

Growth and Activity of Starter and Adjunct Lactobacilli and Lactococci during Ripening of Two Types Bulgarian White Brine Cheese

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Abstract

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The growth and activity of starter and adjunct lactobacilli and lactococci during ripening of two types Bulgarian white brine cheese were studied. The pasteurization of milk used was 72°C for 10 min and 85°C for 10 min. The temperature of cheese ripening was 12°C and 6°C respectively. The count of lactobacilli and lactococci and the changes in nitrogen fractions (NCN/TN - pH 4.6 soluble nitrogen/total nitrogen and NPN/TN - 12% CCl₃COOH soluble nitrogen/total nitrogen) were determined at 10, 17, 24 and 31st day of the ripening of the cheeses. The results obtained showed difference in the growth of lactobacilli and lactococci. The higher temperature of pasteurization of the milk and the lower temperature of ripening of the cheeses had bigger negative influence on the growth of lactobacilli and contrariwise the lower temperature of pasteurization of the milk and the higher temperature of ripening of the cheeses resulted in lower number of lactococci. The NCN/TN and NPN/TN values increased during the ripening of the both types cheese and it was higher for Batch B at all studied stages of ripening.

Key words: growth, proteolytic activity, lactobacilli, lactococci, Bulgarian white brine cheese (BWBC)

Introduction

The biochemical changes in cheese components during ripening are due to the activity of enzymes produced by microorganisms (Norani and Elmer, 1990). That is why the growth of the microorganisms

is essential for the proteolysis, lipolysis and metabolism of lactose, citrate and lactate (Fox et al., 1989). The products of proteolysis are very important for the organoleptic characteristic of produced cheese (Fox, 1989). The microflora of the cheese is presented mainly by the starter lactic

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acid bacteria, which are inoculated into milk and non-starter lactic acid bacteria which contaminate milk after pasteurization (Martley and Crow, 1993). A small percentage of non-starter lactic acid bacteria may survive pasteurization (Turner et al., 1986). They are mainly mesophilic lactobacilli (McSweeney et al., 1993). The lactic starter strain used in cheese manufacture has influence to the rate of growth and final count of non-starter lactic acid bacteria during ripening (Thomas, 1987; Martley and Crow, 1993; Crow et al., 1995).

The aim of the present study was to establish the growth and proteolytic activity of starter and adjunct lactobacilli and lactococci during ripening of two types Bulgarian white brine cheese.

Materials and Methods

Cheese making

The two types white brine cheeses were manufactured in industrial conditions. The cow milk was pasteurized at two different temperatures - 75°C for 10 min (Batch A - Control cheese) and 85°C for 10 min (Batch B - Experimental cheese) and then cooled to 34°C. The milk was inoculated with a mesophilic starter culture, consisted of *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. cremoris* and *Lactobacillus casei*. Standard amounts of CaCl₂ and commercial animal rennet were added. After 60 min (from the addition of the rennet), the curd was cut and leaved to heal for 10 min, then the whey was removed and the curd was pressed until the moisture content reached 64%. The cheeses were put in salt solution (20-22%) for 16 hours and after that the salt content of cheeses was approximately 2.5%. Then the Control and Ex-

perimental cheeses were ripen at 12°C and 6°C respectively, in hermetically closed metal cans filled with brine (10% concentration). The microbiological and physicochemical analyses were conducted at four stages of the ripening process - after 10, 17, 24 and 31 days from the beginning of the ripening.

Physicochemical analysis

The Kashkaval loaves were analyzed for moisture content (IDF Standart 4A:1982), fat (Gerber method; BSI 1955), salt (Reddy and Marth, 1993). Total nitrogen, noncasein nitrogen and nonprotein nitrogen content were determined by the Vakaleris and Price method (1959) modified to suit the specific conditions of the analysis. The total nitrogen content was determined after 20g of cheese was extracted in 10% sodium citrate solution (extract A) and 10 cm³ of the prepared extraction was used for determination by the Kjeldahl method. For the noncasein nitrogen determination 1.4N HCl was added to 80 cm³ of extract A, until pH reached 4.4 - 4.6 and after 10 min was filtered and the filtrate was used for determination by the Kjeldahl method. For the nonprotein nitrogen determination 12% CCl₃COOH was added to 40 cm³ of extract A and after 30 min was filtered and the filtrate was used for determination by the Kjeldahl method. The Kjeldahl method was performed in duplicate using Kjeltac Auto 1030 Analyzer (Tecator Sweden) combined with the Digestion System 20.

Microbiological analysis

The lactococci and lactobacilli count in the cheeses after 10, 17, 24 and 31 days of ripening were determined by cultivations on synthetic culture media (M17 and MRS respectively) and 48 hours incubation at

30°C. The methodology described in IDF Standard 149A:1997 was followed. The samples preparation was according to IDF Standard 122C: 1996 - 10g of the cheese samples were put into container of peristaltic-type blender and 90 cm³ of 20% sodium citrate solution was added. The mixture was stir up until complete dispersion of the samples. The suitable dilutions were made and 1 cm³ of each dilution was put into Petri dishes and the molten media (44±1°C) was added (M17 for lactococci and MRS for lactobacilli determination). After the media became solid the dishes were inverted and incubated at 30±1°C for 48 hours. After incubation all colonies were counted.

Statistical analysis

All statistical analyses were performed using two-way multivariate analysis of variance (MANOVA) and multiple comparison tests were carried out to study the effect of both pasteurization and ripening temperature on the physicochemical characteristics of cheeses and the count of lactobacilli and lactococci (Box et al., 1978). Statistical analyses were carried out on the averages of five results. Differences in the averages and F tests were considered sig-

nificant when the computed probabilities were less than 0.05. All statistical procedures were computed using the Microsoft Excel 2003 and Sigma Plot 2002 software.

Results

The moisture content, pH, fat content, NaCl content, NCN/TN and NPN/TN of the cheeses are shown in Table 1. It can be seen that the moisture content of experimental cheese is higher in all aging stages because of the higher denaturation of serum proteins, provoked by the higher pasteurization temperature, although the differences were not significant ($P < 0.05$). Despite of this the way of alteration was similar for both cheeses. PH initially decreased until the 24th day and after that increased to the 31st day of ripening. This increasing of pH is probably due to the accumulation of alkaline products from the proteolysis of the cheese proteins and partial neutralization of the lactic acid. The pH of Batch A was slightly lower in all aging stages, except the 10th day due to the higher ripening temperature. The NaCl content was slightly higher in the Batch B because of the higher moisture content. NCN/TN and NPN/TN values are good

Table 1
Changes in physicochemical characteristics during ripening of Bulgarian white brine cheese from Batch A

Aging time, days	Moisture content, %	pH	NaCl, %	Fat content, %	NCN/TN	NPN/TN
10	58.6 ± 0.6 ^a	4.47 ± 0.03 ^b	3.16 ± 0.3 ^a	24 ± 0.5 ^c	10.8 ± 0.4 ^a	7.8 ± 0.2 ^b
17	60.6 ± 0.8 ^a	4.32 ± 0.03 ^b	3.98 ± 0.4 ^c	23 ± 0.6 ^c	11.9 ± 0.4 ^a	8.1 ± 0.3 ^b
24	59.7 ± 0.9 ^a	4.10 ± 0.01 ^b	4.33 ± 0.4 ^b	22 ± 0.8 ^c	16.4 ± 0.3 ^b	9.0 ± 0.2 ^a
31	61.6 ± 0.7 ^a	4.27 ± 0.03 ^b	4.27 ± 0.6 ^b	22 ± 0.7 ^c	17.5 ± 0.2 ^c	10.1 ± 0.4 ^c

^{a, b, c} means within same column bearing a common superscript did not differ significantly ($P < 0.05$)

indication of the extent of proteolysis of cheese. The NCN/TN ratio (Tables 1 and 2) shows higher values for the Batch B at all aging stages. This is probably due to the higher proteolytic activity of starter *Lactococcus lactis subsp. lactis* and *Lactococcus lactis subsp. cremoris* at low temperatures. The NPN/TN ratio increased during the ripening of the both types and it was higher for Batch B at all studied stages of ripening. The higher NCN/TN and NPN/TN ratios for Batch B, although not significant ($P < 0.05$), were probably due to the higher amount of serum proteins included in the cheese, dependent on the increased temperature of pasteurization and increased acidity before coagulation of the milk for Batch B.

Through the process of ripening the count of lactococci in the Batch A was lower compared to the Batch B ($P < 0.05$) (Figure 1). The peak of the lactococci growth is at 17th day from the beginning of the ripening process. The count of these microorganisms at this aging stage reached 2×10^7 CFU/g, for the Batch B and 9.6×10^6 CFU/g for the Batch A. After 17th day the count of lactococci decreased for both cheeses and at 31st day they reached 8×10^5 CFU/g and 2.8×10^6 CFU/g

for the Batch A and Batch B respectively (Figure 1). The count of lactobacilli was also higher at 17th day from the beginning of the ripening process. They reached 1.2×10^7 CFU/g for the Batch A and 7×10^6 CFU/g for the Batch B (Figure 2). Similarly to the growth of lactococci the count of lactobacilli decreased significantly ($P < 0.05$) for both cheeses and at 31st day they reached 1.2×10^6 CFU/g and 7×10^5 CFU/g for Batch A and Batch B respectively.

Discussion

From the obtained results (Figures 1 and 2), it is obvious that the growth of lactococci is significant lower ($P < 0.05$) in the cheese produced by milk pasteurized at lower temperature - 75°C for 10 min and ripen at higher temperature - 10°C (Batch A). The number of lactobacilli in the cheese from Batch A was higher than the number in the cheese from Batch B, probably due to the higher sensitivity of lactobacilli for low temperatures and the bigger amount of non-starter lactic acid bacteria in the cheese produced by milk pasteurized at lower temperature. Swearingen (2001) also found that the

Table 2

Changes in physicochemical characteristics during ripening of Bulgarian white brine cheese from Batch B

Aging time, days	Moisture content, %	pH	NaCl, %	Fat content, %	NCN/TN	NPN/TN
10	60.3 ± 0.5^a	4.40 ± 0.01^b	4.33 ± 0.7^c	17 ± 0.6^b	11.2 ± 0.4^a	8.0 ± 0.3^c
17	61.3 ± 0.4^a	4.44 ± 0.01^b	4.41 ± 0.5^c	16 ± 1.0^b	13.6 ± 0.4^b	9.8 ± 0.4^a
24	60.8 ± 0.5^a	4.20 ± 0.04^a	4.41 ± 0.8^c	18 ± 0.9^b	17.8 ± 0.5^c	11.6 ± 0.4^b
31	62.0 ± 0.6^a	4.40 ± 0.02^b	4.54 ± 0.3^c	15 ± 0.8^b	18.1 ± 0.4^c	11.2 ± 0.3^b

^{a, b, c} means within same column bearing a common superscript did not differ significantly ($P < 0.05$)

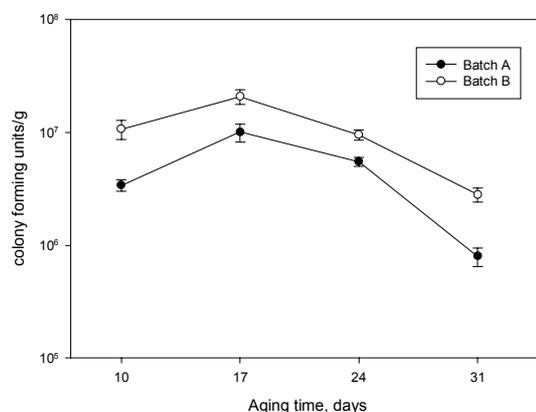


Fig. 1. Growth of lactococci during aging of two types Bulgarian white brine cheese

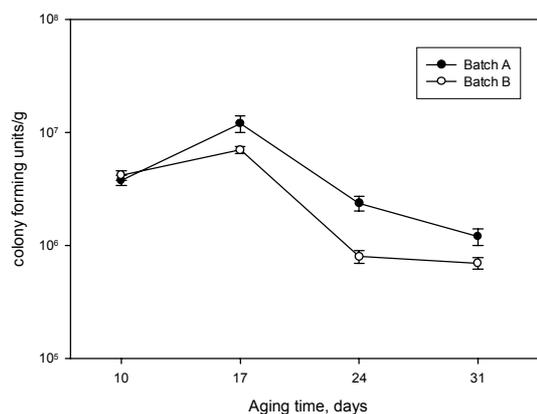


Fig. 2. Growth of lactobacilli during aging of two types Bulgarian white brine cheese

adjunct lactobacilli predominate at the earlier stages of the ripening. Coppola (2003) found that the main microflora of cheese produced from raw milk was presented by lactobacilli. That is why the most probable reason for the differences is probably the bigger amount of adjunct lactobacilli in the cheese from Batch A due to the lower pasteurization and the bigger sensitivity of lactobacilli to the lower temperatures.

Hynes (2001) also found that the temperature and the starter strain have influ-

ence on the growth of non-starter lactic acid bacteria. Although there are a number of contributory factors for proteolytic behaviour in lactococcal bacteria (O'Sullivan et al., 2000), we consider that the bigger NCN/TN and NPN/TN ratios for Batch B shows the bigger proteolytic activity of lactococci which are mainly presented by *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. cremoris*, compared to lactobacilli mainly presented by *Lactobacillus casei* at shown conditions.

Conclusions

There was an evident difference between the growth of the lactic acid bacteria in white brine cheese obtained from milk pasteurized at different temperatures and ripened at different temperatures. The amount of lactococci in the experimental cheese (pasteurization at 85°C, temperature of ripening 8°C) was higher than the amount of lactobacilli. Contrary in the control cheese (pasteurization at 75°C, temperature of ripening 10°C) the amount of lactobacilli is higher than the amount of lactococci. Accordingly, the lactococci/lactobacilli ratio in experimental cheese was higher than in the control cheese. The NCN/TN and NPN/TN values increased during the ripening of the both types cheese and it was higher for Batch B at all studied stages of ripening. By the alteration of the two factors - pasteurization temperature and ripening temperature can be controlled the growth and activity of starter and adjunct lactococci and lactobacilli during cheese ripening.

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