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The application of autochthonous lactic acid bacteria in white brined cheese production

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Summary

The effects of autochthonous strains of lactic acid bacteria on the characteristics of white brined cheeses were studied throughout 90 days of ripening. Cheese A was produced with strains: *Lactococcus lactis* ssp. *lactis* 653, *Lactococcus lactis* ssp. *cremoris* 656, *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* 07 and *Lactobacillus paracasei* ssp. *paracasei* 08 (8:5:5:2) and cheese B with strains: *Lactococcus lactis* ssp. *lactis* 195, *Lactococcus lactis* ssp. *cremoris* 656 and *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* 07 (10:5:5). The lactococci counts in both cheeses and lactobacilli count in cheese A remained at a high level, while lactobacilli count in cheese B increased through the ripening. No significant differences ($P<0.05$) were found in the gross composition of the experimental cheeses, although the pH values were lower in cheese A. Proteolysis was assessed by the water-soluble nitrogen fractions, 5 %-phosphotungstic-acid-soluble nitrogen fractions and SDS-PAGE-electrophoresis. Both experimental cheeses were characterized by a high rate of proteolysis. According to sensory evaluation, experimental cheeses received high total scores. The results show that autochthonous strains of lactic acid bacteria can be successfully applied in white brined cheeses production.

Key words: autochthonous lactic acid bacteria, white brined cheese, ripening

Introduction

White brined cheeses are the most widely produced and consumed cheeses in Serbia (about 60 % of total cheese consumption). There are many different types of Serbian brined cheeses, named according to their production regions as follows: Sjenica cheese, Zlatar cheese, Svrljig cheese, Homolj cheese (Dozet et al., 1996). These cheeses can be very similar, but they also rather differ in respect of the milk type, region of production, manufacturing protocols, composition and sensory properties, etc. Traditionally, they were usually made from cow's, sheep's and goat's milk, but nowadays they are mainly produced from cow's milk. Currently, the cheeses made from ewe`s, sheep's and goat`s milk represent only a small part of the total cheese production in Serbia, because these types of milk participate with only somewhat more than 1 % in the total milk production. Currently, white brined cheeses in Serbia are produced by both traditional and industrial methods. Traditionally, white brined cheeses have usually been made from unpasteurized or medium heat-treated milk, without starter cultures and in small dairy plants and households using simple equipment (Dozet et al., 1996). The characteristics of autochthonous white brined cheeses were extensively investigated in previous studies (Miočinović et al., 1982; Sarić and Bijeljac, 2003; Barać et al., 2006; Maćej et al., 2006).

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Enzymes of naturally present non-starter microflora have a significant role in the numerous events during ripening of traditionally made white brined cheeses. Their growth and activity are completely uncontrolled and unpredictable, resulting in less uniform sensory characteristics and composition than in cheeses made from pasteurized milk with starter cultures (Karakus and Alperden, 1995; Barać et al., 2006). On the other side, autochthonous cheeses mature faster with stronger and richer flavours being obtained from raw milk cheeses (Mc Sweeney et al., 1993; Grappon and Beuvier, 1997).

In an industrial scale, white brined cheeses are made from pasteurized milk with the addition of starter cultures. Different starter cultures, including mesophilic and/or termophilic bacteria, have been used in the production of white brined cheese, but the most important are composed of lactococci *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (Hayaloglu et al., 2002).

The use of commercial starter cultures for cheese production from pasteurized milk results in the loss of typical characteristics of artisan cheeses due to the replacement of complex native microflora with a defined starter culture. The activity of standard starter strains is more controlled, which leads to the development of uniform characteristics of cheese without any flavour and textural defects, due to the controlled conditions used in their production (Johnson et al., 1990).

Cheese ripening is a complex process involving proteolysis, lypolysis and glycolysis as the main biochemical events which lead to the characteristic aroma and texture of each cheese variety. Proteolysis is primarily carried out by the residual coagulant in cheese. Alkaline proteinase of milk, especially plasmin, has also been proven to contribute to proteolysis in some cheese (Somers and Kelly, 2002). The proteolytic activity of starter and non-starter lactic acid bacteria has a very important role in biochemical changes during cheese ripening (Visser, 1993; Mc Sweeney and Fox, 1997).

Microbiological research of traditionally produced cheeses is very important for the development of an appropriate starter for the production of white brined cheeses. Therefore, information about the natural microflora of traditional cheeses may help to define a starter that allows standardization of product quality and safety without changing the basic properties of cheeses. It was observed that lactic acid bacteria present in white brined cheese are predominant throughout ripening (Karakus and Alperden, 1995). Radulović et al. (2006) presented that the most frequent species in the Sjenica cheese are *L. lactis* ssp. *lactis* (35.85 %), *Lb. paracasei* (18.86 %), *Lb. plantarum* (16.99 %), *L. lactis* ssp. *lactis* biovar. *diacetylactis* (7.55 %), *Leuconostoc* spp. (3.77 %), *Lb. curvatus* (1.89 %), *Lb. brevis* (1.89 %) and *Enterococcus* sp. (13.20 %). According to the literature, there is a great number of references concerning the significance and effects of particular starter and non-starter enzymes on the characteristics and ripening of white brined cheese (Dağdemir et al., 2003; Hayaloglu et al., 2004; Goncu and Alpkent, 2005; Psoni et al., 2006; Djerovski et al., 2007).

The aim of this study was to make cheese with characteristics typical for artisanal cheeses using selected autochthonous LAB and heat-treated milk. The effects of different cultures on the chemical, microbiological and sensory characteristics of white brined cheeses during ripening were investigated.

Materials and methods

Bacterial cultures

The lactic acid bacteria used as starter cultures were constituted of five strains: *L. lactis* ssp. *lactis* 653, *L. lactis* ssp. *cremoris* 656, *L. lactis* spp. *lactis* 195, *L. lactis* ssp. *lactis* biovar. *diacetylactis* 07 and *Lb. paracasei* 08, isolated from traditional Serbian (Sjenica) white brined cheese samples. These strains were selected according to their characteristics which are interesting from the biochemical and technological viewpoint, as described by Martinović et al. (2005), and also with regard to the results of the preliminary cheese-making trails carried out at laboratory scale. Before use, each strain was cultivated in M17 broth (Merck, Darmastadt, Germany) for two transfers at 30 °C for 24 h (1 mL inoculum 100 mL^{-1}). For propagation, strains were grown separately in sterile reconstituted skim milk (1 mL inoculum 100 mL^{-1}, heated at 90 °C for 30 min) as described by Fenelon et al. (2000). The cell numbers were approximately $1x10^9$ cfu mL^{-1}. Afterwards, two different starter cultures were prepared by mixing strains as follows: for cheese A: *L. lactis* ssp. *cremoris* 656,

L. lactis ssp. *lactis* 653, *L. lactis* ssp. *lactis* biovar. *diacetylactis* 07, *Lb. paracasei* 08 (8:5:5:2); and for cheese B: *L. lactis* ssp. *cremoris* 656, *L. lactis* spp. *lactis* 195, *L. lactis* ssp. *lactis* biovar. *diacetylactis 07* (10:5:5).

Cheese making

The white brined cheeses were made from fresh cow`s milk in two trials. In each trial, pasteurized (65 °C, 30 min) cheese milk was cooled to 33-34 °C, divided into two equal parts and starter cultures were inoculated to the level of 1 kg 100 kg^{-1}. Food-grade calcium chloride (Merck, Darmstadt, Germany) was added to the cheese milk at the level of 0.2 g L^{-1}. The inoculated milk (32 °C) was held for about 30 min. Then calf rennet (Chr. Hansen, Denmark) was added (0.012 g L^{-1}) and the coagulation took place within 45 min at 31-32 °C. Once curdling was completed, the coagulum was cut into small pieces (2-5 cm) and stirred three times for 5 min during 30 min. The cheese mass was carefully transferred from cheese vats into the mould. After about 1 h of draining (without pressing), the pressure was applied (max. 3 $kg \cdot kg^{-1}$) for 3 h. Then, the cheese curds were cut into pieces of 10x10x7 cm with a knife. The curd blocks were dry salted with 2.4 % NaCl. The next day the cheese was placed in plastic cans and covered with brine (8 % NaCl). The ripening was conducted at 16-18 °C to attain pH~4.9 (first week), and afterwards at 13-14 °C until the end of the 90-day ripening period. Cheese manufacturing was performed in triplicate. The cheeses were sampled and analyzed after 1, 10, 30, 60, 90 days of ripening.

Chemical analysis

Chemical composition of each cheese sample was determined using the following standard methods: total solids by the standard drying method (IDF, 1982), fat content by Van Gulik method (Carić et al., 1997), salt content by Mohr`s method (Pejić and Djordjević, 1963) and total nitrogen content by Kjeldahl method (IDF, 1993). The pH was measured with a pH meter PHM 82 Standard (Radiometer, Copenhagen, Denmark).

Assessment of proteolysis

Determination of nitrogen fractions

Water soluble nitrogen (WSN) was determined in samples of cheese extracts prepared as described by Kuchroo and Fox (1982) and expressed as a percentage of the total nitrogen (WSN/TN). The nitrogen soluble in 5 % phosphotungstic acid (PTA-N) was also determined by Kjeldahl method described by Stadhouers (1960) and expressed as a percentage of TN (PTA-N/TN). All analyses were conducted in triplicate.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE)

Protein changes of the cheese samples after 10, 30, 60 and 90 days of cheese ripening were detected by the SDS-polyacrilamide gel electrophoresis (SDS-PAGE) performed according to Laemmlie (1970) on 12.5 % slab gel. Prior to the electrophoresis, proteins were diluted to 0.002 g mL^{-1} with the sample buffer (pH 6.8). The vertical electrophoresis unit LKB-2001-100 was used in conjunction with the power supply Macrodrive 5 and Multitemp II (LKB, Sweden). Samples (0.017 mL) were run at I=50 mA, Umax=300 V for five hours. The gel was stained with the 0.23 % solution of Coomasie Blue R-250 for 90 min, and distained in the methanol/acetic acid solution (5 % methanol, 7 % acetic acid). Distained gels were scanned using the Scanexpress 12000 (Mustac, Germany). Detected polypeptides were identified using the low molecular weight kit (LKB-Pharmacia, Sweden). β-casein and Na-caseinat (Sigma, USA) were used as control standards.

Microbiological analysis

A 10^{-1} dilution was obtained by homogenization of a each cheese sample (20 g) in 180 mL of sterile sodium citrate 2 % (w/v) for 2 min in Stomacher (Lab Blender 400, Seward Medical, London, UK). Further decimal dilutions were carried out in sterile 0.1 (w/v) Bacteriological Peptone (Oxoid, Basingstoke, UK). Lactobacilli were enumerated following pour plating on MRS agar (Oxoid, CM 361) and incubated at 30 °C for 48 h under anaerobic conditions in gas-pack systems (BBL, Germany); lactococci on M17 agar (Oxoid, CM 785) under aerobic conditions at 30 °C for 48 h. Microbiological analyses were carried out in triplicate.

Sensory analysis

The sensory evaluation of the cheeses was carried out after 10, 30, 60 and 90 days of ripening by nine semi-trained assessors, selected among the staff of the Department of Dairy Technology of the Faculty of Agriculture, Belgrade. Cheeses were evaluated for the following attributes: appearance (exterior-interior), texture and aroma (flavour and odour) and consistency using a 5-point scale (with 1 being the poorest and 5 the best quality). Thus, the scores were multiplied by 1.0, 4.0, 5.0, and 10.0, respectively. The total score for excellent cheese was 100.

Statistical analysis

Statistical analysis was used to study the influence of chemical composition and sensory evaluation by single-factor analysis of variance (ANOVA). The differences between individual means were tested using Turkey's method. Significant differences were considered for $P<0.05$. Calculations were performed with software Statistica for Windows 5.1 StatSoft, 1996 (Tulsa, OK, USA).

Results and discussion

The chemical composition

The chemical composition of white brined cheeses made with different starter cultures during ripening is given in Table 1.

The dry matter (DM) content of experimental cheeses is common for white brined cheese (Hayaloglu et al., 2002; Maćej et al., 2006). According to moisture on free-fat basis (MFFB) content, both cheeses belong to the soft cheese group (Codex Stan, 2003). The DM content increased until the 30th day and then slightly decreased until the end of ripening, but the changes were not statistically differ ($P<0.5$). According to fat in dry matter (FDM) content, experimental cheeses belong to high fat cheeses, but are similar to full fat cheeses (Codex Stan, 2001).

Salt contents varied from 1.73 to 2.15 % in cheese A and from 1.59 to 2.01 % in cheese B during the first 30 days of ripening. The salt contents slightly decreased in the later phase of ripening and at the end of ripening it stood at 1.98 % and 1.74 % in cheese A and B, respectively. The salt contents were significantly different after the 1st and 10th day of ripening in both experimental cheeses ($P<0.05$). The salt contents of examined cheeses were lower than asserted in the data obtained by other authors

Table 1. The chemical composition of experimental cheeses during ripening

Time (days)	DM[a]* (g 100 g^{-1})	Fat[a] (g 100 g^{-1})	MFFB[a]* (g 100 g^{-1})	FDM[a]* (g 100 g^{-1})	Salt[a] (g 100 g^{-1})
			Cheese A		
1	45.62±2.12a	27.50±1.32a	75,00±1.59a	60.28±0.52a	1.73±0.19a
10	46.79±1.71a	28.17±1.04a	74.08±1.55a	60.20±1.12a	2.07±0.15b
30	47.06±1.03a	28.67±1.04a	74.22±0.62a	60.92±1.12a	2.15±0.10b
60	46.67±1.34a	28.67±0.76a	74.76±1.08a	61.42±0.14a	2.07±0.10b
90	46.28±1.43a	28.33±0.58a	74.96±1.45a	61.23±0.84a	1.98±0.12b
			Cheese B		
1	45.36±2.13a	28.00±2.00a	75.89±1.02a	61.73±1.72a	1.59±0.22a
10	46.41±1.70a	28.00±1.80a	74.44±0.60a	60.34±1.72a	1.99±0.09b
30	47.14±1.49a	28.83±1.53a	74.28±0.73a	61.17±1.49a	2.01±0.09b
60	46.44±1.00a	28.83±0.76a	75.26±1.11a	62.09±1.35a	1.91±0.18b
90	45.93±1.12a	28.33±0.58a	75.45±0.97a	61.69±0.32a	1.85±0.14b

[a]Average ± standard deviation for samples from 3 repeated probes
*DM - dry matter; MFFB - moisture on a free-fat basis; FDM - fat in dry matter
a, b - Values within the same column not sharing a common letter were significantly different ($P<0.05$)

(Karakus and Alperden, 1995; Dozet et al., 1996; Hayaloglu et al., 2002, 2004; Maćej et al., 2006).

No significant differences were observed between the mean scores of any parameter of chemical composition of cheese A and B at the similar ripening stages. The values of chemical parameters of investigated cheeses were similar with the literature data for white brined cheeses (Miočinović et al., 1982; Azarnia et al., 1997; Dagdemir et al., 2003; Djerovski et al., 2008). However, it is important to note that the composition of different white brined cheeses varies within a broad interval due to the differences in the composition of raw milk, processing parameters and ripening conditions (Hayaloglu et al., 2002).

It can be concluded that the chemical composition of cheeses was not affected by the use of various starter cultures. References with similar results for other cheeses have been found (Karakus and Alperden, 1995; Hayaloglu et al., 2002; Goncu and Alpkent, 2005; Djerovski et al., 2007). The pH changes are presented in Figure 1.

Lower pH values were determined in cheese A throughout the entire ripening process. In cheese A, the high level of lactococci and lactobacilli, which were added as starter cultures, contributed to the faster development of acidity in the first month of the ripening process. In cheese B, naturally present lactobacilli were on a lower level during 30 days of ripening. Towards the end of ripening, the number of present lactobacilli increased and their faster growth and activity can lead to a better development of acidity (Mc Sweeney et al., 1995).

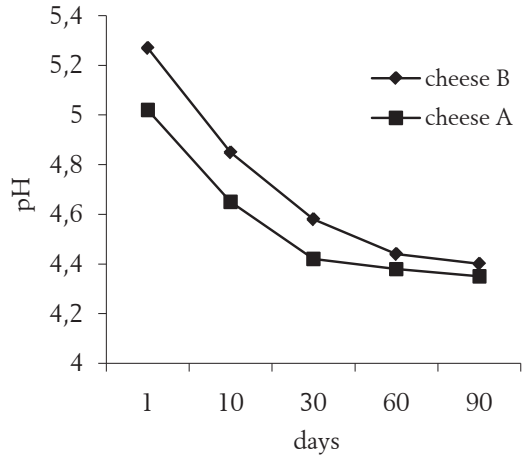

Figure 1. Changes of pH values during the ripening of cheeses A and B

Proteolysis

The total nitrogen (TN) content was not significantly different ($P<0.05$), either between experimental cheeses or between the ripening stages of cheeses A and B (Table 2).

The data on TN content is very similar to the literature data (Azarina et al., 1997; Hayaloglu et al., 2002). The changes in the TN content are related with the changes in the DM content during cheese ripening (Award et al., 1999; Maćej et al., 2006).

Furthermore, the TN content of white brined cheeses decreased during ripening due to the diffusion of certain soluble nitrogenous compounds into the brine (Abd El Salam et al., 1993; Michaelidou et al., 2005).

The proteolysis of white brined cheeses is affected by the type of the coagulant used, salt concentration, storage temperature, ripening period and other conditions. High moisture in the curd and a low degree of heat treatment during processing white brined cheese, caused the coagulant to be retained at a higher level compared with other cheese types (Abd El Salam et al., 1993).

As it was expected, the level of WSN/TN increased in cheeses throughout aging (Table 2). The WSN/TN content increased continuously in cheese A, while in cheese B WSN/TN content showed more significant increase in the later ripening stages. The cheeses contained a small quantity of WSN compounds at the beginning of ripening which indicates that proteolytic changes started during curd processing (Fox et al., 1993).

The WSN/TN content of cheese A and B at the beginning of ripening was 8.54 and 7.87 and then increased to 19.02 and 20.04, respectively. Abd El Salam et al. (1993) reported that the ripening index (WSN/TN) of white brined cheeses usually ranges from 12 to 20 % (max. value is 25 %). Presented results are in agreement with those reported for different varieties of white brined cheese by other researchers (Azarina et al., 1997; Moatsou

Table 2. The changes of nitrogen compounds content during cheese ripening

Time (days)	TN[a]* (g 100 g^{-1})	WSN/TN[a]* (g 100 g^{-1})	PTAN/TN[a]* (g 100 g^{-1})
Cheese A			
1	2.34±0.14a	8.54±1.06a	-
10	2.40±0.11a	11.11±0.85b	1.49±0.22a
30	2.41±0.07a	16.57±1.69c	2.54±0.20b
60	2.37±0.06a	17.75±1.58cd	3.26±0.53c
90	2.33±0.08a	19.02±2.09d	4.10±0.43d
Cheese B			
1	2.35±0.07a	7.87±0.48a	-
10	2.40±0.05a	9.90±0.73ab	1.36±0.28a
30	2.42±0.05a	11.74±1.60b	2.41±0.20b
60	2.37±0.04a	18.68±1.37c	3.32±0.28c
90	2.27±0.04a	20,04±1.00c	4.28±0.53d

[a]Average ± standard deviation for samples from 3 repeated probes
*TN - total nitrogen; WSN/TN - water soluble nitrogen expressed as % of TN; PTAN/TN - nitrogen soluble in 5 % phosphotungstic acid expressed as % of TN
a, b, c, d - Values within the same column not sharing a common letter were significantly different ($P<0.05$)

et al., 2002; Sarić et al., 2002; Goncu and Alpkent, 2005; Maćej et al., 2006). However, it can be concluded that both experimental cheeses were characterized by a high rate of proteolysis.

The PTA-N fraction in cheese contains small peptides and amino acids mainly from the proteolytic activity of starter and non-starter bacteria and to a lesser extent rennet (Abd El Salam et al., 1993). The formation of PTA-N compounds was found to be very extensive during the ripening of both cheeses. The PTA-N/TN content of cheese A and B was 1.49 and 1.36 at 10 days of ripening, respectively. At the end of ripening, the PTA-N/TN content of cheese A and B was 4.10 and 4.28, respectively. The PTAN/TN content increased continuously in both experimental cheeses, but there were no significant differences ($P<0.05$) between cheeses A and B. Abd El Salam et al., (1993), reported that the PTA-N/TN content in white brined cheeses is usually within the range of 3-5 % and presented results correspond with this interval. The increase of PTA-N/TN content illustrates that the secondary proteolysis in both experimental cheeses was intensive, which contributes to a rich flavour and aroma of cheese.

Results of SDS PAGE electrophoresis showed differences in proteolytic changes on β-casein in experimental cheeses. The hydrolysis of β-casein in cheese A is shown in Figure 2 and in cheese B in Figure 3.

The most intensive αs-casein and β-casein degradation was detected up to 30 days of ripening in cheese A, but in the second month of cheese ripening they became more intensive in cheese B. Therefore, at the end of cheese ripening the degree of β- and αs_1-casein hydrolyses was at a slightly higher level in cheese B.

The results observed by SDS-PAGE were in agreement with the dynamic of changes in WSN/TN content, and they both illustrate the rate of primary proteolysis in cheese A and B. These results showed significant proteolytic changes on β-casein, as a consequence of proteolytic ability of starter culture (*L. lactis* ssp. *lactis* 195 and *Lb. paracasei* 08) to hydrolyze β-casein as described by Radulović et al. (2010).

Lee et al. (1990) reported that *Lb. casei* strains possess stronger peptidase activity, as an important factor in proteolytic changes during white brined cheese ripening. For accelerated ripening of Turkish white brined cheeses, Karakus and Alperden (1995) recommended application of *Lb. casei* strains. The application of *Lb. paracasei* 08 resulted

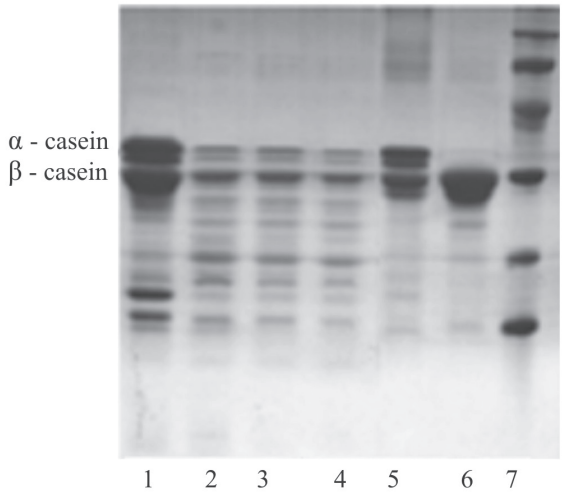

Figure 2. SDS-polyacrilamide gel electrophoresis of cheese A. Lines 1-4: cheese after 10, 30, 60, 90 days of ripening; line 5: Na-caseinate; lane 6: βcasein; lane 7: standard

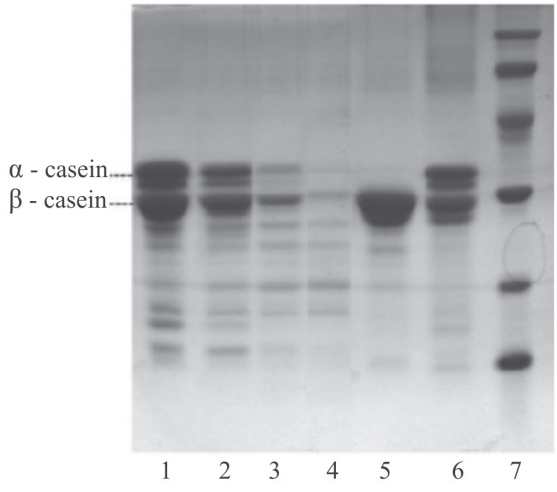

Figure 3. SDS- polyacrilamide gel electrophoresis of cheese B. Lines 1-4: cheese after 10, 30, 60 or 90 days of ripening; line 5: Na-caseinate; lane 6: βcasein; lane 7: standard

in more intensive secondary proteolytic changes in cheese A, which means that besides extracellular proteinases, this strain probably possesses extracellular peptidases.

Proteolytic changes results obtained by investigation during experimental cheeses (A and B) ripening are similar to proteolytic changes in artisan cheese. Content of WSN, PTA-N and ripening index (WSN/TN) in experimental cheeses were approximate data for artisan Sjenica white cheese (Maćej et al., 2006).

Furthermore, SDS PAGE of casein profile throughout the ripening of cheeses A and B is similar with the profile obtained in the traditional Sjenica white cheese. The results of SDS PAGE electrophoresis in artisan Sjenica cheese showed intensive changes of β-casein (Barać et al., 2006).

Viability of lactic acid bacteria through cheese ripening

The changes of lactic acid bacteria (LAB) number during the ripening of white brined cheeses made with different starter cultures are presented in Figure 4 and Figure 5. The high rate of cells was maintained during the whole ripening period. Generally, it was expected that the number of LAB will decrease during ripening. Within 10 days of ripening the maximum number of bacteria and activity were recorded but then declined. Therefore, for cheese A max. count of lactococci was 8.21 log units and for cheese B about 8.35 log units. These results are similar to those obtained for Genestoso Spanish cheese (Arenas et al., 2004), Turkish white cheese (Öner et al., 2006) and Feta cheese (Manolopoulou et al., 2003). During the cheese aging process, their number decreased slightly until the end of ripening, but still remained at a high level. These organisms decreased by 0.59 log units (for cheese A) and 0.69 log units (for cheese B) until the end of the ripening period. The evaluation of lactic acid bacteria during cheese ripening was similar to that described by Requena et al. (1992), Tzanetakis et al. (1995) and Arenas et al. (2004).

The lactobacilli count in cheese A, which on the first day of ripening stood at 6.92 log units, increased by 0.53 log units and reached a maximum 7.45 log units at the end of the ripening period. In cheese B, made without lactobacilli in starter culture, a low level was detected on the first day of ripening (2.02 log units), but throughout the ripening lactobacilli count increased by 5.38 log units and reached a maximum at the end of ripening (7.40 log units). The increase of the lactobacilli count is a consequence of growth and activity of non-starter lactobacilli. Apart

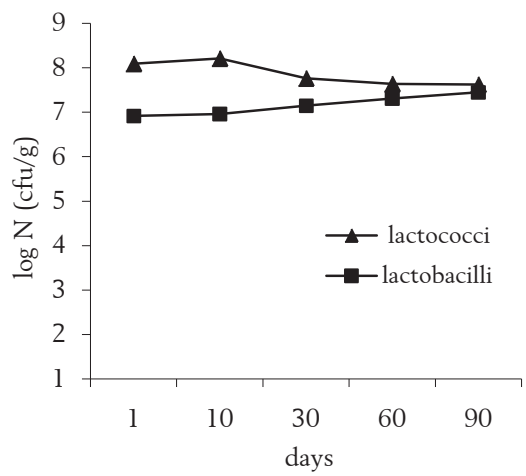

Figure 4. Changes in the lactic acid bacteria count during the ripening of cheese A

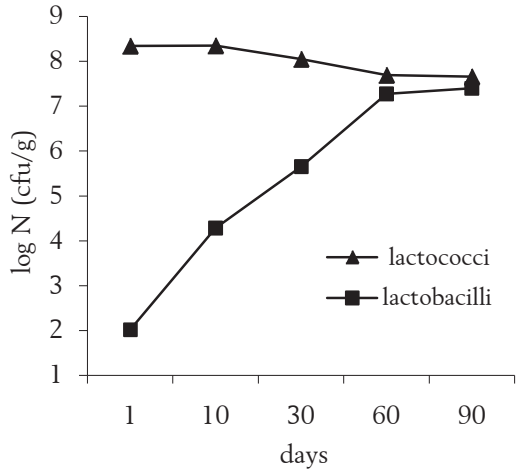

Figure 5. Changes in the lactic acid bacteria count during the ripening of cheese B

from proteolytic agents in cheeses (rennet and plasmin), proteolytic activity of applied lactobacilli in starter had an important role in the beginning of the cheese A ripening process (Djerovski et al., 2007).

Sensory analysis

The data for sensory evaluation of experimental white brined cheeses after 10, 30, 60 and 90 days of ripening is given in Table 3. No significant differences were observed between the mean scores awarded to experimental cheeses obtained by sensory evaluation. However, cheese A received a somewhat higher total score than cheese B, in the early stage of ripening, but the difference was not significant ($P<0.5$). It is interesting to note that after 10 and 30 days of ripening the overall impression score for cheese A was slightly higher than for cheese B, while after 60 and 90 days it was higher for cheese B than for cheese A. Nevertheless, both cheeses were graded as acceptable products with very good sensory char-

Table 3. The sensory evaluation of cheeses A and B

Time (days)	Sensory attribute*				Total score*
	Appearance Exterior[a]	Appearance Interior[a]	Texture[a]	Aroma[a]	
	Cheese A				
10	4.86±0.05a	4.73±0.05ab	4.63±0.11a	4.51±0.05a	91.97±0.76ab
30	4.87±0.08a	4.64±0.10a	4.76±0.10a	4.55±0.13a	93.72±1.07a
60	4.79±0.10a	4.67±0.12ab	4.62±0.15a	4.40±0.10ab	90.21±1.01b
90	4.78±0.06a	4.86±0.05b	4.55±0.15a	4.20±0.05b	89.40±2.42b
	Cheese B				
10	4.93±0.03a	4.60±0.20 a	4.49±0.21 a	4.35±0.13ab	89.32±0.62a
30	4.86±0.05a	4.69±0.07a	4.71±0.20a	4.45±0.23a	91.65±1.89a
60	4.88±0.11a	4.74±0.14a	4.73±0.21a	4.32±0.16ab	90.32±1.48a
90	4.92±0.10a	4.76±0.15a	4.74±0.21a	4.19±0.10b	89.94±3.04a

*Sensory attribute: Appearance - exterior score 0-5; Appearance - interior score 0-20; Texture score 0-25; Aroma score 0-50; Total score max 100

[a] Average ± standard deviation for sensory evaluation by a five member trained panel

a, b - Values within the same column not sharing a common letter were significantly different ($P<0.05$)

acteristics. Off-flavour was not noticed in cheeses in any ripening period. Karakus and Alperden (1995) showed that cheeses made with the selected strains of *Lactococcus* and *Lactobacillus* genera received significantly higher scores than control cheese made with the commercial O type starter obtained by sensory evaluation.

Conclusion

The results of this research indicate that starter cultures prepared from selected autochthonous lactic acid bacteria strains can be successfully used in white brined cheese production. Data related to the chemical composition of cheeses showed no significant differences between cheeses made with different cultures. The course of proteolytic changes during the ripening of experimental cheeses was generally similar to white brined cheese. The experimental cheeses were evaluated by sensory analysis and received high scores. Both cheeses were characterized by specific sensory properties, as well as a high and standard product quality.

Autochthonous lactic acid bacteria applied as starter cultures in white brined cheese production can be important for achieving specific sensory characteristics, typical for traditionally made cheeses.

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Primjena autohtonih bakterija mliječne kiseline u proizvodnji bijelih sireva u salamuri

Sažetak

U radu je praćen utjecaj autohtonih bakterija mliječne kiseline na karaketristike bijelih sireva u salamuri tijekom 90 dana zrenja. Sir A je proizveden sa sojevima: *Lactococcus lactis* ssp. *lactis* 653, *Lactococcus lactis* ssp. *cremoris* 656, *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* 07 i *Lb. paracasei* ssp. *paracasei* 08 (8:5:5:2), a sir B sa sojevima: *Lactococcus lactis* ssp. *lactis* 195, *Lactococcus lactis* ssp. *cremoris* 656 i *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* 07 (10:5:5). Broj laktokoka kod oba sira i broj laktobacila kod sira A održavao se na visokoj razini, dok se broj laktobacila u siru B povećavao tijekom zrenja. Nisu nađene značajne razlike ($P<0.05$) u ukupnom sastavu eksperimentalnih sireva, iako su pH vrijednosti bile niže u siru A. Proteolitičke promjene utvrđene su određivanjem dušičnih frakcija topivih u vodi, dušičnih frakcija topivih u 5 % fosfovolframskoj kiselini i SDS-PAGE elektroforezom. Oba sira su okarakterizirana visokim stupnjem proteolize. Na osnovu senzorske ocjene sireva, utvrđeno je da su oba sira ocijenjena visokim ocjenama. Rezultati su pokazali da se autohtoni sojevi bakterija mliječne kiseline mogu uspješno primijeniti u proizvodnji bijelih sireva u salamuri.

Ključne riječi: autohtone bakterije mliječne kiseline, zrenje bijelih sireva u salamuri

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