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Effect of rennet type and ripening period on chemical properties of Bulgarian white brined cheese

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Abstract

The possibilities of using microbial rennet as an alternative coagulant during production of Bulgarian white brined cheese were investigated. The changes in the chemical properties, proteolysis and free amino acids (FAA) content of Bulgarian white brined cheese made from calf rennet (sample A) and microbial rennet (sample B) were studied during ripening period of 45 days at 12°C. The chemical properties and proteolysis of the cheese samples were determined at the 1st, 15th, 30th and 45th day of ripening. According to the results, until the end of ripening period, the effect of rennet type on some chemical properties (pH, proteins and moisture content) decreased, and titratable acidity, fat and salt content increased. At the end of ripening period in cheese samples was found that the ratio non-casein nitrogen (NCN) and non-protein nitrogen (NPN) as percentage of total nitrogen (TN) values gradually increase compared to initial ratio. At all ripening periods, Leucine, Serine, Phenylalanine, Valine, Lysine and Alanine were the main FAA in cheese samples.

Introduction

Bulgarian dairy industry is known with the unique quality of yoghurt and cheese. White brined cheese is the most popular cheese variety in Bulgaria, representing approximately 80% of the country's total cheese production. It is generally produced from sheep, goat, buffalo or cow's milk or a mixture of them (Edgarian and Panayotov, 2011). Cheese is produced by coagulation of the milk protein - casein. Cheese curd is obtained using rennin (chymosin) or other milk clotting enzymes with similar function.

Milk clotting enzymes originally extracted from the stomach of ruminants are named calf rennet's. In cheese manufacturing the coagulation of milk is traditionally made by calf rennet (Kamber, 2008). Enzymes with similar function, capable of initiating the proteolysis of κ-casein, are known as aspartic proteinases. Sources of these proteinases can be from plant and microbial origin, or different ruminants, other than calf (Kehagias, 2005).

In recent years, microbial proteases are extensively applied in cheese manufacturing. Therefore, other suitable coagulants (bovine, porcine and chicken), including proteinases from microorganisms (*Mucor miehei, Mucor pusillus, Penicillium roqueforti, Penicillium camemberti* and *Cryphonectria parasitica*) have become more popular in the production of cheeses (Vishwanatha *et al*., 2010; Jacob *et al*., 2011).

From technological point of view, ripening is a very important process when cheeses submit specific microbiological and biochemical changes. These changes affect organoleptic and texture properties of the cheese (Pachlova *et al.*, 2012). The main role of proteolysis is the liberation of amino acids as precursors for a complex series of catabolic reactions, related to production of flavor compounds (Katsiari *et al.*, 2000a, 2000b; Fox and McSweeney, 2004; Bontinis *et al*., 2011; Hayaloglu and Karabulut, 2013). The aim of the present work is to study the effect of rennet type and ripening period on chemical properties of Bulgarian white brined cheese.

Materials and Methods

Cheese preparation

White brined cheese samples were produced by a classical technology, according to Bulgarian National Standard (BNS) 15-2010. White brined cheese was prepared from cow's milk. The milk was standardized to a fat content of 3.6% (w/w) (casein to fat ratio 0.72), pasteurized at 72-74°C for 15 min, and cooled to 32-34°C. Then 30 cm3 of 50% (w/v) CaCl2 solution was added and inoculated with 0.5% (v/v) starter culture (*Lb. bulgaricus, Lb. casei, S. thermophilus* and *Lc. Lactis,* more than 9.5×10^9 cfu/g), obtained from LB Bulgaricum Laboratory,

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Table 1. Changes in chemical properties during ripening

Analysis	Cheese samples							
	A				B			
	Days of ripening				*Days of ripening*			
	1st	15th	30th	45th	1st	15th	30th	45th
pH	4.72±0.13[a]	4.59±0.06[b]	4.48±0.06[c]	4.20±0.15[d]	4.75±0.14[a]	4.61±0.15[b]	4.45±0.16[c]	4.18±0.1[d]
TA[1](%l.a.)	1.64±0.10[a]	1.76±0.10[b]	2.03±0.17[c]	2.39±0.15[d]	1.63±0.07[a]	1.77±0.06[b]	2.08±0.18[c]	2.43±0.1[d]
Moisture(%)	60.1±0.3[a]	59.3±0.4[b]	57.7±0.4[c]	55.5±0.7[d]	60.4±0.2[a]	58.7±0.3[b]	57.0±0.3[c]	54.9±0.4[d]
Fat(%)	21.0±0.3[a]	21.5±0.1[b]	22.0±0.3[c]	23.5±0.3[d]	21.0±0.3[a]	21.5±0.1[b]	22.0±0.3[c]	23.0±0.3[d]
Protein(%)	15.3±0.1[a]	15.2±0.2[a]	14.5±0.2[b]	14.4±0.3[b]	15.3±0.1[a]	15.1±0.2[a]	14.4±0.1[b]	14.3±0.1[b]
FDM[2](%)	52.7±0.2[a]	52.9±0.3[a]	52.8±0.2[a]	52.8±0.3[a]	52.1±0.1[a]	52.2±0.2[a]	52.3±0.1[a]	52.3±0.2[a]
NaCl(%)	3.3±0.1[a]	3.4±0.1[b]	3.6±0.1[b]	3.6±0.1[b]	3.4±0.1[b]	3.5±0.2[b]	3.6±0.2[b]	3.6±0.1[b]

[1]TA – titratable acidity;represented as l.a. - lactic acid; 2FDM - Fat in Dry Matter
a,b,c,d Means with different letters within a row are significantly different ($p<0.05$)

Sofia, Bulgaria. The milk was divided in two equal portions (20 L each) - the first batch (sample A) was coagulated with calf rennet (activity~1000 IMCU/cm3) and the second batch (sample B) was coagulated with microbial rennet - Formulase (activity~1000 IMCU/cm^3). The coagulum obtained during the enzymatic coagulation was cut into cubes having size of 3 cm and then drained. The cheese curd was divided to batches, lined with cheese cloth to drain whey and pressed for about 3-3.5 h at 20 ± 2°C. Then, the curd was cut into segments having dimensions 11.8 x 11.8 cm. The shaped curd was put into solution containing 22% NaCl (w/v) at 14-16°C for 12-15 h. After pre-brining period, the cheeses were packaged in plastic cups (1 kg) containing brine 8% NaCl (w/v) and transferred to ripening room (12°C). The ripening of white brined cheese samples (A and B) was respectively performed for 45 days. The chemical analyses were conducted at four stages of the ripening period: in the 1st, 15th, 30th and 45th day.

Chemical analysis

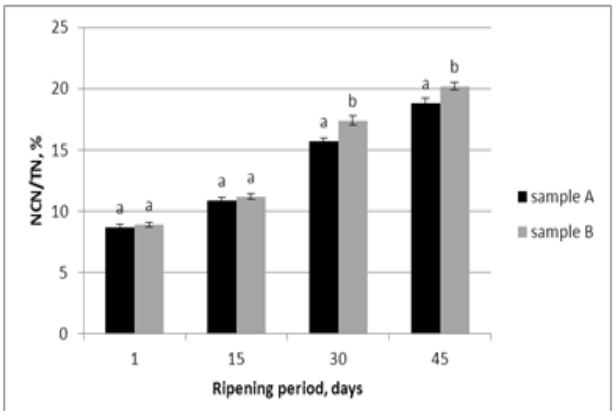
Titratable acidity (TA) of cheese samples was determined by the Thorner's method (BNS 1111-80) and represented as percentage of lactic acid. The pH of cheese samples was measured using a pH meter (model MS 2000, Mycrosist, Plovdiv, Bulgaria) with a glass electrode (Sensorex, Garden Grove, USA) standardized at 20°C in the range 7.01 – 4.01. Total solids of cheese samples were determined by BNS 1109-80. The total fat and fat-in-dry matter (FDM) content of the cheese samples were determined by the Gerber (BNS 1671-89). Salt content of the cheeses was determined by Mohr titration method (BNS 8274-82). The total nitrogen content of the cheeses was determined by Kjeldahl method. Non-protein nitrogen (NPN) and non-casein nitrogen (NCN) contents were determined according to Vakaleris and Price (1959) and represented as percentage of total nitrogen (TN) content. Ripening index (RI) was estimated by using the formula: (NPN/TN and NCN/TN) x 100, as proposed by Alais (1984). Free amino acids (FAA) were measured by the Pico-TAG method (Milipore) (Waters Associates, USA) according Cohen *et al*. (1989).

Statistical analysis

Computer processing of the results is performed using the program Microsoft Excel 2010 (ANOVA). Multiple comparisons are made by Lowest Standard Deviation (LSD) method for all analyzed parameters. The results are presented as mean value ± SD (n=3).

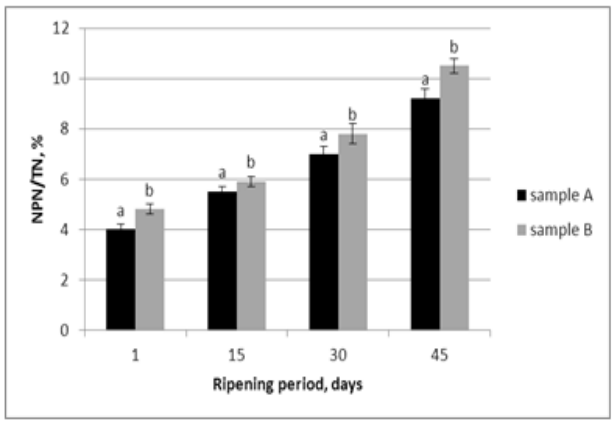
Results and Discussion

The changes of chemical properties during ripening of Bulgarian white brined cheese manufactured from cow's milk using calf rennet and microbial rennet are given in Table 1. The data reveals that the different types of milk-clotting enzymes had not significant effect on titratable acidity, pH, moisture and salt content in cheese during the ripening period at 12°C ($p>0.05$). In both cheese samples (A and B), titratable acidity increases up to day 45 and the pH decreased until day 45 of ripening. The variation of the two parameters represented the development and dynamics of fermentation process in the studied cheese samples. It is well known that lactic acid fermentation starts



a,b Means with different letters within a column are significantly different ($p<0.05$)

Figure 1. Changes in ratio NCN/TN during ripening of white brine cheeses produced using different milk-clotting enzymes



a,b Means with different letters within a column are significantly different ($p<0.05$)

Figure 2. Changes in ratio NPN/TN during ripening of white brine cheeses produced using different milk-clotting enzymes

during curd treatment and continues during ripening (Pappa *et al*., 2006; Shahab *et al*., 2012; Balabanova *et al*. 2014). The process is conducted by the starter and nonstarter lactic acid bacteria in the curd. Similar results were reported by Kestenova *et al*., (1982) and Ivanov *et al*., (2015). Salt content increased during ripening with about 0.3% (w/w) for sample A and 0.2% (w/w) for sample B, respectively. These results of diffusion of salt from the surface into the center of the cheese were observed when samples were taken for the determinations, because of the different moisture content. The total fat content of the both cheese samples at the beginning of ripening period was 21.0% (w/w) and increased to 23.5% (w/w) for sample A and 23.0% (w/w) for sample B ($p<0.05$), presented in Table 1. Changes in fat content could be due to a decrease in moisture content (Shahab *et al.,* 2012). It was found that the fat-in-dry matter steady in both cheese samples throughout the ripening period ($p>0.05$), due to an increased dry matter, presented in Table 1. Total protein content of the cheeses samples did not change significantly ($p>0.05$) over the 1st to 45th day, presented in Table 1.

The main biochemical process during cheese ripening and one of the most important factors for the development of specific cheese flavor and texture is proteolysis. The changes on the ratio NPN/TN and NCN/TN during ripening of white brined cheeses samples produced using different rennet types are given in Figure 1 and Figure 2.

At the first day of ripening the ratio NPN/TN and NCN/TN in cheese samples was similar ($p>0.05$). The ratio NPN/TN and NCN/TN increased slightly to 15th day of ripening between the two samples, and after the 30th day the ratio NPN/TN and NCN/TN in the sample B was the highest. This is an indicator for the hydrolysis of casein related to the effect of the microbial rennet activity and the proteases from starter culture and nonstarter lactic acid bacteria observed during the ripening period (Irigoyen *et al*., 2001). The cheeses produced with calf rennet (sample A) showed lower ratio NPN/TN and NCN/TN than these recorded in sample B, at the end of the ripening.

The free amino acid content in cheese samples is presented in Table 2. The total amount of FAA during ripening period increased from 53.4 to 139.1 mg/100 g cheese for sample A and from 54.5 to 154.8 mg/100g cheese for sample B. The main FAA in cheeses at the first stages of ripening were Glutamine (Glu), Leucine (Leu), Proline (Pro), Lysine (Lys), Phenylalanine (Phe) and Alanine (Ala). The results are in agreement with reports about amino acid content of white cheeses (Eren-Vapur and Ozcan, 2012a, 2012b). Amino acid catabolism is a main process for the flavor formation in cheeses. The principal FAA, including Leucine (Leu), Glutamic acid (GluA), Phenylalanine (Phe), Valine (Val) and Lysine (Lys), were presented in the 60-day old Turkish White brined cheese made from pasteurized cow's milk (Ucuncu, 1981; Michaelidou *et al*., 2003).

Previous authors Alichanidis *et al*., (1984); Katsiari *et al*., (2000a); Michaelidou *et al*., (2003) have shown that Leu, Glu, Val and Lys were major FAA in Feta cheese made from cow's milk. At the end of the ripening, the basic FAA in both cheese samples were Leucine (Leu), Serine (Ser), Phenylalanine (Phe), Valine (Val) and Lysine (Lys). The amount of Pro and Glu decreased until the end of ripening for both cheese samples. The amino acid Cysteine during ripening was found to be absent, and the amount of Arginine for this period remained low.

Table 2. Content of free amino acids (FAA) during ripening

Free amino acids (mg/100 g)	Cheese samples			
	A		B	
	1st	45th	1st	45th
Glycine	1.2±0.1a	3.4±0.3b	1.3±0.1a	3.7±0.2b
Valine	2.5±0.2a	14.1±0.4b	2.5±0.3a	15.8±0.5c
Leucine	8.1±0.3a	32.6±0.4b	8.5±0.2a	34.5±0.3c
Isoleucine	0.8±0.1a	1.9±0.3b	1.0±0.1a	2.4±0.3b
Threonine	0.6±0.1a	1.9±0.3b	0.7±0.2a	2.6±0.3c
Serine	2.9±0.3a	20.0±0.4b	3.2±0.1a	22.7±0.3c
Proline	7.2±0.2a	5.2±0.3b	7.1±0.1a	5.9±0.3c
Asparagine	1.2±0.1a	8.4±0.3b	1.1±0.1a	9.1±0.4b
Aspartic acid	1.6±0.2a	1.9±0.3b	1.5±0.1a	2.3±0.2b
Methionine	0.6±0.2a	3.7±0.3b	0.4±0.1a	4.1±0.2b
Glutamic acid	1.4±0.1a	5.6±0.3b	1.5±0.1a	6.4±0.4c
Phenylalanine	4.1±0.1a	16.0±0.3b	4.0±0.1a	17.3±0.4c
Glutamine	9.3±0.1a	2.3±0.2b	9.3±0.1a	2.7±0.3b
Lysine	5.5±0.1a	10.0±0.4b	5.7±0.2a	11.8±0.6c
Histidine	0.0±0.0a	1.2±0.1b	0.0±0.0a	1.1±0.1b
Tyrosine	2.4±0.1a	1.3±0.2b	2.6±0.1a	1.5±0.1b
Tryptophan	0.6±0.1a	3.8±0.3b	0.5±0.1a	4.4±0.3b
Cysteine	0.0±0.0a	0.1±0.0a	0.0±0.0a	0.0±0.0a
Arginine	0.2±0.1a	0.3±0.1a	0.2±0.1a	0.4±0.1a
Total:	53.4	139.1	54.5	154.8

a,b,c,d Means with different letters within a row are significantly different ($p<0.05$)

Conclusion

The obtained results indicated that the type of rennet had not significant effect ($p>0.05$) on the chemical properties of white brined cheese. No significant ($p>0.05$) differences in the ratio of NPN/TN and NCN/TN were observed between the cheeses manufactured with microbial and calf rennet. It was concluded that the rennet coagulant used in cheese-making during ripening do not change the proteolysis process in cheese samples. According to the obtained results, the proteolysis increased the amount of free amino acids during ripening. Therefore, the microbial rennet could be considered as an alternative of the calf rennet and successfully used in the manufacture of white brined cheese.

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