

PROPERTIES OF LOW-FAT ULTRA-FILTERED CHEESES PRODUCED WITH PROBIOTIC BACTERIA

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Abstract - Probiotics are live microorganisms that in certain numbers may confer a health benefit on the host. Nowadays, there are many dairy products on the market, especially fermented milks, with probiotics, and their popularity is rising. The aim of this article was to investigate the viability of commercial probiotic bacteria (*Lactobacillus acidophilus* LAFTI®L10 i *Bifidobacterium lactis* LAFTI®B94, DSM, Netherland) as well as their influence on the changes of composition, pH, proteolysis, microbiological status and sensory properties of low-fat ultra-filtered (UF) cheeses within 2 months of ripening. Low-fat cast ultra-filtered (UF) cheeses were produced according to the defined production procedure by mixing UF milk protein powder, skim milk and cream, without (control cheese A) and with adjunct probiotic culture (cheese B). The compositional parameters (milk fat, proteins and dry-matter content), pH, proteolysis parameters (water soluble nitrogen, nitrogen soluble in 5% PTA, urea and SDS PAG electrophoresis), as well as the numbers of starters and probiotic bacteria, were determined during ripening. In addition, sensory evaluations of cheeses were performed throughout the ripening time. A significant influence of probiotic strains on the composition, pH and primary proteolysis of cheese during ripening was not found. The counts of commercial probiotic bacteria were maintained at high levels ($>10^7$ cfug⁻¹) during the overall ripening period, as a prerequisite of their therapeutic effects. The adjunct probiotic cultures enhanced the rate of secondary proteolysis, which was shown by the significantly higher levels of PTAN/TN of experimental compared to the control cheeses. The sensory evaluation showed that the overall aroma of low-fat cheeses was remarkably improved by the addition of the probiotic cultures used. Based on the results it can be concluded that the low-fat UF cheeses differ in good dietetic and functional properties as well as very acceptable sensory properties, and can be used as carriers of probiotics.

Key words: Low-fat UF cheeses, probiotic bacteria, viability, proteolysis, sensory properties

INTRODUCTION

In the past 20 years, the popularity and commercialization of food that could be beneficial to human health have significantly increased around the world. Milk products, including cheeses, and especially those with a lower fat content, represent a good base for the development of new products with functional properties. The most popular functional dairy products are produced with the addition of probiotic bacteria.

Probiotic bacteria are live microorganisms that in certain numbers show numerous positive effects on consumer health (Mattila-Sandholm et al., 2002). The therapeutic benefits of probiotics include the treatment of conditions including gastrointestinal disorders, hypercholesterolemia and lactose intolerance, suppression of procarcinogenic enzymes, immunomodulation and the treatment of food-related allergies (Begley et al., 2005, Huebner et al., 2007). It is recommended that products

with probiotics should contain at least 10^7 live microorganisms per g or ml (Ishibashi et al., 1993) in order to achieve their positive effects on consumer health.

The fortification of dairy products through the addition of probiotic bacteria is predominantly present in the production of fermented dairy milk products. However, in the last decade numerous research studies have been done on the possibility to incorporate and achieve the viability of probiotics during the production, ripening and storage period of different kinds of cheeses. To achieve the required probiotic viability in the product and their positive effects, it is necessary to find the proper selection of cheese type and technological production, ripening and storage conditions.

In the production of cheeses with probiotics, the type and strain of probiotic bacteria and their basic characteristics must be taken into account, with the aim of achieving the desired cell viability and metabolic activity during production and consumption of products. In addition, it is important to determine the effect of probiotic bacteria in the profile and flow changes during ripening, as well as the sensory properties and quality of cheeses (Gomes and Xavier, 1999, Mattila-Sandholm et al., 2002).

Probiotic bacteria can be added as a primary starter or adjunct culture. The first method is less suitable because of the low ability of probiotics to produce sufficient amounts of lactic acid during fermentation. Therefore, the addition of probiotics in the form of adjunct culture, along with starter cultures, is a much more reliable and affordable solution. However, special care must be taken in the choice of combination of probiotics and starter and their characteristics (Gomes and Xavier, 1999, Ross et al., 2002).

Significant success has been achieved in incorporating probiotics in the production of acid coagulated cheeses due to their similarity to fermented milks (Panić, 2004, Roy et al., 1997, Vinderola et al., 2000). However, much research into the incorporation of

probiotics in different kinds of cheese, such as cheddar (Ong et al., 2007, McBreatry et al., 2001), Edam (Tucović et al., 2004), brined cheeses (Kasimoglu et al., Yilmaztekin et al., 2004, Sabbagh et al., 2010), semi-hard (Bergamini et al., 2006), etc. has also been undertaken. The obtained results indicate that the cheeses possess a significant potential as carriers of probiotic bacteria.

UF cheeses are a very popular cheese group in our country and the Mediterranean region. They are usually produced as full-fat cheeses, but in the last several years, low-fat variants also exist on the market. Low-fat cheeses belong to dietetic products and represent a good base for creating products that may be classified as functional food (Miočinović et al., 2011).

The aim of this study was to investigate the possibility to incorporate probiotics in low-fat UF cheeses. In addition, the influence of probiotics on the composition, proteolysis and sensory properties of cheese, as well as their viability during the ripening and storage periods, was determined.

MATERIALS AND METHODS

Materials

The formulation and production of low-fat UF cheeses were carried out with the following raw materials: milk protein powder Promilk 852 (Ingredia, France), as a source of protein component, skim milk powder (Dairy plant Subotica, Subotica, Serbia), as a source of lactose component, and cream (Polimark, Belgrade, Serbia), as a source of lipid component of UF milk. Water was used as a solvent for the hydration of the milk protein isolate and skim milk powder, as well as for the dry-matter standardization of UF milk. For the production of cheeses, a mix of *Lactococcus lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* (LL50A, DSM, Netherlands) was used as primary starter culture, and *Lactobacillus acidophilus* (LAFTI®L10, DSM, Netherlands) and *Bifidobacterium lactis* (LAFTI®B94, DSM, Netherlands) was used as adjunct probiotic culture.

Cheese manufacture

In order to obtain the desired cheese composition, all raw materials (milk protein powder, skim milk powder and cream) were mixed at a ratio 18:17:7.5. Dissolution, hydration and intensive mixing of milk powders were performed at 50°C for 1 h, followed by heating of the concentrate obtained at 85°C for 5 min and then cooling at an inoculation temperature of 35°C. Inulin (1.5 g kg⁻¹; Cosucra, Belgium) was added before UF concentrate inoculation. Two variants of low-fat UF cheeses were produced: cheese A was produced with commercial starter cultures consisting of *Lactococcus lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* (LL50A, DSM, Netherlands) and cheese B was produced with the same commercial starter culture and commercial probiotic bacteria *Lactobacillus acidophilus* LAFTI®L10 and *Bifidobacterium lactis* LAFTI®B94 (DSM, Netherlands). An adequate amount of rennet (Fromase®, DSM, Netherlands) was added and then the UF concentrate was poured into packaging units (0.5 kg). Coagulation and fermentation was at 29-30°C for 17-18 h. Cheeses were dry salted by the addition of 20 g kg⁻¹ salt (Kristal So, Belgrade, Serbia) consisting of NaCl and KCl at a 3:1 ratio. Cheese ripening was at 12°C and 5°C during 7 days and 7 weeks, respectively. Cheeses were made in three replications.

Analytical methods

Sampling, determination of composition, proteolysis parameters, electrophoresis and sensory properties of low-fat UF cheeses were performed after fermentation (day 0), and after 7, 21, 35 and 56 days of ripening.

Cheese composition

Grated cheese samples were analyzed in duplicate for dry matter (DM) by the oven drying method at 102 ± 2°C (IDF, 1982), fat (MF) by the Van Gulik method (IDF, 1986), and total protein (TP) by the Kjeldahl method (IDF, 2002) on a Kjeltex System (Tecator 1002, Sweden).

Microbiological analysis

Two 10 g slices from each cheese sample were transferred to sterile saline diluents containing 1% peptone, and homogenized (Stomacher 400 Seward, London, UK). Starter bacteria counts were determined on M17 agar (Oxoid, CM 785) aerobically at 30°C for 48 h. *Lb. acidophilus* and *Bifidobacterium* sp. were determined on MRS-IM agar with 2% of maltose and MRS-IM agar with 2% glucose and dichloxallin, LiCl and cysteine hydrochloride solutions (Chr Hansen, Denmark), respectively, microaerophilic incubated (Gas Pack, BBL, Germany) at 37°C for 3 days. Microbiological data were transformed into logarithms of the number of colony-forming units (cfug⁻¹).

Assessment of proteolysis

Cheeses were analyzed for water-soluble (WSN) and 5% (v/v) phosphotungstic acid (PTAN) nitrogen content according to the methods of Kuchroo and Fox (1982) and Stadhousers (1960), respectively. Both of them were expressed as a percentage of the total nitrogen (WSN/TN and PTAN/TN).

Urea polyacrylamide gel electrophoresis (urea PAGE) was performed according to Andrews (1983). Electrophoresis was performed using a vertical slab unit TV200YK (Consort, Belgium) with 100x200x1 mm slabs using Tris-glycine electrode buffer, at a constant current of 60 mA, a maximum voltage of 300V, for 3 h, in a 4% stacking gel (pH 7.6) and 12% separating gel (pH 8.9).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) was performed according to Laemmli (Laemmli, 1970). Electrophoresis was performed with the same equipment as urea PAGE, Tris-glycine electrode buffer, using a 4% stacking gel (pH 6.8) and a 15% separating gel (pH 8.9), at a constant current of 80 mA, maximum voltage of 300V for 4 h.

α- and β-casein (Sigma, USA) and phosphorylase B (94000), bovine serum albumin – BSA (67000), ovalbumin (43000), carbonic anhydrase (30000),

trypsin inhibitor (20100) and α -lactalbumin (14400) (LKB-Pharmacia, Sweden) served as standards.

The gels were stained with 0.23% Coomassie Brilliant blue R-250, 3.9% trichloroacetic acid, 17% methanol, 6% acetic acid for 1.5 h, and destained in 8% acetic acid, 18% ethanol. The gel images were recorded using a scanner Bear Paw 2448TA+ (Mustek, Germany).

Sensory analysis

The sensory evaluation of the cheeses was carried out by six experts after 7, 21, 35 and 56 days of ripening. Four selected characteristics were evaluated, including exterior and interior appearance, body and texture, and flavor (odor and taste) using a 5-point scale, with 1 being the worst and 5 the best quality. Depending on the importance of attributes, they were corrected and multiplied by 1, 4, 10 and 5, respectively. The sum of the corrected scores gave the “percentage of total sensory quality”, the maximum being 100 (Radovanović and Popov-Raljić, 2001).

Statistical analysis

Data were analyzed using STATISTICA 6.0 (StatSoft, USA) data analysis software. The LSD test was used to determine differences among the cheeses at a 0.05 statistical level.

RESULTS AND DISCUSSION

Composition of the cheeses

The changes in low-fat UF cheese composition during the ripening period are shown in Table 1.

There were no significant differences ($P>0.05$) in the gross composition of control and experimental low-fat UF cheeses. As expected, all cheeses belonged to the group of soft and low-fat products (Regulative, 2010).

Results of previous studies based on the use of probiotics showed no significant influence of ad-

juvant culture addition on the composition of different types of cheeses (Kasimoğlu et al., 2004, Ong et al., 2006, 2007, Miočinović et al., 2012). None of the cheese composition parameters changed significantly ($P>0.05$) during 8 weeks of ripening. Similar to our results, UF-Feta cheeses are characterized by a close structure and a very low rate of syneresis during ripening (Karami et al., 2009).

Microbiological analysis

Starter and probiotic bacteria counts during the ripening of the low-fat UF cheeses are shown in Table 2.

The numbers of starter bacteria cells were maintained on the high level throughout the ripening period. As expected, their numbers were not significantly different between the cheeses, due to use of the same type and amount of starter culture.

A high viability of *Lb. acidophilus* (above 10^7 cfug⁻¹) was found during the ripening period of low-fat UF cheese B produced with commercial probiotic bacteria. However, a reduction in the number was observed during the ripening period, probably because of the low temperature of ripening (Kasimoğlu et al., 2004).

At the beginning of the study period, the number of bifidobacteria was significantly lower than the number of *L. acidophilus*, probably because of the ratio of these two strains used in the commercial starter culture. During the ripening period, the viability of bifidobacteria was maintained at a relatively constant level. The significant reduction in their number was found at the end of investigated ripening period ($< 10^7$ cfug⁻¹), probably due to their sensitivity to low pH values. McBreaty et al. (2001) indicate that the viability of bifidobacteria significantly depends on the type of the strains used. Improving the viability of probiotic bacteria during cheese ripening, especially very sensitive bifidobacteria, can be achieved by a number of microencapsulation techniques (Boylston et al., 2004, Özer et al., 2009). Nonetheless, these results suggest that this type of cheese can be a good carrier of both

Table 1. The composition parameters of low-fat UF cheeses produced without (A) and with (B) probiotic bacteria during ripening.

Days of ripening	Cheese	Dry matter (%)	Milk fat	Total proteins (%)	Moisture in non-fat substances (%)	Fat in dry matter (%)
0	A	24.69±0.42a	3.83±0.24a	15.94±0.33a	78.31±0.61a	15.54±1.19a
	B	24.61±0.16a	3.83±0.29a	16.63±0.44a	78.53±0.17a	15.58±1.27a
7	A	27.03±0.59a	3.83±0.24a	16.44±0.75a	75.88±0.75a	14.20±1.10a
	B	27.04±0.52a	3.83±0.29a	16.96±0.38a	75.87±0.75a	14.19±1.31a
35	A	26.57±0.18a	3.83±0.24a	16.73±0.14a	76.36±0.26a	14.43±0.89a
	B	26.23±0.28a	3.67±0.29a	16.24±0.29a	76.58±0.07a	13.97±0.95a
56	A	26.11±0.26a	4.00±0.00a	16.08±0.19a	76.97±0.27a	15.32±0.15a
	B	26.05±0.76a	4.00±0.00a	16.44±0.82a	77.03±0.80a	15.37±0.46a

*Results are expressed as mean ± standard error of means; means of each parameter in the same column with the same letter do not differ significantly ($P>0.05$).

Table 2. Viability of starter and probiotic bacteria ($\log \text{cfug}^{-1}$) during ripening of low-fat UF cheeses made without (A) and with (B) probiotic bacteria.

	Days of ripening			
	0	7	35	56
<i>Lactococcus</i> sp.				
A	8.46±0.18a	8.55±0.28a	6.43±0.23a	6.82±0.12a
B	8.41±0.20a	8.20±0.12a	7.11±0.16b	7.11±0.17b
<i>L. acidophilus</i>				
B	8.36±0.22	7.75±0.25	7.43±0.23	7.15±0.49
<i>B. lactis</i>				
B	7.49±0.10	7.43±0.13	7.54±0.30	6.74±0.17

strains of probiotics due to their high level during the ripening period, which is necessary in order to achieve positive therapeutic effects on the health of consumers.

Proteolysis parameters

Nitrogen fractions

The content of WSN/TN, PTAN/TN and PTAN/WSN of low-fat UF cheeses during ripening are shown in Table 3.

The content of WSN/TN marked a constant increase during cheese ripening, especially after 7 and 21 days ($P<0.05$). The content of WSN/TN of cheeses A and B was 11.12 and 11.30%, respectively, at the end of ripening, which is significantly lower compared to WSN/TN (12-20%) of brined cheeses made by conventional manufacturing process (Abd El-Salam and Alichanidis, 2008). The addition of an adjunct probiotic culture showed no significant influence ($P>0.05$) on the rate of primary proteolysis determined by WSN/TN content. Similar results were presented by Ong et al. (2006, 2007) who investigated the impact

Table 3. Proteolysis parameters of low-fat UF cheeses produced without (A) and with (B) probiotic bacteria during ripening, WSN/TN – water soluble nitrogen as part of total nitrogen; PTAN/TN and PTAN/WSN – phosphotungstic acid soluble nitrogen as part of total and water soluble nitrogen, respectively.

Days of ripening	Cheese	WSN/TN	PTAN/TN	PTAN/WSN
0	A	8.55±0.07a	0.90±0.09a	10.48±1.07a
	B	8.65±0.09a	0.86±0.12a	9.99±1.30a
21	A	10.35±0.27a	1.41±0.04a	13.61±0.68a
	B	10.50±0.59a	1.62±0.05b	15.44±0.97a
35	A	10.46±0.10a	1.38±0.06a	13.21±0.66a
	B	10.79±0.22a	1.62±0.12b	14.95±0.46a
56	A	11.12±0.26a	1.76±0.12a	15.84±0.75a
	B	11.30±0.59a	1.70±0.15b	15.05±0.56a

*Results are expressed as mean ± standard error of means; means of each parameter in the same column with the same letter do not differ significantly ($P>0.05$).

Table 4. The sensory analysis of low-fat UF cheeses produced without (A) and with (B) probiotic bacteria during ripening.

Days of ripening	Cheese variant	Exterior appearance	Interior appearance	Texture and consistency	Aroma	% of Max. quality
21	A	4.97±0.05a	4.97±0.05a	3.78±0.17a	3.49±0.15a	78.62±2.53a
	B	4.94±0.10a	4.97±0.05a	3.89±0.11a	3.83±0.14b	83.14±1.65b
35	A	4.89±0.02a	4.92±0.14a	3.50±0.00a	3.46±0.07a	76.66±0.63a
	B	4.92±0.07a	4.92±0.07a	3.65±0.30a	3.82±0.19b	82.71±2.95b
56	A	4.89±0.07a	4.92±0.13a	3.50±0.13a	3.46±0.19a	76.66±2.03a
	B	4.96±0.07a	4.96±0.07a	3.77±0.13a	3.78±0.21b	81.93±5.03b

*Results are expressed as mean ± standard error of means; means of each parameter in the same column with the same letter do not differ significantly ($P>0.05$).

of different probiotics on the properties of Cheddar cheese during 6 months of ripening. These results were expected due to fact that primary proteolysis of most cheeses takes place under the action of residual rennet and starter proteinases. Hence, starter cultures, including probiotics, usually have different peptidase systems which do not assume a significant role during primary proteolysis, but influence the secondary proteolytic changes (Sousa et al., 2001).

The content of PTAN/TN increased constantly throughout ripening. A statistically significant increase of PTAN/TN ($P<0.05$) was observed after 7 and 21 days. It is important to note that the cheese made with probiotics (cheese B) showed a significantly higher content of PTAN/TN after 21 days of ripening compared to control cheese A. This relatively intensive rate of secondary proteolysis is presumably the result of specific peptidolytic activity of

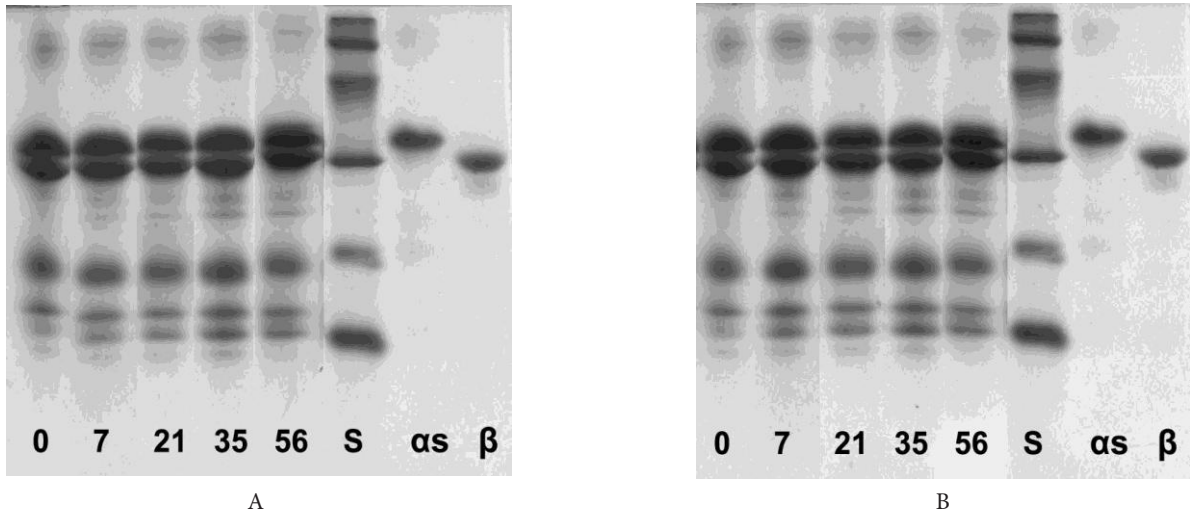


Fig. 1. Urea electrophoretograms of low-fat UF cheeses produced without (A) and with (B) probiotic bacteria after production (0) and 7, 21, 35 and 56 days of ripening; α_s – standard of α_s casein and β – standard of β casein (Sigma, USA).

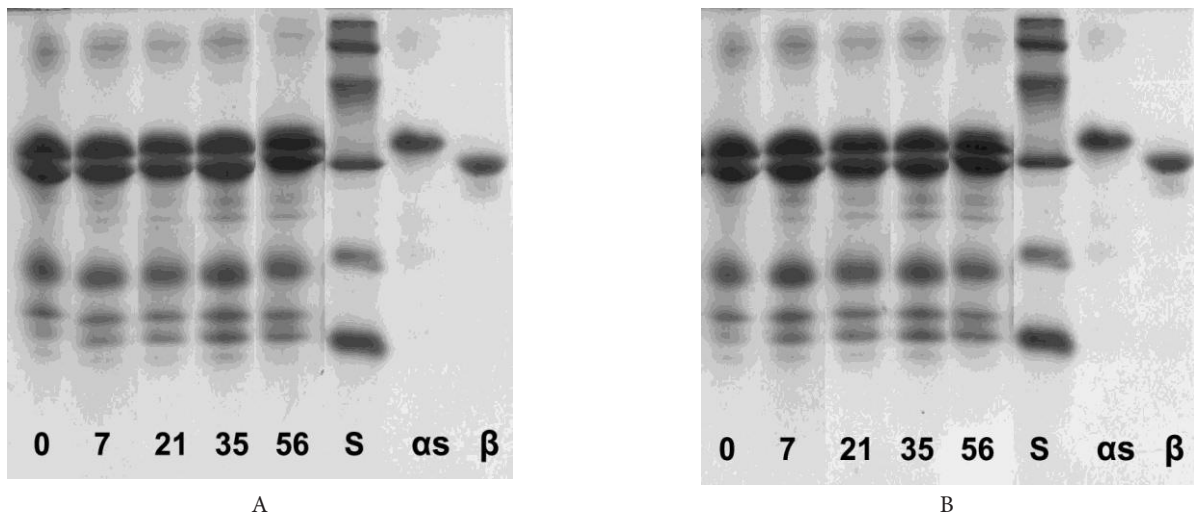


Figure 2. SDS electrophoretograms of low-fat UF cheeses produced without (A) and with (B) probiotic bacteria after production (0) and 7, 21, 35 and 56 days of ripening; S – molecular weight standard; α_s – standard of α_s casein and β – standard of β casein (Sigma, USA).

adjunct probiotic culture. The higher rates of secondary proteolysis due to the addition of probiotics were also found during studies of different kind of cheeses (Ong et al., 2006, Bergamini et al., 2006, Kasimoğlu et al., 2004, Miočinović et al., 2012).

The content of PTAN/TN was about 1.76% at the end of the ripening period of 56 days. It was significantly lower than the PTAN/TN of traditionally made cheeses, which is often within 3-5% (Abd El-Salam and Alichanidis, 2008). These results confirm

the facts about slower proteolytic changes during the ripening of UF cheeses (Bech, 1993).

PAG electrophoresis

Urea and SDS electrophoretograms of low-fat UF cheeses produced without (A) and with (B) probiotics during ripening are shown in Figs. 1 and 2. At all ages, the PAGE patterns for the control and the experimental cheeses were similar, suggesting that the mode and rate of casein breakdown were simi-

lar in both cheeses. It is also evident that the rate of hydrolysis of the two caseins was different. The hydrolysis of α_{s1} -casein was more intensive than that of β -casein. This indicates a more pronounced activity of residual chymosin than of plasmin. According to the results of proteolysis and casein degradation, it can be concluded that proteolytic changes of low-fat UF cheeses are weak, as already shown by determination of the soluble nitrogen fractions in Table 3.

The sensory evaluation

The results of the sensory assessment of cheese quality during ripening are shown in Table 4. The appearance as well as textural quality of both low-fat UF cheeses was considered very satisfactory at all sampling ages with no significant differences ($P > 0.05$) between the cheeses. The experimental low-fat UF cheeses made with commercial probiotic culture received significantly ($P < 0.05$) higher flavor scores than the control cheese, which influenced the total sensory quality expressed as % of maximum quality (Table 4). The results are attributed to the significantly ($P < 0.05$) higher concentrations of PTAN (small peptides and free amino acids) in the experimental cheese B than in control cheese A made just with starter lactococci. It is of note that the addition of the adjunct culture significantly improved the sensory quality of the low-fat cheese due to specific peptidolytic enzymes that are presumably responsible for the formation of flavor compounds. The enhancement of flavor of various low-fat cheese varieties due to the addition of an adjunct culture, including probiotics, is in agreement with the results of numerous research studies (Kasımoğlu et al., 2004, Ong et al., 2006, Sabbagh et al., 2010)

CONCLUSION

Low-fat UF cheese represents a good vehicle of probiotic bacteria that maintains a satisfactory viability during ripening period. A high number of *Lb. acidophilus* throughout the ripening period was found which is necessary for therapeutical effects. The use of probiotic bacteria as an adjunct contributes to a higher rate of secondary proteolysis, which improves

the sensory properties of low-fat UF cheese. Low-fat UF cheeses, produced according to the established production procedure, are distinguished by very acceptable sensory quality and satisfactory dietetic and functional properties, due to a low fat and salt content, adequate fiber-inulin content, as well as probiotic viability.

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