Changes in some Microbiological and Chemical Parameters during the Ripening of Sheep Cheese at Different Temperatures

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Abstract

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The changes in microbiological and chemical parameters during the fermentation and ripening of sheep cheese were observed. The cheese was produced from raw sheep's milk at different temperatures on the sheep farm. The following parameters were determined: total plate count, coliform count, *Staphylococcus aureus* count, pH value, titrimetric acidity, and the amount of lactic acid. As our results indicate, the fermentation at temperatures of between 18–22°C reduces the number of undesirable microorganisms due to the presence of lactic acid being produced by lactic-acid bacteria. During the fermentation at lower temperatures (below 18°C) the amount of lactic acid is insufficient, and the undesirable microorganisms can survive.

Keywords: sheep cheese; fermentation; ripening; inhibition; coliforms; *Staphylococcus aureus*; pH; lactic acid; titrimetric acidity

The production of sheep milk and cheese has had a long tradition in the Slovak republic. Some 419 thousands sheep were bred in Slovakia by 1 January 1999. In this year that figure is supposed to increase to 481 000 sheep, which includes 295 000 ewes (PALO 1998). One sheep is able to produce between 80 and 150 litres of milk (depending upon the breed) within the period of lactation (MARGETÍN 1999). Almost 90% of sheep's milk in Slovakia is used for the production of sheep cheese (PALO & KALÁB 1984). Although sheep cheese is considered to be a hazardous food, it is still popular among consumers. Poor milking hygiene and the direct processing of milk on sheep farms mainly cause the risk for consumers (FILO 1997).

For the time being, sheep cheese is mainly produced from raw (non-pasteurised) milk. For that reason it is necessary to be as hygienic as possible when milking. As recorded in research studies, the total bacterial count in milk obtained under hygienic conditions consists of 70% of milk fermentation bacteria and 30% of undesirable microorganisms. In general, the lower is the milking and milk processing hygiene on the sheep farm, the higher is the number of undesirable microorganisms in cheese (KRČÁL *et al*. 1988).

During the production and storage of sheep cheese, the development and multiplication of bacteria naturally present in milk can be observed. Apart from desirable milk fermentation bacteria, various undesirable bacteria (including pathogens) can be detected in milk (PREKOPPOVÁ & KRČÁL 1990). Among undesirable microorganisms, *Staphylococcus aureus* is very dangerous for the health of consumers. As required by the Slovak Codex Alimentarius (1998), when a sample contains 10^3 cells of *Staphylococcus aureus* in 1g, it must be examined for the presence of thermostable enterotoxins.

The aim of this study was to observe the influence of different fermentation temperatures on the microbiological and chemical parameters of sheep cheese.

MATERIAL AND METHODS

The sheep cheese used in the experiment was produced on a sheep-farm in accordance with the prescribed technological procedure. After manufacture, the cheese was divided into two samples and stored at different temperatures as follows:

Sample A: at 21°C for three days and then at 14°C for up to 7 days,

Sample B: at 17°C for three days and then at 14°C for up to 7 days.

The microbiological examination included the determination of the following:
– total plate count (TPC) using agar with glucose, tryptone, and yeast extract incubated at 30°C for 72 hrs (STN ISO 4833, 1997)
– coliform count using agar with crystal violet, neutral red, bile salts, and lactose incubated at 30°C for 48 hrs (STN ISO 4832, 1997)
– *Staphylococcus aureus* count determined on the surface of agar according to Baird-Parker after a 48-hr incubation at 37°C (STN ISO 6888, 1997).

During the chemical examination the following parameters were determined:
– pH value with the help of pH-meter,
– titrimetric acidity according to Soxhlet-Henkel,
– the amount of lactic acid using the method of capillary isotachophoresis (ŽABKOVÁ *et al*. 1986).

RESULTS AND DISCUSSION

As the results in Table 1 demonstrate, during the fermentation and ripening of sheep cheese (sample A) the total plate count increased from 10^5 to 10^8 CFU/g within 24 hrs, and to a value of 10^{10} CFU/g within 72 hrs. Furthermore, 96–168 hrs after the production, the count of bacteria was reduced to 10^8 CFU/g. As to sample B, the total plate count increased from 10^5 CFU to 10^7 CFU/g within 24 hrs, then it gradually rose reaching the peak value of 10^{11} CFU/g 120 hrs after manufacture, and, finally, it dropped again to 10^9 CFU/g.

The coliform count in sample A was 10^2 CFU/g immediately after production, after 24 hrs it reached a value of 10^5 CFU/g, and the maximum value was recorded after 48 hrs (10^6 CFU/g). Then the number of coliforms gradually decreased to the value of 10^2 CFU/g after 168 hrs. In sample B, a smaller increase was observed after 24 hrs (from 10^2 to 10^3 CFU/g) and changes in the count of coliforms during the cheese fermentation and ripening were less conspicuous compared with sample A. The highest values were recorded after 96 hrs (10^5 CFU/g), then they decreased to 10^4 CFU/g after 168 hrs.

A rapid increase in the *Staphylococcus aureus* count (from 10^2 to 10^5 CFU/g) was also recorded within 24 hrs after the cheese production (sample A). A further increase (to a value of 10^6 CFU/g) was noticed after 48 hrs, and then the values gradually dropped to 10^2 CFU/g after 160 hrs. In sample B, the *Staphylococcus aureus* count gradually grew over 120 hrs after the cheese production, and then it slowly decreased to a value of 10^4 CFU/g (144–168 hrs after manufacture).

Comparing the results of microbiological examination we found that in sample A (cheese produced under the optimum temperature) the coliform counts started to decrease 48 hrs after manufacture, and after 168 hrs they dropped to a value of 6.8×10^2 CFU/g. However, in sample B (cheese produced at a temperature of 17°C) the coliform counts still increased up to 72 hrs after manufacture (to a value of 7.8×10^5 CFU/g) and subsequently decreased very slowly (7.2×10^4 CFU/g after 168 hrs). Similar differences between both samples were also found in the counts of *Staphylococcus aureus*.

The Slovak Codex Alimentarius (1988) requieres the examination of five samples of sheep cheese with the following limits: the maximum coliform count 10^4 CFU/g (in 2 samples 10^5 CFU/g is allowed) and the maximum *Staphylococcus aureus* count 10^2 CFU/g (in 2 samples 10^3 CFU per g is allowed). Based upon the above-mentioned requirements we state, that sample B did not meet the given requirements for the counts of *Staphylococcus aureus*.

The presence of *Staphylococcus aureus* is well documented in the literature. FEDERIČOVÁ *et al*. (1991) isolated 200 strains of *Staphylococcus aureus* from the samples of sheep cheese taken during common veterinary-hygiene supervision. 12 of these were reported to produce enterotoxins as follows: 8 strains enterotoxin C (4%), 2 strains enterotoxin D (1%), and 2 strains enterotoxin A (1%). BURDOVÁ *et al*. (1994) isolated 52 strains of *Staphylococcus aureus* from cow's milk, 14 of them (26.9%) produce

Table 1. Changes in total plate count (TPC), coliform count, and *Staphylococcus aureus* count during sheep cheese ripening (CFU/g)

Raw milk	TPC 9.3×10^5		Coliforms 9.5×10^2		*Staphylococcus aureus* 3.1×10^2	
Time after production (hrs)	sample A	sample B	sample A	sample B	sample A	sample B
24	3.2×10^8	4.7×10^7	7.5×10^5	4.7×10^3	1.8×10^5	2.6×10^3
48	7.2×10^9	6.2×10^8	1.1×10^6	1.5×10^4	4.2×10^5	6.7×10^3
72	4.3×10^{10}	1.1×10^{10}	5.6×10^5	5.8×10^5	7.5×10^4	4.6×10^4
96	8.6×10^9	3.4×10^{11}	1.4×10^4	7.8×10^5	4.2×10^4	2.3×10^5
120	2.0×10^9	2.4×10^{11}	4.7×10^3	4.7×10^5	7.0×10^3	6.8×10^5
144	7.8×10^8	9.2×10^{10}	9.6×10^2	3.6×10^5	9.8×10^2	5.8×10^4
168	3.4×10^8	4.8×10^9	6.8×10^2	7.2×10^4	2.6×10^2	1.4×10^4

Table 2. Average values of pH, titrimetric acidity, and the amount of lactic acid during sheep cheese ripening

Raw milk	pH 6.6		Titrimetric acidity (°SH) 9.5		Lactic acid (g/100 g) 0.20367	
Time after production (hrs)	sample A	sample B	sample A	sample B	sample A	sample B
24	6.5	6.4	18.5	20.5	0.4857	0.5536
48	5.9	6.2	78.0	62.0	3.9486	2.6762
72	5.6	6.1	98.0	71.0	4.8232	3.2115
96	5.4	5.9	102.0	76.5	5.4245	3.7309
120	5.1	5.6	115.0	87.0	5.8324	4.2105
144	4.9	5.4	119.0	93.0	6.3678	4.3273
168	4.7	5.3	121.0	96.0	6.6172	4.5057

enterotoxins. DUDRIKOVÁ *et al*. (1999) investigated the samples of sheep's milk produced by18 producers. The milk coming from 2 producers contained 900 and 200 cells of *Staphylococcus aureus* per 1 ml. These counts were not sufficient to produce enough enterotoxins to endanger human health. These facts serve as the evidence of favourable epizootic and enzootic situation on all the farms tested, as well as of a high level of milking hygiene.

The activity of lactic acid bacteria is mainly influenced by temperature. The optimum temperature for their growth and multiplication ranges from 18 to 22°C (KRČÁL *et al*. 1988; PREKOPPOVÁ & KRČÁL 1990; FILO 1997).

The changes in chemical parameters are recorded in Table 2. As shown there, the ripening of cheese resulted in a decrease in pH-value – in sample A from 6.5 (24 hrs after manufacture) to 4.1 (168 hrs after manufacture), in sample B from 6.4 (24 hrs after manufacture) to 4.7 (168 hrs after manufacture). On the other hand, the titrimetric acidity increased during ripening – in sample A from 18.5°SH (24 hrs after manufacture) to 129°SH (168 hrs after manufacture), in sample B from 20.5°SH (24 hrs after manufacture) to 102°SH (168 hrs after manufacture). An increase was also noticed in the amount of lactic acid – in sample A from 0.4857 g/100 g (24 hrs after manufacture) to 6.6172 g/100 g (168 hrs after manufacture), in sample B from 0.5526 g/100 g (24 hrs after manufacture) to 4.5057 g per 100 g (168 hrs after manufacture).

According to the Slovak Codex Alimentarius (2000) the pH-value of sheep cheese has to fall between 4.9 and 5.2. The titrimetric acidity should be between 75 and 110°SH or 119°SH respectively (FILO 1997; GRIEGER *et al*. 1979, respectively).

Many authors also referred to those changes in the microbiological and chemical parameters of sheep's milk that were caused by the fermentation process (PREKOPPOVÁ 1976; GRIEGER *et al*. 1979; KRČÁL & PREKOPPOVÁ 1987; KRČÁL *et al*. 1988; PREKOPPOVÁ & KRČÁL 1990; FILO *et al*. 1992).

CONCLUSION

As follows from our results, the fermentation of cheese under optimum conditions leads to a rapid development of desirable lactic acid bacteria at the beginning of the ripening. This results in an intense production of lactic acid and thus in the inhibition of undesirable microorganisms. However, the fermentation of cheese under unsuitable conditions follows in an insufficient multiplication of lactic acid bacteria, and thus the amount of lactic acid produced is not large enough to inhibit the undesirable bacteria. This leads to a decrease in the quality of the final product, as well as to an incerased health risk for consumers.

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Výnos Ministerstva pôdohospodárstva SR a Ministerstva zdravotníctva SR z marca 2000 č. 363/1/200-100, ktorým sa vydáva hlava Potravinového kódexu SR upravujúca mlieko a výrobky z mlieka, tretia časť, šiesta hlava, desiaty oddiel, § 57., vestník Ministerstva pôdohospodárstva SR, ročník XXXII, 30. máj 2000, čiastka 12.

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Súhrn

PAŽÁKOVÁ J., PIPOVÁ M., TUREK P., NAGY J. (2000): **Zmeny niektorých mikrobiologických a chemických ukazovateľov v priebehu zrenia ovčieho hrudkového syra pri rôznych teplotách**. Czech J. Food Sci., **19**: 121–124.

Sledovali sme zmeny mikrobiologických a chemických ukazovateľov počas kysnutia a zrenia ovčieho hrudkového syra, ktorý bol vyrobený z nepasterizovaného ovčieho mlieka priamo na salaši, pri rôznych teplotách. Stanovovali sme celkový počet mikroorganizmov, počet koliformných baktérií a počet *Staphylococcus aureus*, ďalej pH, titračnú kyslosť a kyselinu mliečnu. Ako vyplýva z výsledkov štúdie, pri dodržaní teplotných podmienok počas kysnutia (18–22 °C) dochádza ku znižovaniu počtu nežiadúcich mikroorganizmov pôsobením kyseliny mliečnej, ktorá vzniká činnosťou baktérií mliečnej fermentácie. Pri nedodržaní tejto teploty (pod 18 °C) rozvoj kyslomliečnych baktérií nie je dostatočný, netvorí sa dostatočné množstvo kyseliny mliečnej a nežiadúce mikroorganizmy prežívajú.

Kľúčové slová: ovčí hrudkový syr; fermentácia; zrenie; inhibícia; koliformné baktérie; *Staphylococcus aureus*; pH; kyselina mliečna; titračná kyslosť

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