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Physicochemical, sanitary and safety indicators changes during the ripening of Bulgarian white brined cheese from local farms

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Abstract

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The present study aims to determine the physicochemical and microbiological changes of white-brined cheese from local farms during manufacturing and ripening. Milk pasteurization for white-brined cheese production leads to a severe reduction of microorganisms in milk. Thus, after pasteurization the total number of microorganisms decreases from 480 000 to 810 cfu/cm^3, *Salmonella* spp. from 800 to 2 cfu/cm^3 and *E. coli* from 4000 to 0 cfu/cm^3. Ripening processes lead to a drastic reduction of cheese microflora with prevalence of specific lactic microflora (lactobacilli and lactococci) on the 45th day and complete annihilation of *E. coli* and *Salmonella* spp. These changes in the cheese microflora made the final product safe for consumption. On the other hand, the experimental data shows a strong multiplication of *Salmonella* spp. on the 7th day (10 cfu/cm^3 at the 24th hour reached 0 cfu/cm^3 on the 7th day) and insufficient decrease of the number of other microorganisms, making fresh white-brined cheese at its early ripening stages unsafe for consumption. Ripening of the cheese brings about an increase of the dry matter percentage (from 33.5% at 24th hour to 38.5% at 45th day), the fat content (from 13.3% to 16.4%), salt content (from 4.1% to 5.8%) and total protein content (from 13.7% to 16.7%) and reduction of moisture in non-fat substance (from 76.7% to 73.8%) of the final product. These changes are in accordance with the accepted standards for white-brined cheese production.

Keywords: white-brined cheese; physicochemical properties; microbiology; safety

Introduction

The white-brined cheese is a high quality food product, very popular in the Balkan and East Mediterranean countries, in particular Bulgaria, Greece and Turkey (Enikova, 2010; Pintado et al., 2015). It is distinguished by the high nutritional value, mainly due to the high content of total protein (17-18%) and fat (22-25%). It is rich in minerals, especially calcium, and various vitamins (mostly A and D). White-brined cheese stimulates the proper functioning of digestive system (Peychevski, 1983; Baltadjieva, 1993). The production of cheese is based on complex biochemical and microbiological processes, accompanied by degradation of the main components of the product (Tverdohleb et al., 1991). In biochemical ripening of the cheese a large number of decomposition products are formed, which are absorbed easily by the body (Kestenova et al., 1982; Grappin et al., 1985; Madkor et al., 1987). Technological condi-

tions and parameters strongly influence the formation of consistency, texture, aroma and taste of the cheese. Temperature, humidity, sanitation, processing of the cheese, storage conditions and other factors form the characteristics of the resulting product (Peychevski, 1983; Kestenova, 1985).

The microbiological quality and safety of white-brined cheese can be affected by variety of factors, including the quality of the milk, the use of pasteurization or thermization, the technological parameters and the level of microbial contamination occurring throughout the production and storage of the cheese (Bintsis & Papademas, 2002). Pasteurization of the milk used for cheese production largely ensure the lack of pathogenic bacteria of tuberculosis, brucellosis, salmonellosis, listeriosis and other infections associated with the dairy products consumption (Enikova, 2010). Microbiological processes during cheese ripening are very complex, poorly researched, run under the threat of primary and secondary microbial contamination of raw material with a variety of microorganisms originating from both the ecosystem and anthropogenic (Beresford & Williams, 2004; Temelli et al., 2006). The dynamics of the white-brined cheese microbiological processes is important because the fermentation and ripening of cheese are fundamental prerequisites for the disposal of unwanted microflora, particularly the pathogens (Bintsis & Papademas, 2002; de Souza et al., 2003; Enikova, 2010). The aim of the present study is to follow the dynamics of basic physicochemical parameters during ripening and determine the changes in some important sanitary and safety indicators through monitoring the microbial load of main groups according Bulgarian State Standard for production of white-brined cheese.

Material and Methods

Material

Assessment of the physicochemical and microbiological changes during the ripening of white-brined cheese is conducted. In the experiment were included four batches of white-brined cheese. They were produced in dairy farm, located 15 km from Nova Zagora town, Bulgaria. The dairy farm processed its own milk as well as aggregate milk from the region of Nova Zagora Municipality.

Milk

The study was performed with cow milk, obtained from cows, reared in the private farm in Nova Zagora village. Milk samples were obtained in the morning and evening, proportionally to the milk yield, according to EN ISO 707:2008**.**

Cheese

White brined cheese was prepared from cow milk. The milk was pasteurized at 72°C for 10 min, and cooled to 32 to 35°C. *Streptococcus salivarius* subsp*. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were used as starter culture. The cheese was made as described by Peichevski et al. (1988).

The cheese produced in the dairy farm, was placed under appropriate ripening conditions in the laboratory of Department of Dairy Science in Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria.

Methods

Microbiological analysis

25 g of each sample was taken from the cheese interior, homogenized with pastille in a sterile mortar and transferred into 225 ml of a sterile 2% (w/v) trisodium citrate solution. Serial 10-fold dilutions of cheese homogenate and milk sample were prepared in sterile 0.1% (w/v) peptone water (Oxoid, Basingstoke, UK).

For the quantitative determination of the aerobic mesophilic microorganisms, sanitary-indicator microorganisms (*E. coli*, Coliforms and *Enterobacteriaceae*) and pathogens (*Salmonella* spp.) in the milk and cheese samples, medium sheets (Rida®Count Total; Rida®Count *E. coli*/Coliform; Rida®Count *Salmonella*/Enterobacteriaceae, R-Biopharm AG, Germany) coated with selective, chromogenic culture medium were used.

The transparent cover film was opened and 1 ml of the sample homogenate or dilutions had been applied onto the nonwoven fabric of the medium sheet with pipet. The sheets were inoculated in duplicate, incubated at 35°C for 24-48 h and the colonies were counted. The results were expressed in colony forming units – CFU/ml for milk samples or CFU/g for cheese respectively. Specific microorganisms are forming colony with different colors on the specific test cards.

Quantitative determination of the lactoflora (lactobacilli and lactococci) – 10 grams of each sample were homogenized in 90 ml of sterile saline (0.85% NaCl, w/v), supplemented with peptone (0.1%, w/v; Oxoid®, UK) and then serial dilutions from homogenates were prepared. One ml aliquot of the 10-3, 10-4, and 10-5dilutions were pour-plated in MRS agar (Oxoid®, UK) for enumeration of lactobacilli (each sample was plated in duplicate) and in M17 agar for lactococci respectively. After incubation at 37°C for 48 h, resulted colonies were counted and the results are expressed as CFU/g.

Physicochemical methods

Dry matter, fat, total proteins were determined for all samples according to the Bulgarian standard (BDS), ISO standards and International Dairy Federation standards (IDF):

• content of dry matter – by oven drying to constant weight at 102°C ± 2°C (Bulgarian standard (BDS) 1109:1989);

• fat – ISO 2446:2008(IDF 226:2008)

• solids nonfat – by calculation based on the dry matter and milk fat; total protein were determined by Kjeldal method according to Bulgarian standard (BDS) EN ISO 8968-1:2014;

• density – according to Bulgarian standard (BDS) 1110:1973;

• titratable acidity by Thorner method (Bulgarian standard (BDS) 1111:1980);

• sodium chloride (NaCI) – Bulgarian standard (BDS) 8274- 82 (1982);

• moisture in non-fat substance and salt in moisture (S/M) were calculated according to Lawrence and Gilles (1980).

Statistical analysis

Statistical analysis was done by Statistica 7 software, graphics by Microsoft Excel 2007.

Results and Discussion

Composition and properties of processed milk

Data on the composition and properties of cow milk processed into white cheese are presented in Table 1. The milk is close in composition and properties to that used by Peychevski et al. (1988) for the production of white-brined cheese from cow milk. Its physicochemical parameters meet the requirements of Regulation (EO) 853/2004.

Table 1. Composition and properties of cow's milk processed into white cheese (n = 4)

Parameters	Raw milk	Pasteurized milk
	$\bar{x}^* \pm S\bar{x}^{**}$	$\bar{x}^* \pm S\bar{x}^{**}$
Dry matter, %	12.21 ± 0.056	13.6 ± 0.523
Fat, %	3.38 ± 0.502	4.76 ± 0.093
Non-fat substance, %	8.83 ± 0.058	8.85 ± 0.070
Total protein, %	3.24 ± 0.024	3.44 ± 0.014
Density, °G $_{20/4°C}$	30.6±0.367	29.6 ± 0.778
Titratable acidity, °T	15.1 ± 0.048	14.9 ± 0.141
Active acidity, pH	6.38 ± 0.106	6.36 ± 0.024

*$\bar{x}$ is the average velue; **$S\bar{x}$ is the standard deviation of the average value

After pasteurization (76-78°C/25 min) there is an increased percentage of dry matter, milk fat, non-fat substance and total protein, due to the reduction of water content in milk. The proportion between milk fat/total proteins in pasteurized milk is 1.4, which is in the optimal range recommended for milk intended for cheese production (Shayfel, 2010).

Hygiene indicators

The total number of mesophilic aerobic microorganisms in raw cow milk used for production of white-brined cheese (Table 2) is 248 000 cfu/cm^3, which don't exceeds requirements of Regulation (EC) No 853/2004 – ≤ 300 000 cfu/cm^3. The highest contamination level of the raw milk microflora occupies lactococci group, followed by the *Enterobacteriaceae* and coliforms. There is a relatively small number of *Salmonella* spp. (800 cfu/cm^3) and *E. coli* (4000 cfu/cm^3), respectively. After pasteurization (76-78°C/25 min), the microbial load of milk is greatly reduced. Total aerobic mesophilic count and the number of lactococci drastically decreased and reached 810 cfu/cm^3 and 30 000 cfu/cm^3, respectively. The presence of coliforms and *Salmonella* spp. in the pasteurized milk is limited to acceptable levels that meet practical microbiological criteria for heat-treated milk for cheese production. Our results confirmed the conclusions of many authors about the reducing potential of heat treatment on various microbial species (Bintsis & Papademas, 2002; Beresford & Williams, 2004; Pintado et al., 2015)

Table 2. Number of microorganisms in cow milk processed into white-brined cheese, cfu/cm^3 (n = 4)

Parameters	Raw milk	Pasteurized milk
Total aerobic count	248 000	810
Lactococci	2 700 000	30 000
Lactobacilli	30 000	100
Enterobacteriaceae	860	20
Salmonella spp.	800	2
Coliforms	25300	100
E. coli	4 000	0
L. monocytogenes	0	0

Biochemical processes in the cheese manufacture

The cheese ripening process involves changes in its physicochemical properties accompanied by the development of a characteristic flavor (Oner et al., 2006). Dry matter content in cheese has been changed by many factors as milk quality, conditions of cheese production and degrees of ripening. The fresh cheese contains 33.5% of dry matter as compared to the maturation beginning and reaches 38.5% at the end (Table 1). The low dry matter content and respectively high water content of cheese may be attributed to the effect of high temperature pasteurization on kappa (κ)-casein forming complex with β-lactoglobulin which increase water-holding capacity (Lucey & Kelly, 1994, Fox et al., 2000).

The protein and fat are the most important ingredients responsible for the texture of cheese (Othman, 2011). The

Table 3. Composition and properties of the produced white-brined cheese (n = 4)

Indices	24 hour	7th day	15th day	30th day	45th day
	$\bar{x}^* \pm S\bar{x}^{**}$	$\bar{x}^* \pm S\bar{x}^{**}$	$\bar{x}^* \pm S\bar{x}^{**}$	$\bar{x}^* \pm S\bar{x}^{**}$	$\bar{x}^* \pm S\bar{x}^{**}$
Dry matter, %	33.5± 0.995	34.6 ±2.37	34.0 ± 1.835	35.06 ± 3.350	35.5 ± 3.530
Fat, %	13.3 ± 1.115	16.0 ± 2.64	17.3 ± 3.18	17.4 ± 3.450	16.4 ± 3.071
Fat in dry matter, %	39.7 ± 1.374	46.25 ± 0.125	51.0 ± 1.325	50.0 ± 0.785	47.0 ± 2.546
Total protein, %	13.7 ± 1.466	14.0 ± 1.110	14.0 ± 1.179	13.9 ± 0.880	13.7 ± 1.115
Salt, %	4.1 ± 0.659a	4.9 ± 0.505b	5.7 ± 0.373a	5.8 ± 0.192ab	5.8 ± 0.111ab
Moisture in non-fat substance, %	77.0 ± 0.458	78.0 ± 1.245	80.0 ± 0.958	79.0 ± 0.895	78.0 ± 2.214
Salt in moisture, %	6.17 ± 0.142	7.50 ± 0.325	8.64 ± 0.415	8.94 ± 0.957	9.00 ± 0.748
Titratable acidity, °T	199 ± 44.39	214 ± 61.37	225.3± 34.5	230.33±30.55	229.67±30.99
Organoleptic assessment	Normal taste and smell of fresh curd	–	–	–	Moderately strong aroma with pronounced acidity

$^*\bar{x}$ is the average velue; $^{**}S\bar{x}$ is the standard deviation of the average value

fat content increased approximately 3% during the ripening while the protein remained relatively constant.

The moisture in the non-fat substance of the cheese is one of the factors that influence the growth of microorganisms (Pierre et al., 1999). The moisture in the non-fat substance increased from 77% at 24th hour to 80% on the 15th day, and then decreased again at the end of the ripening period making conditions for microbial growth unfavorable.

The high salt content in the total weight of cheese justify rapid acid formation. Titratable acidity affects the rate of ripening and consistency of the product. It is increasing rapidly in the first hours and days of ripening and subsequently slows its dynamics. The titratable acidity of cheese produced on the 24th hour was 199.0°T. This acidity is higher than the obtained from Iliev (1996) for white-brined cheese from cow milk – 147.2°T. Titratable acidity of the cheese during ripening increased until 45th day and reached 229.7°T. These results are similar to these obtained by Mas et al. (2002) of goat cheese and a little lower than obtained from cow feta cheese by Peychevski et al. (1988).

In terms of organoleptic assessment, mature white-brined cheese has a soft texture and a pronounced lactic acid taste.

Microbiological processes for the production and ripening

During the ripening of cheese significant changes were observed in terms of the parameter total aerobic count. The number of mesophilic microorganisms decreased dramatically – from 45 000 cfu/cm^3 in the fresh cheese to 1000 cfu/cm^3 in the ripe one (Table 4). During the fermentation of cheese, comprising the first 7-15 days, specific microflora and in particular lactococci strongly prevailed and became predominant. Their number was nearly 400 times more on the 7th day when compared to the 24th hour. Similar change in the lactococci level was observed by de Souza et al. (2003) in the first weeks of cheese ripening. On the other hand, the dynamics of lactobacilli number was not very pronounced. At the end of the 30-days period, a strong decrease in the number of lactobacilli was observed, then on the 45th day it increased again, which is probably due to the reduction of the impact of residual microflora on the starter cultures. Similar results were obtained by Enikova (2010).

The numbers of *Salmonella* spp. do not vary very much. Still, before the 7th day these microorganisms reached comparatively high levels, making the consumption of the product during this period extremely high risk factor. Although specific lactic microflora in cheese (lactococci and lactobacilli) reach relatively high concentrations in the product, in the early stages of ripening they cannot be full antagonists of unwanted microflora and in particular some of the representatives of *Enterobacteria-*

Table 4. Dynamics of lactic acid and pathogenic microflora during the ripening of cheese, cfu/cm^3 (n = 4)

Microorganisms	24th hour	7th day	15th day	30th day	45th day
Total aerobic mesophilic bacteria	45 000	36 000	20 000	4200	1000
Lactococci	500 000	185 000 000	85 000 000	300 000	200 000
Lactobacilli	100 000	96 000	93 000	13 000	50 000
Enterobacteriaceae	120	60	0	0	0
Salmonella spp.	20	0	0	0	0
E. coli	260	100	0	0	0
L. monocytogenes	0	0	0	0	0

ceae. Another reason for the reduction of *Enterobacteriaceae* numbers in the cheese is the lower pH resulting from the ripening process (Bintsis & Papademas, 2002).

The results in Table 4 revealed that the microorganisms of *Enterobacteriaceae* were active during the first 7 days of ripening. This confirms the conclusions made after extensive long-term studies (Enikova, 2010), as well as other authors' data (Bintsis & Papademas, 2002; Pintado et al., 2015).

The presence of *E. coli* in food products is considered as a criterion for the hygiene of the production processes. The data presented in Table 4 shows that the ripe cheese does not suffer residual *E. coli*, although initially high concentrations of this microorganism were presented, which highlights the antimicrobial effect of the cheese starter (*Str. lactis* and *Lb. casei*) and the added yogurt starter milk (*Str. thermophilus* and *Lb. bulgaricus*) against these representatives of pathogenic microflora. Similar results established Enikova (2010) in the study on the impact of different types of white-brined cheese starter cultures on the presence of coliforms in the finished product. The absence of coliforms and *E. coli* in particular in the final product is a reliable efficiency indicator of the ripening processes impact on the riskiest indicator group and pathogenic bacteria.

These results prove the thesis that the cheese in the early stages of its ripening not only does not have any antimicrobial effect, but on the contrary, there is active propagation of many pathogenic bacteria in the period up to the 15th day, therefore there is a high health risk from fresh cheese consumption. It arises primarily because of secondary milk contamination after pasteurization – from the working equipment, from anthropogenic sources, and in each case of gross violations of production hygiene (Temelli et al., 2006).

A requirement in Bulgarian standard (BDS) 15:2010 is included to determine the presence of *Listeria monocytogenes* as a criterion for the safety of white-brined cheese, although the traditional technology exclude the possibility of its development in the product. This is because of the combination of a heat treatment of the raw material and the strong ripening process that leads to accumulation of organic acids in concentrations incompatible with the development of *Listeria monocytogenes* (Enikova, 2010). In the present study this microorganism is not found in the pasteurized milk and in any stage of ripening.

By means of water activity (a_w) parameter is determined the relationship between the presence of accessible to the microorganisms water in the product and the likelihood of development of some microflora in the cheese.

Water activity during the ripening remained almost equal up to the 30th day and then fell sharply and from 0.98 to 0.81. Water activity significantly helps the control of the metabolic activity and growth of cheese microorganisms. The minimum requirement for the development of lactic acid microorganisms from the starter culture is 0.98 (Weber & Ramet, 1987). Besides the use from the microorganisms the reduction of a_w during the cheese ripening is due to the hydrolysis of proteins and peptides, amino acids and triglycerides to glycerol and fatty acids, which includes the holding of one water molecule for each peptide or an ester bond in the hydrolysis. Another factor which influences the reduction of a_w is the proteolysis that occurs during the cheese ripening. The loss of water from the microorganisms because of the low a_w environment contributes to the decrease of the bacteria number through the subsequent plasmolysis and cell lysis (Hickey et al., 2013).

Conclusion

Milk pasteurization leads to a strong reduction in the total count of aerobic mesophilic microorganisms and the pathogenic microorganisms in the milk. The processes during ripening caused a drastic reduction of microflora with observed in 45th day prevalence of specific lactic microflora (lactobacilli and lactococci) and complete annihilation of *E. coli* and *Salmonella* spp. These changes in the cheese microflora make the final product safe and confirms that this type of cheese must be preceded by a maturation period before consumption. On the other hand, the experimental data showed a strong multiplication of *Salmonella* spp. on the 7th day and insufficient decrease in the number of other microorganisms, making fresh cheese at this stage of ripening risk product for immediate consumption. Ripening leads to increasing of the dry matter percentage, fat, salt content and total protein and to reducing of the water content in the finished product, what is in accordance to accepted standards for the production of white- brined cheese from cow milk.

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