According to ABRAMOVIČ *et al.* (2007), the lower stability of α-tocopherol in comparison to γ- and δ-tocopherol is due to the fact that tocopherols reacts faster with peroxy radicals formed in the process of autooxidation.

CONCLUSIONS

As consumers become increasingly aware of the nutritional value of foods and interersted in a healthier lifestyle, the positive nutritional information on cantaloupe melons will help consumer to make informed decision on consuming this nutrition food. The current data demonstrated the importance of not only temperature but also time of storage for the cantaloupe fruits quality parameters. To preserve fruits bioactive compounds it is recommended to keep them in storage for maximum of 7 days at 18°C.

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PAPER

REMOVAL OF BITTER COMPOUNDS FROM CITRUS BYPRODUCTS

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ABSTRACT

Bitter compounds such as limonoids, which consist of oxygenated triterpenoid compounds, are present in citrus fruits. Removal of limonoids from citrus fruits is of considerable importance for the citrus industry. Researchers have removed limonoids from citrus fruits and citrus byproducts using organic solvents. The objective of this study was to use a simpler method to remove limonoids from citrus byproducts.

- Keywords: alkaline solution, citrus byproducts, extraction, limonoids -

INTRODUCTION

Bitterness in citrus fruits is mainly attributed to the presence of limonoids (triterpenes) and flavanone glycosides (flavonoids) such as limonin and naringin, respectively (RIBEIRO *et al.*, 2002). Bitterness from limonin develops gradually in fruit juices, a phenomenon referred to as "delayed bitterness" (HASEGAWA, 1989). Citrus bitterness, which was a major factor contributing to losses of up to $90 million in California from 1992 to 2006, limits marketability; certain commercial citrus varieties (e.g., navel orange) are primarily used as table fruits instead of juice sources (MANNERS, 2007). In Sicily, the main producer of citrus products and byproducts in Italy, the limonin content of blood orange cultivars is approximately 18 ppm (SCORDINO *et al.,* 2005), which constitutes a problem for cattle feed that contains citrus byproducts. Furthermore, citrus byproducts (approximately 350,000-420,000 ton/yr) represent an environmental and economic problem because pulp, pulp wash, and yellow water are difficult to digest. Additionally, citrus byproducts are relatively resistant to microbial degradation (high COD and BOD_5 indexes) due to their high content of bioactive compounds with antimicrobial activity, e.g., ascorbic acid, limonoids, and polyphenols.

The bioactivity of limonoids has been reported in several studies. Limonoids, which are natural fruit compounds consumed by humans and animals, are highly biocompatible (CUI *et al.*, 1997; MANNERS, 2007). Recently, it has been reported that bioactive compounds in citrus fruits have immunomodulatory and anti-inflammatory properties. These properties, which may be partly due to flavonoids (i.e., naringenin, esperidin, nobiletin, and tangeretin), may be attributed to inhibitory effects on macrophages (LI *et al.*, 2008; BODET *et al.*, 2008) and lymphocytes (FANG *et al.*, 2010; LI *et al.*, 2008). Limonin has anti-inflammatory properties in animal models (MATSUDA *et al.*, 1998) and inhibitory effects on lymphocyte proliferation (KIM *et al.*, 2009) depending on the dose; there was a significant increase in lymphocyte proliferation at 5-50 μg/mL limonin, whereas at higher concentrations (1,000 μg/mL limonin), lymphocyte proliferation was inhibited (ROBERTO *et al.*, 2010). In a mouse model of human skin graft, obaculacton inhibited Th1 effector cells and enhanced regulatory T cells (GONG *et al.* 2010). Results obtained from mouse models suggest that limonoids have anti-inflammatory and immunosuppressive properties; however, there are no studies on the effects of limonoids on human cells. It has been reported that limonin is a potential colon cancer inhibitor (GUTHRIE *et al.*, 2000) and that it has effects on the phase I cytochrome P450 enzyme system and the phase II glutathione S-transferase (GST) enzyme in the liver and small intestine of rats (KELLY *et al.*, 2003). Citrus limonoids might be useful for the prevention of different types of cancers in humans (MORABITO *et al.*, 2012).

Several studies have focused on the extraction of limonoids from citrus juice and citrus pulp (for human and animal consumption, respectively) to reduce bitterness; few studies have focused on the in vitro recovery of limonoids from peel waste. Citrus byproducts are good sources of limonoids, especially limonin and nomilin. Limonin synthesis takes place at low pH in a reaction catalyzed by limonin D-ring lactone hydrolase (MANNERS, 2007). At 6 ppm, limonin confers a bitter taste to juices and citrus byproducts (GUADAGNI *et al.,* 1973). Researchers have attempted to remove limonin from juices and molasses (PIFFERI *et al.*, 1993; BIANCHI *et al.*, 1995). Currently, the main limonin extraction method involves the use of an organic solvent. Recently, LIU *et al.* (2012) extracted limonin using an alkaline solution; however, the authors did not report the optimum extraction conditions such as pH, solid-solvent ratio, and temperature.

MATERIALS AND METHODS

Samples

Citrus byproducts (orange peel) were supplied from a local industry (Ortogel S.P.A). Juices and byproducts from several orange cultivars were analyzed for pH, °brix, and dry weight. All determinations were performed in triplicate. Unless otherwise specified, all reagents were obtained from Sigma.

Limonoid extraction

Limonoid extraction was performed using a discontinuous extraction method. To assess the optimum extraction conditions, different extraction parameters were assessed: the solvent-to-solid ratio ranged from 2:1 to 20:1, the extraction temperature ranged from 10° to 40°C, and the extraction time was performed for 60 minutes. Furthermore, different pH values (pH 7-11) of the alkaline solution (potassium hydroxide) were assessed. The extraction method was repeated three times; by the third extraction, >90% of limonin had been recovered. A comparative extraction was performed with dichloromethane, an organic solvent. The extraction method was repeated three times. Data were analysed by Student's *t*-test.

Limonoid determination

Limonoids and their precursors, i.e., limonoid aglycones and limonoid glycoside, were quantified by HPCL according to the method reported by BREKSA III *et al.* (2009). The HPLC sys-

Table 1- Analysis of orange byproducts.

	Composition of orange peel		
	Mean	(% of DW) Range	
Ash	6.39	2.55	13.22
Sugar	7.69	3.52	10.07
Fat	1.77	0.52	4.00
Protein	8.92	6.55	12.51
Limonoids	0.20	0.16	0.24
Flavonoids	5.32	1.51	11.00
Pectin	11.97	2.58	23.02
Lignin	10.12	7.52	14.73
Cellulose	28.92	20.74	39.00
Hemicellulose	8.36	5.59	11.05

tem (Shimadzu, Japan) had two pumps (LC-10A), a control system (SCL-10A), an injector (Rheodyne with 20 uL loop), a photodiode detector (SPD-M10A), a C18 Altima ODS Hypersil column (250 × 4.6 mm I.D.; Milan, Italy), and a packed pre-column. The mobile phase consisted of acetonitrile and water (65:35) and the temperature was maintained at 25°C. Sample elution was performed at 1 mL/min using the isocratic program. Samples were filtered through PTFE filters (0.45 μm) prior to HPLC analyses. Sample peaks were identified by comparing them to standard peaks. Samples were quantified by the internal standard method.

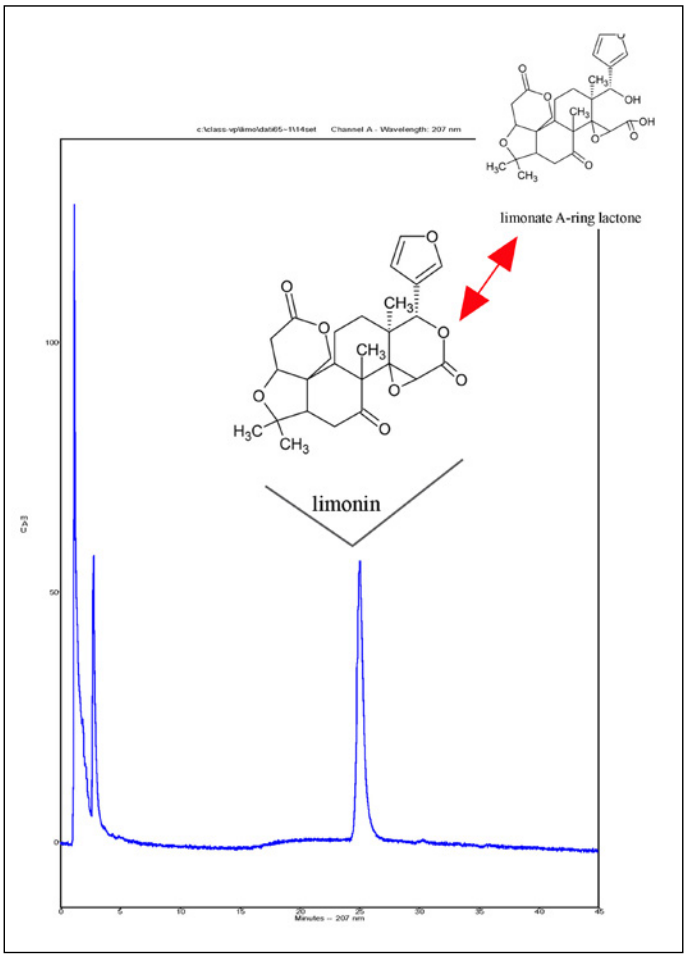


Fig. 1 - HPLC chromatogram of limonin and limonoate A-ring lactone structure.

RESULTS AND DISCUSSION

The composition of orange peel is shown in Table 1. Limonin content in orange peel is not very high; however, it contributes to bitterness in citrus byproducts. The most important citrus byproducts in Sicily is called "pastazzo", which is used in cattle feed. However, following the removal of bitter compounds, dried peel can be used as sources of fiber for human consumption. The limonin extraction method used in this study can be applied in the food/citrus industry because it is solvent-free and the extraction temperatures are compatible with industrial processes. Limonin is the main limonoid present in orange juice byproducts. Limonin is insoluble at low pH (<5) while at high pH (approximately 9) its solubility increases as a result of limonoate A-ring lactone formation (Fig. 1). Fig.1 shows the HPLC chromatogram of limonin extracted from orange peel with the alkaline solution at pH 9. Fig. 2 shows the extraction kinetics of the alkaline solution compared with dichloromethane, Cl_2CH_2. The highest limonoid extraction yield was obtained with Cl_2CH_2. However, the alkaline solution at pH 9 was effective in extracting limonoids from orange peels. The determination of limonin was performed at pH 4 to allow the A-ring of limonoate A-ring lactone to close. The alkaline solutions had good extraction yields (> 90% of limonin) after 20 min. Subsequent extraction methods were performed for 20 min at pH 9. Fig. 3 shows the limonin extraction yield relative to the solvent-to-solid ratio. Cl_2CH_2 was the best extraction solvent; at a solvent-to-solid ratio of 5:1, Cl_2CH_2 resulted in an extraction yield that was 30% higher than that of the alkaline solution. With increasing Cl_2CH_2-to-solid ratio, the extraction yield progres-

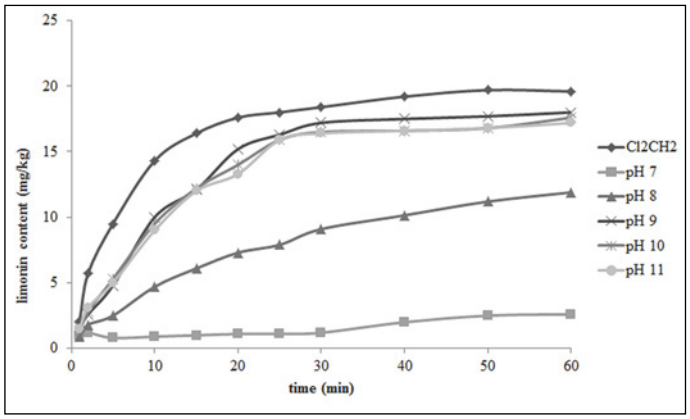


Fig. 2 - Extraction of limonin from orange juice processing byproducts with alkaline solution at different pH value compared to organic solvent at different time of extraction.

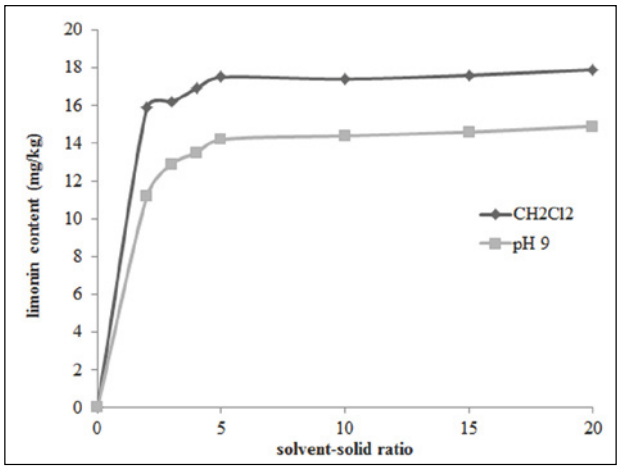


Fig. 3 - Limonin extraction at different solvent-solid ratio comparing organic solvent and alkaline solution.

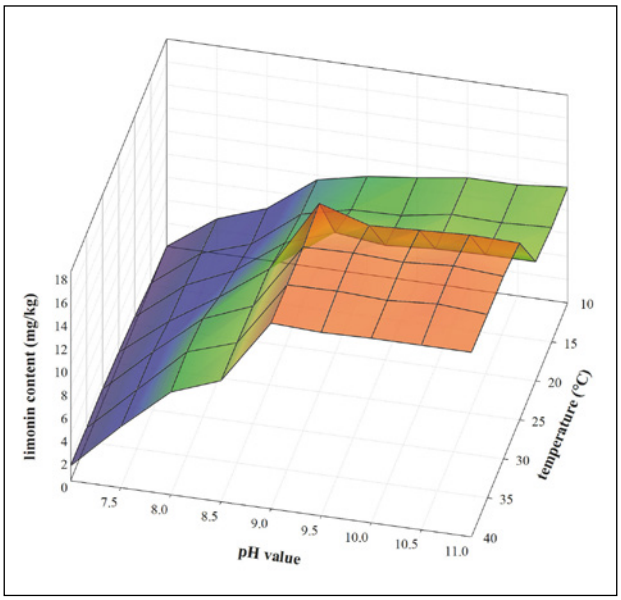


Fig. 4 - Limonin extraction at different pH value and temperature of alkaline solution.

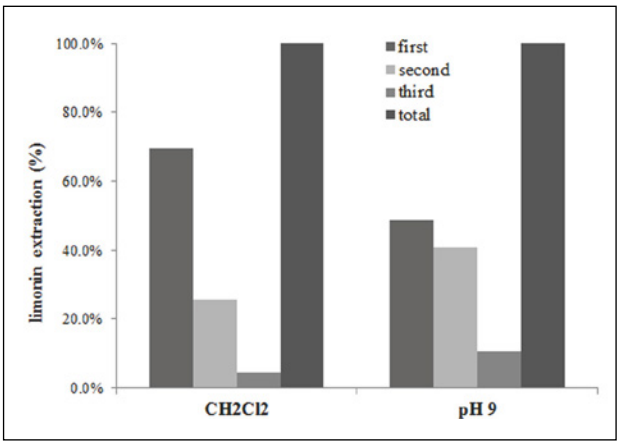


Fig. 5 - Extraction tests comparing organic solvent and alkaline solution at pH 9.

sively increased; however, with alkaline solution-to-solid ratios >5, extraction yield slightly increased. Therefore, all subsequent experiments were carried out at this ratio. Solvents were tested under different extraction temperatures and pH values (Fig. 4). Limonoid extraction yield increased with all alkaline solutions at 10°-40°C. With increasing temperature, limonoid extraction yield increased probably as a result of higher limonin solubility and higher diffusion rate from the inner to outer surface of the peel (CACACE and MAZZA, 2003). As shown in Fig. 4, the alkaline solution at pH 9 and 25°C resulted in the highest limonoid extraction yield.

After determining the optimum pH value and temperature (pH 9 and 25°C, respectively) for limonin extraction, we assessed limonin extraction yield from each extraction step. Following the first extraction, Cl_2CH_2 resulted in a 70% extraction yield and the alkaline solution resulted in a 50% extraction yield (Fig. 5). However, following the second extraction, a 25% extraction yield was obtained with Cl_2CH_2 and a 40% extraction yield was obtained with the alkaline solution. With the third and last extraction, 5% of limonin was extracted by both methods. The total limonin extraction yield following the second extraction was 95 and 90% for Cl_2CH_2 and alkaline solution, respectively. The identification of limonin was performed by comparing the sample retention times (RT) with those of standards (BREKSA *et al.*, 2008).

The extraction method used in this study has been applied for anthocyanin extraction (CACACE and MAZZA, 2003; TODARO *et al.*, 2009). The extraction conditions are suitable in the citrus industry. The extraction of limonin from citrus fruits could have a great economic impact in the citrus industry. In the past decades, the citrus industry has invested its efforts in removing bitter compounds from citrus products and byproducts. The method used in this study is both simple and inexpensive. Following limonoid extraction, citrus byproducts could be used as source of fiber for human consumption.

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PAPER

FREEZE DRYING OF YOGHURT WITH CANDIED CHESTNUT PUREE: SURVIVAL OF LACTIC ACID BACTERIA AND DETERMINATION OF PHYSICAL PROPERTIES

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ABSTRACT

Yoghurts with varying candied chestnut puree contents were freeze dried to produce a novel product: yoghurt powder containing candied chestnut puree. The amount of surviving lactic acid bacteria in the products was determined.

The results showed that increasing the percentage of candied chestnut puree resulted in a decrease of moisture content, water activity, ash content, and *L** values of the powder. Yoghurt with candied chestnut puree could be satisfactorily freeze dried with a minimum level of 8.93 log cfu/g dry matter survival rates for the lactic acid bacteria.

- Keywords: lactic acid bacteria, yoghurt powder, candied chestnut puree, freeze drying -

INTRODUCTION

Yoghurt is a smooth, semisolid fermented milk product produced as a result of the fermentation of milk by the action of symbiotic cultures of *Streptococcus salivarus* subsp. *thermophilus* and *Lactobacillus delbruecki* subsp. *bulgaricus*. The starter microorganisms should be viable, active, and abundant in the product up to the date of minimum durability. The shelf life of yogurt is one day when stored at ambient condition (25°-30°C) and around 5 days at 7°C (KUMAR and MISHRA, 2004). Improvements in the shelf life of yoghurt can be brought about by dehydrating and converting it into a shelf-stable powder. Yoghurt can be dried by freeze, spray, microwave or convective drying methods, and yoghurt powder can be used as an ingredient for the manufacturing of many food products such as confectioneries, bakery foods, yoghurt drink mixes with fruits or vegetables, instant drink mixes, soup bases, dips, and sauces. It can also be consumed directly after reconstitution (KOÇ *et al.*, 2010). Freeze dried yoghurt can be stored for 1-2 years at 4°C, while spray dried yoghurt cultures, when stored under dry and cool conditions, are guaranteed to maintain activity up to 1 year (KOÇ *et al.*, 2009). The quality of yoghurt powder is mainly affected by the viable yoghurt starter bacteria count. Although freeze drying is the best method for drying yoghurt in terms of survival of yoghurt starter bacteria, the high cost of the process is a disadvantage (KOÇ *et al.*, 2009; TAMIME, 1989; KUMAR and MİSHRA, 2004).

The chestnut is one of the most popular nuts in the world. Unlike most other tree nuts, chestnuts are low in protein (2-4%) and fat (2-5%), but high in carbohydrates (up to 70%). Chestnuts also have fibrous substances, minerals (potassium and phosphorus) and vitamins (B and C) (RIBEIRO *et al.*, 2007).

Chestnuts are typical seasonal fruits that maintain their optimum commercial quality for a comparatively limited period because of their high water activity and sugar content (ATTANASIO *et al.*, 2004).

There are different methods of consuming and processing chestnuts. They are commonly roasted, boiled, and fried. However, new products such as flours, creams, purees, canning, and pastries are also gaining importance. Chestnuts can be incorporated into a wide range of dishes such as soups, poultry stuffing, pancakes, muffins, and pastries. Turkey has a large share of the world's chestnut production. Most of the harvested produce is consumed fresh, but because chestnuts are highly perishable due to molding and rotting caused by fungi and the larval development of insects, the remaining crop must be preserved. For this reason, production and consumption of candied chestnuts is very common in Turkey. Candied chestnuts are a sweet product produced by soaking the chestnut shell and thin skin, then adding saccharose, glucose, glucose syrup, or invert sugar syrup, along with other additives according to the production technique. This product protects the nutritional value of fresh chestnuts and adds energy value due to the addition of sugar. The broken kernels are also marketed as puree. Chestnuts having a high content of nondigestable tissue, and health benefits include the development of the intestinal flora, the expulsion of harmful substances from the body, and effectiveness in lowering blood cholesterol levels (PARLAK and BILIŞLI, 2004).

Food powders have an increasing importance in food processing due to their ease of storage and transportation, and their suitability for use in innovative formulations. For these reasons, there have been several studies to determine powder properties of dried products (HOGEKAMP and SCHUBERT, 2003; NIJDAM and LANGRISH, 2006; CHEN and PATEL, 2008; FANG *et al.*, 2008; GONG *et al.*, 2008; JINAPONG *et al.*, 2008; JANGAM and THORAT, 2010; KOÇ *et al.*, 2011).

The physical and biochemical properties of food powders determine their functionality and quality as food products. Flowability, which determines a product's handling properties, is an important characteristic of powdered products. Poor flowability is typically explained by the idea that small-particle sizes have more surface contact area and thus more frictional forces resistant to flow (JINAPONG *et al.*, 2008). The flowing behavior of a powder not only depends on its physical properties, but also on a combination of parameters inherent in the powder, and on the ambient conditions and equipment used (TURCHIULI *et al.*, 2005). Powder products are reconstituted before use. In the case of dried powdered foods, a number of properties influence the overall reconstitution characteristics (KOÇ *et al.*, in press). Reconstitution of food powders follows the following steps: wetting of particles, sinking of particles into solution, dispersion of particles in solution, and complete dissolution of particles (CHEN and PATEL, 2008).

Compared to spray drying methods, freeze drying generally results in better survival of starter culture and improved reconstitution properties (KUMAR and MISHRA, 2004). The reconstituted freeze dried yoghurt has a high content of microorganisms, and the *Lactobacilli-streptococci* ratio is no different than the original yoghurt (KUMAR and MISHRA, 2004). Yoghurt powder used as a functional food can also be mixed with dried fruit particles to formulate a novel product and improve the reconstitution properties of the yoghurt.

In this study, yoghurt and candied chestnuts, which are individually considered healthy products, were combined to obtain a novel product with beneficial properties of both. The aim of the study was to determine physical properties such as moisture content, water activity, ash content, color (L^*, a^*, b^* values) and color change (ΔE) ,

handling properties such as bulk density and flowability, and reconstitution properties such as wettability and solubility, along with determining of the survival of lactic acid bacteria in yoghurt powder.

MATERIALS AND METHODS

White, plain yoghurt was purchased from a local producer (Pinar Dairy Products Inc., Turkey) and kept at 4°C until used on the day of purchase. Though the company makes other yoghurts, the white, plain variety, because of 14.2% (wb) total solids and 1.5% fat was selected as the test material. Candied chestnut puree containing 46.8% total sugar, 0.3% fat, 24.8% starch, and 0.9% protein was purchased in a local supermarket (Kardelen Inc., Turkey).

Preparation of yogurt mix

Yoghurts both plain and mixed with candied chestnut puree at 5, 10, and 20% by weight were prepared by mixing the ingredients and poured on metal Petri plates. Sample thickness was kept at a constant 3 mm by measuring with a digital caliper.

Freezing and freeze drying

The samples were frozen in an air-blast freezer (Frigoscandia, Helsinborg, Sweden) at -18°C for 2 hours, and freeze dried for 8 hours in laboratory scale equipment (Armfield Model-FT33 Vacuum Freeze Drier, England) at 13.3 Pa absolute pressure with a condenser temperature of -45°C. Samples were dried to constant weight. All freeze drying experiments were replicated twice.

Methods of analyses for powder properties

Moisture content of both fresh yoghurt and dried powders were determined gravimetrically with a modified method in an oven (Ecocell 222, Germany) at 105°C for 4 hours (PARLAK and BILIŞLI, 2004). The water activity (a_w) values of yoghurt powders were measured using a water activity measurement device (Testo AG 400, Lenzkirch, Germany) with a ±0.001 sensitivity. Ash contents were determined by dry ashing in a muffle furnace at 550°C for 8 hours (GÜLER and PARK, 2009). Analysis of powder properties for the products was made according to following methods: *Flowability*: 2 g of powder was transferred to a 50 mL graduated cylinder. The bulk density was calculated by dividing the mass of the powder by the volume occupied in the cylinder. The samples were dropped 50 times in order to evaluate their tapped densities. Then Carr Index and Hausner Ratio values were calculated by using Equation (1) and (2) respectively (JINAPONG *et al.*, 2008; KOÇ *et al.*, 2011).

$$CI = \frac{(\rho_{tapped} - \rho_{bulk})}{\rho_{tapped}} \times 100 \quad (1)$$

$$HR = \frac{\rho_{tapped}}{\rho_{bulk}} \quad (2)$$

Wettability: A measured amount of distilled water (100 mL) at 25°±1°C was poured into a 250 mL beaker. The powder sample (10 g) was placed in the beaker and the time it took for it to become completely wetted was recorded. *Solubility*: 2 g of yoghurt powder was added to 50 mL of distilled water at 25°±1°C. The mixture was agitated vigorously with a magnetic stirrer (Heidolph MR Hei-Standard, Germany) at 500 rpm. The time required for the material to dissolve completely was recorded (GOULA and ADAMOPOULOS, 2005; KOÇ *et al.*, 2010). *Color:* The color values of plain yogurt, prepared frozen-yoghurt samples, freeze dried yoghurt powders, and reconstituted yoghurt samples (*L**, *a**, and *b** values) were measured with a Minolta Chroma Meter CR-400 (Minolta Co., Ltd, Japan). The total color change of reconstituted yogurt powders, with respect to fresh yoghurt samples, was calculated by using Equation 3 (HUNTER, 1975).

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (3)$$

All experiments on powder properties were replicated twice.

Enumeration of lactic acid bacteria

For the enumeration of lactic acid bacteria, 1 g of yoghurt for the initial load, or yogurt powder, was diluted with 9 mL of 0.1% peptone water and mixed uniformly with a vortex mixer (Elektro-M17, Turkey). Subsequent serial dilutions were prepared and viable numbers were enumerated using a pour-plate technique (DAVE and SHAH, 1997). MRS Agar (de Man Rogosa Sharpe Agar, pH 5.4, Oxoid, Hampshire, UK) in a double layer was used for *L. bulgaricus* and incubated at 37°C for 72 hours. M17 Agar (pH 7.2, Oxoid, Hampshire, UK) was used for the enumeration of *S. thermophilus* under aerobic incubation at 37°C for 48 hours. The average counts from the plates were calculated and the results were expressed in log cfu/g dry matter. Experiments on enumeration of lactic acid bacteria were performed in triplicate.

Statistical analysis

The data were analyzed by using a statistical-package program (SPSS 14.0 for Windows evaluation version; SPSS Inc., Chicago, Ill) with a general linear model procedure. Differences were considered significant at $p < 0.05$. All analyses were carried out with parallels.

RESULTS AND DISCUSSION

Powder properties

The results of the analysis for physical, handling, and reconstitution properties of the powders are shown in Table 1. The moisture and water activity values of all yoghurt powders decreased with increased amounts of chestnut puree due to the increased amount of carbohydrates. Therefore, both the lowest moisture content and water activity values were found for yoghurt with 20% by weight chestnut puree. In another part of this study, in which rheological behavior and sensory analysis of the candied chestnut yoghurt at the same compositions were investigated, the most acceptable products were determined to be yoghurt with 10% by weight chestnut puree (SAKIN-YILMAZER *et al.*, in press). Ash content (%) values of yoghurt powder were found between 6.85 and 2.60 in wet basis. Ash content decreased with the addition of chestnut puree (46.8% total sugar) due to the increased amount of carbohydrates that leave no ash upon burning.

The bulk and tapped densities of yoghurt powders are also given in Table 1. The results of this study show that the addition of chestnut puree increased the bulk and tapped densities of the powders. KOÇ *et al.* (in press) reported values of 538 and 746 kg/m^3 respectively for plain yoghurt dried with spray drying, compared to bulk and tapped densities of 210 and 220 kg/m^3 for plain yoghurt dried by freeze drying in this study. The bulk densities of milk and whey were also reported to be 400 and 530 kg/m^3, respectively. JINAPONG *et al.* (2008) studied the spray drying of soymilk and reported the bulk and tapped densities at 210 and 350 kg/m^3. For the determination of the flowability behavior of the powdered samples, Carr Index (CI) and Hausner Ratio (HR) values were calculated and the results are shown in Table 1. These results show the candied chestnut yoghurt powders have very good flowability behavior (JINAPONG *et al.*, 2008). A CI value of 27.93% was observed for spray dried yoghurt powders, indicating fair flowability (KOÇ *et al.*, in press). In a study by JINAPONG *et al.* (2008), the CI value was reported at 40% (bad flowability). The reason for this flowability was the small-particle size and large surface-area-per-unit mass of the powder, leading to more contact surface between powder particles and increased frictional forces that resisted flow. For the yoghurt powders cohesiveness values based on HR were low. The cohesiveness was observed to be in the intermediate level for the spray dried plain yoghurt powder (KOÇ *et al.*, in press).

The reconstitution properties of solubility and wettability are also given in Table 1. The increase in content of chestnut puree resulted in reduced times for solubility and wettability of the powders. At 31.8 s, the wettability value of plain yoghurt is very good, compared to 374 s for the spray dried yoghurt powders (KOÇ *et al.*, in press).

Color is important for marketability of the products and consumer acceptance. Even though a functional food could provide several health benefits to consumers, without being visually attractive it would not be marketable. Thus, the color of the supplemented products should, ideally, remain unchanged after production and during storage (ZARE *et al.*, 2011). The *L**, *a**, and *b** values of the yoghurt powders at different chestnut puree concentrations are shown in Table 2. The addition of chestnut puree decreased the *L**, and increased the *a** and *b**, values of the powders, except for a slight decrease in *b** values of the powders with 5% and 10% of addition. The values of color changes, determined as the change in *L**, *a**, and *b** values between reconstituted yogurt powder and

Table 1- Physical properties of yoghurt powder at different concentrations.

Chestnut ratio (%)	Moisture content (% wb)	Water activity	Ash content (% wb)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr Index CI	Hausner Ratio HR	Solubility (s)	Wettability (s)
0	5.19 (±0.06)	0.28 (±0.01)	6.85 (±0.22)	0.21 (±0.03)	0.22 (±0.01)	4.50	1.05	15.20 (±0.28)	31.80 (± 0.71)
5	4.94 (±0.00)	0.10 (±0.02)	4.85 (±0.46)	0.22 (±0.02)	0.23 (±0.15)	4.35	1.05	13.30 (±0.42)	30.15 (± 1.34)
10	4.90 (±0.44)	0.09 (±0.00)	3.78 (±0.17)	0.25 (±0.11)	0.26 (±0.05)	3.80	1.04	10.00 (±1.13)	28.20 (± 0.28)
20	4.79 (±0.18)	0.08 (±0.00)	2.60 (±0.45)	0.32 (±0.06)	0.35 (±0.01)	8.57	1.09	9.65 (±0.35)	26.55 (±0.49)

Table 2 - The measured values of *L**, *a**, *b** and calculated ΔE values of yoghurt powder with different concentrations.

Chestnut ratio(%)	*L* values	*a* values	*b* values	ΔE
0	92.92 (±0.20)	-1.96 (±0.02)	8.16 (±0.12)	18.53
5	89.79 (±0.15)	-0.87 (±0.04)	7.98 (±0.16)	15.91
10	86.76 (±0.17)	-0.12 (±0.01)	7.82 (±0.11)	17.19
20	85.57 (±0.35)	0.26 (±0.03)	8.24 (±0.13)	9.37

Table 3 - Populations of lactic acid bacteria in yoghurt and yoghurt powder containing chestnut puree.

Chestnut concentration (%)	Populations of lactic acid bacteria (log cfu/g dry matter)[1]					
	Streptococcus thermophilus			*Lactobacillus bulgaricus*		
	Yogurt	Yogurt Powder	Reduction	Yogurt	Yogurt Powder	Reduction
0	9.83 (±0.17)a	9.45 (±0.40)b	0.38	7.69 (±0.98)c	6.31 (±1.32)d	1.38
5	9.73 (±0.15)a	9.46 (±0.38)b	0.27	8.23 (±1.62)c	6.45 (±0.69)d	1.78
10	9.81 (±0.18)a	9.31 (±0.31)b	0.50	7.37 (±1.10)c	6.27 (±0.78)d	1.10
20	9.59 (±0.14)a	8.93 (±0.62)b	0.66	7.27 (±1.03)c	5.77 (±0.64)d	1.50

[1] Values are the mean of three replicates and two parallels. Standard deviations are shown in table.
Values that are not followed by the same letter are significantly different ($p<0.05$)

fresh yoghurt samples by using Equation 3, are also given in Table 2. KOÇ *et al.* (2010) reported that the total color change increased with increasing outlet air temperature in spray drying, ranging from 3.06 to 30.36. In a study by KUMAR and MISHRA (2005), mango- and soy-fortified yoghurt was dried in a recirculatory convective dryer in 4 mm-thick forms using hot air at 50° C and an air velocity of 2.5 m/s with 80% air recirculation. The value of color change was calculated as 8.02.

Survival of lactic acid bacteria

Survival ratios of lactic acid bacteria in both plain and candied chestnut yoghurt samples are given in Table 3. All of the results are given in dry basis in order to compare the number of bacteria in yoghurt and yoghurt powder. The numbers of *S. thermophilus* (9.93 - 9.59 log cfu/g) were significantly higher than the numbers of *L. bulgaricus* (8.23 - 7.69 log cfu/g) in yoghurt samples with or without candied chestnut puree ($p<0.05$). Similar to our observations, ZARE *et al.* (2011) reported the numbers of *S. thermophilus* as 8.39 log cfu/mL, and *L. bulgaricus* as 7.69 log cfu/mL in plain yogurt.

Freeze drying of candied chestnut yoghurt significantly reduced the number of *S. thermophilus* and *L. bulgaricus*, about 0.27-0.66 log and 1.10-1.78 log cfu/g dry matter, respectively. The survival ratio of lactic acid bacteria is shown in Fig. 1. VENIR *et al.* (2007) indicated that the freeze drying process of yoghurt samples with added sucrose-blueberry reduced the numbers of *S. thermophilus* and *L. bulgaricus* about 1.52 and 2.33 log, respectively. According to the Turkish Food Codex Communique on Fermented Milk (No: 2009/25), yoghurt must contain at least a minimum of 7 log cfu/g total specific microorganisms (ANONYMOUS, 2009). In our study, yoghurt powder with or without chestnut-candy puree meet this criteria, with a minimum total lactic acid bacteria count of about 8.93 log cfu/g dry matter.

No significant differences in the number of lactic acid bacteria were obtained in the different concentrations of chestnut candy ($p>0.05$). VENIR *et al.* (2007) observed that freeze drying resistance appeared to be similar for *S. thermophilus* and *L. bulgaricus* in plain yoghurt; on the other hand, they observed that the addition of sucrose-blueberry to yoghurt increased the survival of *S. thermophilus* and *L. bulgaricus*.

Freeze drying is considered a suitable dehydration process for bacteria when the ultimate goal is achieving a solid and stable final formulation (CARVALHO *et al.*, 2004). CAPELA *et al.* (2006) pointed out that different species of *Bifidobacterium* and *Lactobacillus* survived in the freeze drying process at about 52.21 (84.99%) and 40.93 (85.73%), respectively. In this study, it was observed that *S. thermophilus* was more resistant

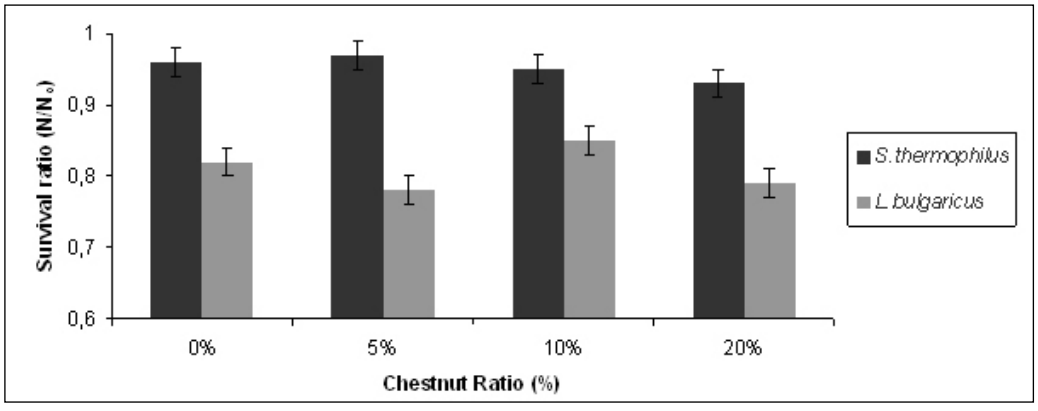


Fig. 1- Survival ratio (N/N_0) of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in yogurt powder containing chestnut puree.

than *L. bulgaricus* in the freeze drying process. The survival of *S. thermophilus* was greater after the freeze drying process compared to *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus casei* subsp. *pseudoplantarum* (TO and ETZEL, 1997). KIM and BHOWMIK (1990) observed that *S. thermophilus* was also more resistant to spray- and freeze drying processes than *L. bulgaricus*. In another study, the survival rate of *S. thermophilus* was found to be higher than that of *L. bulgaricus* during spray drying of yoghurt samples (KOÇ *et al.*, 2010). It is concluded that the freeze drying process can cause membrane injury of *L. bulgaricus*, which results increase in membrane permeability (CASTRO *et al.*, 1997).

CONCLUSIONS

Yoghurt, plain or with candied chestnut puree, could be satisfactorily freeze dried with the determined physical, handling, and reconstitution properties. Increasing the percentage of candied chestnut puree resulted in a decrease of moisture content, water activity, ash content, and *L** values of the powder product. Very good flowability and low cohesiveness were observed, considering the reconstitution properties of the powders. Total lactic acid bacteria survived at a minimum level of 8.93 log cfu/g dry matter in freeze dried, candied chestnut yoghurt samples, meaning that it meets Turkish Food Codex Communique on Fermented Milk criterion of higher than 7 log cfu/g. Further studies are needed for the survival of lactic acid bacteria in the storage conditions of yoghurt powder.

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Acknowledgments. Acknowledgments of assistance are appropriate provided they are not related to analyses or other services performed for a fee. Financial support, thanks for assistance, artic1e number or thesis fulfilment may be included.

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