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Application of common packaging materials in the probiotic fresh cheese production

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Abstract

The aim of this work was to investigate the application of common packaging materials polypropylene (PP) and polystyrene (PS) in the probiotic fresh cheese production packaging. Probiotic and traditional cheeses were produced from milk with standardized milk fat content of 2.3 g/100 g including the application of two cultures (probiotic and traditional). The samples were packed in the PP and PS cups and stored at 4 °C for 30 days. The observed permeability of gases through the two applied packaging materials was significantly different. Cheese samples were analysed for microbiological properties whereby lactic acid bacteria, *Bifidobacterium* sp. and aerobic mesophilic bacteria (AMB) were determined. Packaging materials showed no significant effect on the content of ascorbic acid which is known to be sensitive to the presence of oxygen.

Key words: fresh cheese, probiotics, packaging materials, barrier properties, shelf-life

Introduction

Fresh acid cheeses (called lactic cheeses) are unripened cheeses, such as Quarg, Cottage and cream cheeses manufactured by the coagulation of milk, cream or whey using acid, a combination of acid and rennet or a combination of acid and heat. Many processing factors (milk pre-heat treatment, rate and temperature of acidification, level of gelforming protein, pH, etc.) influence the coagulum structure which in turn affects rheological and physico-chemical stability as well as the nutritive value of the Quarg (Fox et al., 2000; Milanović et al., 2004; Iličić et al., 2012; Lucey, 2011). The applied processing steps and the milk composition provide the specific cheese texture, while the applied lactic acid bacteria generate acid and are usually responsible for the characteristic flavour of cheese. Microorganisms play an essential role in cheese production since they mostly contribute to the development of sensory properties by their metabolism. Besides microorganisms also generate several low molecular weight antimicrobial compounds and affect the complex microflora which contributes to the microbiological safety of the product and production of (Grattepanche et al., 2008).

Milk fermentation by probiotic starters, especially genera *Bifidobacterium* and *Lactobacillus*, is very important in the production of probiotic cheeses. Their valuable influence on human health is still under investigation, although many positive effects have already been confirmed (Gomes et al., 1995; Roy et al., 1997; Stanton et al., 1998; Goetti et al., 1998; Vinderola et al., 2000; Mc Brearty, 2001; Boylston et al., 2004; Medici et al., 2004; Burity et al., 2005b; Erdogrul and Erbilir, 2006; Ong et al., 2007). Cheeses are suitable food matrixes

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for the addition of probiotic bacteria due to their solid consistence, low acidity and high protein and fat content, which allows the probiotic survival during the storage (Cruz et al., 2009; Felicio et al., 2016). Felicio et al. (2016) studied the effect of partial substitution of sodium chloride by potassium chloride and addition of arginine on microbiological, physicochemical, rheological and sensory characteristics in probiotic Fresh Minas cheese. Thereby sodium reduction and addition of arginine did not constitute a hurdle to lactic and probiotic bacteria survival with presented value of about 9 log CFU/g, ranging from 7.11 to 9.21 log CFU/g, respectively. Generally, the sodium reduction and addition of arginine, and the supplementation of Minas cheese with probiotic cultures might lead to development of a product with potentially positive effects on cardiovascular health.

The influence of milk fat and starter cultures (probiotic and traditional) on nutrient characteristics (protein, phosphorus, calcium and cholesterol content) was analysed by Iličić et al. (2015). The combination of traditional and probiotic cultures in Quarg production contributed to a greater cholesterol content per gram fat compared to samples produced by a traditional starter culture.

Proteolytic enzymes from lactic acid bacteria caused the degradation of casein and peptides, leading to the production of free amino acids that contribute directly to the basic taste of cheese and indirectly to cheese flavour, as the precursors for other catabolic reactions (Esriche et al., 1999; Irygoyen et al., 2007; Hannon et al., 2007). These reactions and side-chain modifications may generate keto-acids, ammonia, amines, aldehydes, acids and alcohols, which are essential contributors to cheese taste and aroma. For example, bitterness is due to hydrophobic peptides, rancidity to fatty acids, and fruitiness to esters (Hannon et al., 2007). Volatile fatty acids in fresh cheese are the products of various metabolic pathways, mostly microbial. Their further degradation leads to the generation of the very important groups of compounds - aldehydes and ketones. Besides β -oxidation of fatty acids, they can be synthesized by direct oxidation of hydrocarbons (Panseri et al., 2008; Iličić et al., 2012).

The main volatile compounds in fresh Quarg cheese are hexadecanal, 2-pentadecanone, 2-tridecanone and 2-undecanone (Iličić et al., 2012). Authors concluded that probiotic cheeses contained less quantity of the main volatile compounds (hexadecanal) than the traditional cheeses (Iličić et al., 2012). Some authors associated the presence of hexadecanal with the waxy, floral aroma of cheese.

Numerous factors impact cheese quality during its shelf life, including product and processing characteristics, packaging and storage conditions. Peterson et al. (2002) specified packaging characteristics of cream cheese, investigating the impact of different packaging materials containers of PP/PE, PET/PE, and PS/EVOH/PE, top web of PET/Al-oxide/PE, as well as different draw depths of the containers 25 mm, 50 mm, and 70 mm.

Along with other factors, the choice of the packaging material plays an important role in maintaining quality of products containing probiotics as well as the viable counts of probiotic microorganisms at an optimal level throughout the shelf-life (Mattila-Standholm et al., 2002; Da Cruz et al., 2007). Polymers (PP and PS) are very often used packaging materials. Many authors have found that high polymers are materials with acceptable physical, mechanical and barrier properties related to the permeability of light and gases (Brown, 1992; Robertson, 1993; Lazić and Curaković, 1997; Pajin et al., 2006).

However, there is very little evidence regarding probiotic cheeses. It is very important to keep the level of oxygen within the package as low as possible, for the long shelf life probiotic product. Therefore, low permeability of packaging material is more than desirable. This problem was investigated in several studies to develop alternatives that minimize negative effects of oxygen. The most promising are those evaluating the addition of antioxidants, such as ascorbic acid (Champagne and Gardner, 2005). Generally, ascorbic acid content in cheeses is very low, because of its lost into the whey as hydrosoluble vitamins associated with the non-fat, aqueous fraction (Renner et al., 1989).

In this paper, the effect of two common packaging materials: PP and PS on probiotic fresh cheese functional characteristics after one month storage was investigated.

Materials and methods

Milk

Milk with 10.74 g/100 g dry matter, 2.3 g/100 g fat, 2.93 g/100 g protein, 4.82 g/100 g lactose, 0.69 g/100 g ash, and pH=6.7 taken from AD Novi Sad Dairy, Novi Sad, Serbia, was used for the production of all samples of fresh cheeses.

Starter cultures

Starter cultures (Chr. Hansen, A/S, Denmark) for inoculation of pasteurized milk were as follows the probiotic culture (P)- DVS- Probio- TecTM ABT-1 (*Lb. acidophilus*- 5, *Bifidobacterium*- 12, *Streptococcus thermophilus*), and the traditional culture (T) - FD (*Lc. lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Lc.* subsp. *lactis biovar diacetylactis*).

Fresh cheese production

Fresh cheese samples were produced according to the procedure Milanović et al. (2004) at the Laboratory of Dairy Technology, Faculty of Technology, Novi Sad. Six parallel experiments were performed using 6x15 L partially skimmed milk, which was previously homogenized and pasteurized at 71 °C for 15 s. After that, three batches of milk were inoculated with the traditional starter culture at 28 °C, while the remaining three batches were inoculated with the probiotic starter at 32 °C. Both starters were applied at a level of 0.01 g/100 g, together with 0.005 g/100 g enzyme Hannilase L 2235 (protease from Rhyzomucor miehei, Chr. Hansen, A/S, Denmark). Fermentation lasted 9.25 hours until pH≈4.5-4.3 was achieved. Subsequently, the coagulum was cut, heat-treated by gently stirring at 60 °C, quickly cooled for 5 min and drained for 6.5 hours. Cheese samples were homogenized by mixing and packed in aerobic conditions in polypropylene

and polystyrene cups of 0.18 L, using printed aluminium lids \emptyset 75.5 mm with thermo lacquer. The samples were stored at 4 °C for 30 days. Technological parameters of processing are presented in Table 1.

Methods

Packaging material thickness was measured according the standard SRPS G.S2.733. Mass per surface was determined according to the standard (SRPS G.S2.702). Tensile strength (TS) and elongation at break (EB) of films were measured on the Instron Universal Testing Instrument Model No 4301 (Instron Engineering Corp., USA), according to ASTM standard method D882-10. Permeability of gases (CO₂, O₂ and N₂) through two applied packaging materials (PP and PS) was measured by the method of Lyssy, according to DIN-53380, using the apparatus Lyssy GPM-200 (Systech Instruments, United Kingdom), gas chromatograph Gasukuro Kogyo GC-320 (Gasukuro Kogyo, Japan) and integrator HP 3396A (Hewlett-Packard, USA). Air permeability was estimated by calculation (Lyssy, 1984). Values for gas transmission rates were determined at 23 °C and 50 % RH.

Chemical analysis of milk and cheese was performed by employing standard methods: dry matter content after drying at 105 °C; fat according to Gerber (in milk) and Van Gulik (in cheese); total nitrogen according to Kjeldahl; lactose in milk by titrimetry and ash after mineralization at 550 °C (Carić et al., 1997). pH was measured by an electric pH-meter (ph Spear, Eutech Instruments, Oakton, USA).

Ascorbic acid in cheese samples was determined using HPLC with UV-DAD detection (Agilent 1100, USA). Ascorbic acid was extracted with metaphosphoric acid and extract was clarified and prepared for HPLC analysis by centrifugation and fine filtration (Malbaša et al., 2006; Malbaša et al., 2007). After packing, cheeses were stored at 4 °C for 30 days.

Table 1. Processing parameters

	Processing parameters						
Cheese sample	Coagulation time (h)	pН	Milk volume (m ³)	Cheese mass (kg)	Cheese yield (g/100 g)		
Traditional	9.25	4.5	15.0	2.64	18.0		
Probiotic	9.25	4.3	15.0	3.58	24.0		

Microbiological analysis

Viability of total lactic acid bacteria and total aerobic mesophylic bacteria were monitored after the manufacturing process.

For this purpose, 20 g portions of duplicate cheese samples were blended with 180 mL of sterile physiological solution. Total lactic acid bacteria were determined by pour-plating 1 mL of each dilution in MRS agar, prepared as a basal medium containing maltose, as described by the International Dairy Federation after 3 days of anaerobic incubation at 30 °C for both cheeses (Škrinjar, 2001).

Total aerobic mesophilic bacteria were counted by surface the colony count technique at 30 °C for enumeration of microorganisms (ISO, 4833).

Results and discussion

Characteristics of packaging materials

Results of physico-mechanical and barrier properties testing for selected packaging materials and packaging are presented in Table 2. Thickness of packaging material is important property that influences mechanical, as well as barrier characteristics of packaging material and packaging, as well as quality of packaging formation. Besides mean values for the material thickness variation, scattering of thickness values is also of great importance. Thickness values of two packaging materials were approximate and consistent, with no major discrepancies. Measured mass per surface showed similar values for PP and PS. Tensile properties are important and they show suitability of packaging material for selected application, predict behaviour during packaging filing and closing, transport, manipulation and storage.

According to the obtained results (Table 2), PP showed better tensile properties, tensile strength and elongation at break compared to PS. Tensile strength for the lids was similar in both directions measured. Filled cups were inspected visually and no obvious faults (displaced lid, unsealed spots and seal defects) were detected. All polymer packaging materials permeate gases in certain extent, why characteristic of the packaging material must be considered before application. Permeability for gases depends on the nature of material, its thickness, temperature and relative humidity and gas concentration gradient on the material surfaces (Lazić et al., 2010). Barrier characteristics of the applied packaging materials were assumed to be directly related to the cheese shelf-life. It is the most important to keep the level of oxygen within the package as low as possible for anaerobic and microaerophilic strains in probiotic products. Exposure to dissolved oxygen during storage is highly detrimental to

Table 2. Physical-mechanical and barrier properties of the packaging materials and packaging

		PS	РР	Al-lids	
Thickness (µm)		$(1.25 \pm 0.008)10^{3*}$	$(1.54 \pm 0.005)10^{3*}$	50.55±5.75	
				Print	4.62 ± 0.39
Mass per surface (g/m²)		1415 54 + 62 55*	1412.81±39.81*	Al	80.73±0.49
		$1415.54 \pm 62.55^*$	1412.81±39.81	Thermo lacquer	9.18±0.52
				Total	94.53 ± 0.44
Tensile strength (N/15 mm)	Longitudinal MD	-	-	38.2±3.48	
	Perpendicular TD	87.04±7.00**	108.21±11.17**	36.3±2.05	
Elongation at break (%)	Longitudinal MD	-	-	-	
	Perpendicular TD	19.70±11.87**	44.86±1.64**	-	
Gas permeability (mL/(m² 24 h bar))	CO ₂	867.3±44.70*	31.2±0.44*	-	
	O ₂	232.3±12.78*	31.4±2.84*	-	
	N ₂	76.2±4.19*	25.6±1.75*	_	
	Air	$108.1 \pm 5.95*$	26.9±3.66*	-	

MD- Machine direction; TD- Transverse direction; *data for the extruded tape, **data for the cup

B. bifidum and Lb. acidophilus. Being strictly anaerobic, Bifidobacterium is more sensitive to oxygen than Lb. acidophilus, although the sensitivity depends on the specific strain used (Talwalkar and Kallasapathy, 2003). The absence of an electron transport chain results in the incomplete reduction of oxygen to hydrogen peroxide. In addition, probiotic bacteria do not produce catalase, which causes the breakdown of hydrogen peroxide, thus leading to an accumulation of derived toxic metabolites, such as superoxide anion, the hydroxide radical and hydrogen peroxide in the cell, causing its death (Da Cruz et al., 2007; Talwalkar and Kallasapathy, 2003). In laboratory conditions, permeability of polypropylene and polystyrene to oxygen and nitrogen was measured and the obtained results are presented in Table 2. As expected, permeability of polypropylene was found significantly lower, regarding all tested gases, than the permeability of polystyrene.

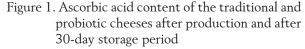
Nutritive characteristics of fresh cheese

The analysis of Quarg samples for nutrient parameters and energy value of samples are presented in Table 3. Traditional fresh cheese sample had slightly lower level of water (73.0 g/100 g), while sample produced with probiotic culture contained 79.48 g/100 g water. Fat content in samples ranged from 9 % (probiotic sample) to 11.5 % (traditional sample), while fat content in total solid varied from 43.86 % (probiotic sample) to 45.12 % (traditional sample). On the basis of water and fat content on a dry matter (total solids), all samples fulfilled the criteria to be classified as soft Quarg. Total proteins ranged from 9.63 % (w/w) (probiotic sample) to 12.5 % (w/w) (traditional sample). The obtained differences in protein content among cheese samples produced with different starter cultures were statistically significant (P<0.05). Protein content directly affect the minerals content and their ratio (phosphorus and calcium). The phosphor content varied

between Quarg samples ranging from 131.5 mg 100 g^{-1} (P) to 137.9 mg 100 g^{-1} (T). The concentration of calcium is very important for cheese quality. It is evident from Table 3 that the calcium content varies from 58 mg/100 g (T) to 91.7 mg/100 g (P).

The fat content affects the cholesterol content in Quarg samples. From the data in Table 3, it is apparent that the cholesterol content increased with higher fat content in dry matter. Such results were in accordance with literature data (Iličić et al., 2015). The authors concluded that the cholesterol content and cholesterol in energy increased with higher fat content in samples, while the cholesterol in fat decreased with the increase of fat content in probiotic Quarg cheese (Iličić et al., 2015). The energy value of Quarg samples produced from semi skim milk was on average 546.9 kJ/100 g.

Negligible effect of packaging material on a decrease of ascorbic acid content could be noticed (Figure 1). The average content of ascorbic acid in traditional cheese was as follows: $215 \ \mu g/100 \ g$ (after production), and $180 \ \mu g/100 \ g$ (after 30 days).



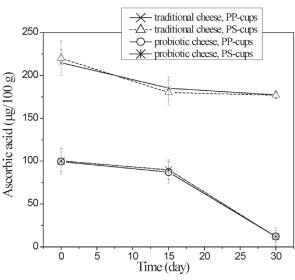


Table 3. Chemical compositions of the traditional and probiotic cheeses after production

Sample	Water (g/100 g)	Fat (g/100 g)	Protein (g/100 g)	Phosphorus (mg/100 g)	Calcium (mg/100 g)	Cholesterol (mg/100 g)	Energy value (kJ/100 g)
Traditional	73.00 ± 0.40	11.50 ± 0.20	12.50 ± 0.18^{a}	137.9 ± 2.76	58±1.16	39.0 ± 0.58	546.9 ± 10.9
Probiotic	79.48 ± 0.42	9.0 ± 0.18	9.63 ± 0.18^{a}	131.5 ± 2.63	91.7±1.83	32.7 ± 0.65	546.9 ± 10.0

 a Different letter for the same column indicate significant differences (P<0.05)

Also, average content of ascorbic acid in the probiotic cheese was: $100 \ \mu g/100 \ g$ (after production), and $10 \ \mu g/100 \ g$ (after 30 days). Water content in cheeses affected the level of ascorbic acid. Average value of water content in traditional cheese was 73.0 g/100 g, while the level of water in the probiotic cheese was 79.48 g/100 g, after production (Table 3).

Significant loss of ascorbic acid content in the probiotic product during the tested shelf-life might be associated with its degradation in the presence of oxygen. Ascorbic acid in milk is predominantly present in the form of L-ascorbic acid. In the presence of oxygen, it is reversibly oxidized to dehydroascorbic acid, which is further irreversibly oxidized to the biologically inactive 2, 3-Diketo-L-gulonic acid (Shephard et al., 1999). The intensity of ascorbic acid degradation within PP-cups was equal to the degradation within PS-cups, which implies that the difference in oxygen permeability of two materials did not affect degradation.

Microbiology of fresh cheese samples

The average number of LAB+BB was 2.4×10^8 CFU/g in traditional fresh cheese and 3.4×10^7 CFU/g in the probiotic cheese after the production. Relatively small difference in counts of cells with microaerophilic and anaerobic characteristics should be associated with the significant difference in microbiological characteristics of the initial starters. Higher count of aerobic bacteria cells in probi-

otic samples can be related to the antimicrobial and enzyme activities of probiotic starter as well as to higher production of organic acids (Figure 2a).

After a 30-day storage period of the traditional cheese, the average number of LAB+BB remained at the initial level (2.3x10⁸ CFU/g in PP-cups and 2.15x10⁸ CFU/g in PS-cups), despite the fact that the applied packaging materials possessed different permeability to oxygen (Figure 2b). The count of the LAB+BB cells, present in the probiotic cheese, remained almost unchanged (6x107 CFU/g in PP-cups and 2.5x107 CFU/g in PS-cups), approving very high level of their viability, regardless of the packaging material. Number of aerobic mesophilic bacteria significantly increased during storage in traditional cheese (1.4x108 CFU/g in PP-cups and 1.05x108 CFU/g in PS-cups). However, the probiotic cheese exhibited significant decrease of aerobic bacteria (5x106 CFU/g, in PP-cups, and 3.8×10^6 CFU/g, in PS-cups).

Conclusions

On the basis of the obtained results it can be concluded: Polypropylene and polystyrene with permeability to oxygen of 31.4 mL/m²/24 h/1 bar and 232.3 mL/m²/24 h/1 bar respectively, showed to be appropriate materials for packaging fresh probiotic cheese, in the defined storage period. Both packaging materials enabled high level of lactic acid

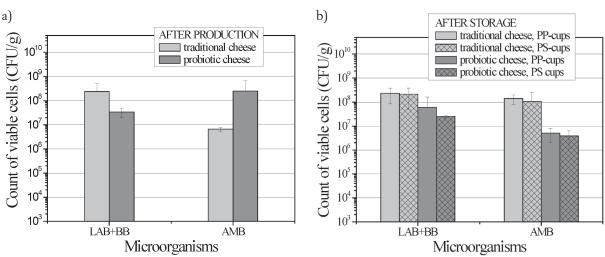


Figure 2. Total number of lactic acid bacteria and *Bifidobacteria* (LAB+BB) and aerobic mesophilic bacteria (AMB) in the traditional and probiotic cheeses: a) after the production and b) after 30 days of storage

bacteria viability during cheese storage. During the storage period there was a considerable number of microorganisms present in the traditional Quarg fresh cheese compared to probiotic cheese. Both packaging materials enabled high level of probiotic microorganisms viability during cheese storage. There was a two times higher content of vitamin C in a traditional Quarg than in probiotic fresh cheese during storage. Ascorbic acid content decreased in both packages, but at faster rate in probiotic fresh cheese during storage.

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Primjena uobičajenih materijala za pakiranje u proizvodnji probiotičkog svježeg sira

Sažetak

Cilj ovog rada bio je ispitati mogućnost primjene uobičajenih materijala za pakiranje - polipropilena (PP) i polistirena (PS) u proizvodnji probiotičkog svježeg sira. Probiotički i tradicionalni sir proizvedeni su iz mlijeka s 2,3 g/100 g mliječne masti primjenom probiotičke i tradicionalne starter kulture. Uzorci su pakirani u PP i PS-čaše i skladišteni 30 dana na 4 °C. Propusnost plinova kroz dva primijenjena materijala za pakiranje bila je značajno različita. U uzorcima sira uzetih za mikrobiološku analizu određene su bakterije mliječne kiseline, *Bifidobacterium* i aerobne mezofilne bakterije (AMB). Utjecaj materijala za pakiranje na sadržaj askorbinske kiseline, osjetljive na prisustvo kisika, nije bio značajan.

Ključne riječi: svježi sir, probiotici, materijali za pakiranje, barijerna svojstva, rok trajanja

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