



CHANGES IN SELECTED PARAMETERS OF STEAMED CHEESE DURING STORAGE

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ABSTRACT

Parenica is a steamed, lightly smoked or unsmoked cheese wound into roll. The aim of this work was to evaluate selected microbiological and chemical parameters of smoked and unsmoked parenica cheeses from industrial and farm dairy after production and after 7 days of storage at 6 °C. Yeasts and molds, as an indicator of hygiene production conditions, were cultivated for 5 days on DRBC agar at 25 °C. The dry matter content was determined by drying to constant weight. Determination of NaCl was performed using the Chloride Analyzer 926. Counts of yeasts in unsmoked cheeses after production were comparable and reached average value 1.65 log CFU.g⁻¹ from farm dairy and 1.57 log CFU.g⁻¹ from industrial dairy. Yeasts weren't detected in samples of smoked cheeses from the farm dairy, however their presence was found in 50% of smoked cheeses from industrial dairy with average value 2.67 log CFU.g⁻¹. Average count of yeasts in unsmoked cheeses from the farm dairy after storage was 3.48 log CFU.g⁻¹ and 2.04 log CFU.g⁻¹ from industrial dairy (yeasts were present in 83.3% of cheeses). The molds appeared in cheeses only sporadically after storage. Dry matter content in all analyzed samples was in accordance with value declared by the producers. No samples of unsmoked parenica cheeses and only 33% of smoked parenica cheeses from farm dairy were in accordance with declared content NaCl (2%). 33% of the unsmoked cheeses and 25% of smoked cheeses from an industrial dairy were in accordance with declared data (NaCl 1.8%).

Keywords: steamed cheese, parenica, yeasts and molds, dry matter, NaCl

INTRODUCTION

Steamed cheese or *Pasta-filata* cheese originate primarily in the greater northern Mediterranean regions of Italy, Greece, the Balkans, Turkey, and East Europe. The term *pasta-filata*, which is derived from an Italian phrase that literally means "spun paste" or "stretched curds", refers to a unique plasticization and stretching process shared by all *pasta-filata* cheeses that provides this diverse group with a common identity. This type of cheeses includes soft or semisoft cheeses consumed fresh (e.g. fresh Mozzarella) or after a short aging, whereas other varieties are hard or semihard ripened cheeses aged for longer before consumption (e.g. Kashkaval) (McMahon and Oberg, 2017).

The production of steamed cheese has a long tradition in Slovakia too. These cheeses, together with sheep cheeses, sheep's lump cheeses and "žinčica" (sheep whey drink), are considered typical Slovak dairy specialties. Therefore, the *Slovenská parenica*, *Zázrivské vojky*, *Zázrivské* and *Oravské korbáčiky* as well as the *Slovenský oštiepok* (this may be also unsteamed) were registered in the EU Register as products with Protected Geographical Indication (Drončovský et al., 2017).

Slovenská parenica is a steamed, lightly smoked or unsmoked cheese wound into two opposed rolls 0.06-0.08 m in diameter and 0.05-0.08 m high, forming "S"-shape bulk. Moreover, the rolls are bound with cheese string. Prior to rolled up, the cheese strips are 0.002-0.003 m thick, 0.05-0.08 m wide and 4-6 m long (Supeková et al., 2008).

The basis of the steamed cheese, resp. parenica cheese production is lump cheese from cow's or sheep's milk, which may be raw or pasteurized. The microbiota of raw milk can vary considerably in number and species distribution depending on the contamination of the raw milk. Cheese production from raw milk means an increased risk of survival of pathogenic bacteria and their incorporation into cheese (Zimanová et al., 2016). Starter cultures to restore microbial life and to ensure acidification of lump cheese should be added to pasteurized milk for cheese production. Thermophilic lactic acid bacteria (such as *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactobacillus helveticus*) are used for steamed cheese production. Mesophilic lactic acid

bacteria (such as *Lactococcus lactis* ssp. *cremoris*) can also be used although differences in cheese flavor profile would be expected. The principal role of starter culture is to sufficiently acidify the milk and subsequently curd via conversion of lactose into lactic acid (Zimanová et al., 2016; McMahon and Oberg, 2017). Lump cheeses for the production steamed cheeses should have an ideal value of pH between 5.1 and 5.3 (Drončovský et al., 2017). Steamed cheeses are characterized by stretching phase, which follows the curd acidification. The stretching phase consists in submitting curd to elongation in presence of hot water (Tripaldi et al., 2018). Steaming water with a temperature of 65 to 85 °C is used in the production of parenica. When the lump temperature rises to about 50-55 °C, it obtains stretchable and formable consistency. Casein aggregates are bound and form long chains, which entraps fat and moisture (Zimanová et al., 2016; Drončovský et al., 2017; Tripaldi et al., 2018). It is supposed that the ability of the cheese to stretch and rearrange in hot water its structure to unidirectional fibers is mainly due to the amount of casein-associated calcium available to cross-link the amorphous para-casein structure at the time of lump steaming. Two key parameters determine the amount of calcium bound on casein at the time of steaming: (i) the total calcium content of the lump cheese; and (ii) the distribution of total calcium between the soluble and insoluble (casein bound) form. Therefore, control of acidification, demineralization and dehydration together with reaching the critical pH value of lump cheese at the time of steaming are key technological parameters for the production of all steamed cheeses (Roginski, 2002; Fox et al., 2004).

After steaming, forming and cooling, the next stage in the production of steamed cheeses is salting. Salt plays an essential role in cheese making. The salt content of the cheese directly affects the taste of the cheeses, promotes the syneresis of the cheese curd and thus regulates the water content of the cheese, decreases the water activity of the cheese, affects the activity of the native milk enzymes, rennet enzymes and enzymes of cultural and noncultural microorganisms. The salt also regulates the fermentation of residual lactose and hence the pH value in the young cheese as well as the ripening process and the quality of the ripened cheeses (Fox et al., 2004; Bähler et al., 2016; Zimanová et al., 2016). Sodium chloride is reported to change the confirmation of casein proteins and modify the

solubility of the different casein fraction. Since cheese is a soft gel of concentrated casein micelles with embedded fat globules and a continuous serum phase, the swelling of this concentrated protein network increases in the presence of sodium chloride. In particular, the voluminosity (size) of the casein micelles increases (Bähler et al., 2016). Also Everett and Auty (2008) reported that salt limits the action of bacteria in cheese, as well as has secondary effect of flavor enhancement. Saltiness is one of the most important flavor attributes of cheese and is directly correlated to overall desirability by consumers. Ganesan et al. (2014) reported that a drop in salty flavor decreased levels of brothy, buttery, lactone/fatty acid, sulfur, umami, and sour flavors (even though steamed cheese pH was the same). The steamed cheeses may be salted during the steaming process (hot salt solution is used as the steaming water), the salt may be added to the steamed dough at the exit of the steaming machine or cheeses may be salted in a cold salt bath, to keep their shape. Presently, direct dry salting of cheeses is also preferred because of the risk of yeast and mold contamination of the salt bath (Walstra et al., 2006; Zimanová et al., 2016). Locci et al. (2012) reported that adding sodium chloride during steaming and stretching process increases the yield.

Salt concentration is an important topic in cheese production and has received increased attention due to efforts towards sodium reduction in human nutrition. However, salt reduction in pasta filata cheese is not simple because the sodium chloride content influences cheese moisture and hence affects ripening, techno-functional properties and sensory perception (Ganesan et al., 2014).

The next stage in the production of steamed cheeses can be smoking. Fellows (2017) state that the classic smoking of cheese by wood has a long tradition in Slovakia. When smoking softer cheeses, wood from fruit trees is used.

Smoking of cheeses is just one way of enhancing their sensory properties (partly of taste, color, texture) and also contributes to preserving and thereby prolonging the shelf life of the cheeses (Semjon et al., 2017). Smoke is produced by increasing the temperature of the wood and with limited air access to prevent combustion. The smoking may be accomplished in several ways. The main smoking techniques include cold or hot smoke, but electrostatic smoking or liquid smoke treatments are also used. The principle of extending the shelf-life of a food is to combine the reduced water activity and the capture of bacteriostatic, bactericidal and antioxidant smoke components on the food surface (Zimanová et al., 2016). Preservation of food by smoking is attributed to antimicrobial properties of smoke components such as organic acids. Of the reported organic acids found in smoke condensates, acetic acid, propionic acid and benzoic acid are credited with possessing the most antimicrobial potential; the antimicrobial potential of organic acids is accredited to the influence on overall pH and the undissociated form of the acid. The cell membrane lipid bilayer can be easily penetrated by organic acids in their undissociated forms. Also, carbonyls in smoke materials have role as an antimicrobial. However, their efficacy when in the form of smoke condensates can be inferred based on the 133 different aldehydes and ketones present in smoke. Of these carbonyls, formaldehyde and acrolein have proven toxicity against microorganisms. Carbonyls inhibit

microbial growth by penetrating the cell wall and inactivating enzymes located in the cytoplasm and the cytoplasmic membrane. It was documented from long time ago that commercial liquid smoke preparations are effective against various types of spoilage and pathogenic microorganisms (Ismail, 2016).

Higher mentioned several factors in the production of steamed parenica cheese affect the quality of steamed cheeses, including microbiological quality. The aim of this work was to evaluate and compare the selected microbiological and chemical parameters of smoked and unsmoked parenica cheeses produced from pasteurized milk in industrial dairy and farm dairy after production and after storage.

MATERIAL AND METHODS

Samples of unsmoked and smoked parenica cheeses were taken once a month from February 2018 to March 2019 from one industrial and one farm dairy located in mountain region of Slovakia. Parenica cheeses from farm dairy were packaged under vacuum and cheeses from industrial dairy were in modified atmosphere (with nitrogen and carbon dioxide). Samples of steamed cheese were analyzed day after production and after 7 days of storage at 6 °C (7 days - shelf life declared for cheeses from farm dairy). Microbiological quality of parenica cheeses was determined by cultivation method. Yeasts and molds in cheeses, as an indicator of hygiene production conditions, were cultivated for 5 days on DRBC agar (Merck, Germany) at 25 °C (ISO 21527-1, 2008).

The dry matter content was determined by oven drying method (ISO 5534, 2004) by drying to constant weight at 102±2 °C. Determination of NaCl in steamed cheeses samples was performed using the Chloride Analyzer 926 (Sherwood Scientific Ltd., UK) and pH using pH meter Hanna (HANNA instruments, Czech Republic).

Analyses were replicated twice and from obtained values were calculated – mean values, standard deviation, coefficient of variation.

The obtained results were processed by variation-statistical method in ANOVA of Statistica CZ9.1 software (Stat Soft Ltd., Czech Republic). The differences were considered significant at the P<0.05 level.

RESULTS AND DISCUSSION

The microbiota of steamed cheeses consists of various representatives not only of bacteria but also of yeasts and molds. The presence of yeasts and molds in cheeses can be considered as an indicator of hygiene level in cheese production establishments. The role of molds and yeasts in cheese quality is variable. This microbiota is usually considered undesirable in soft cheeses; however, it could have a positive effect on the aroma of traditional cheeses (Fuentes et al., 2015).

The results of yeasts counts in parenica cheeses samples after production are shown in Fig. 1.

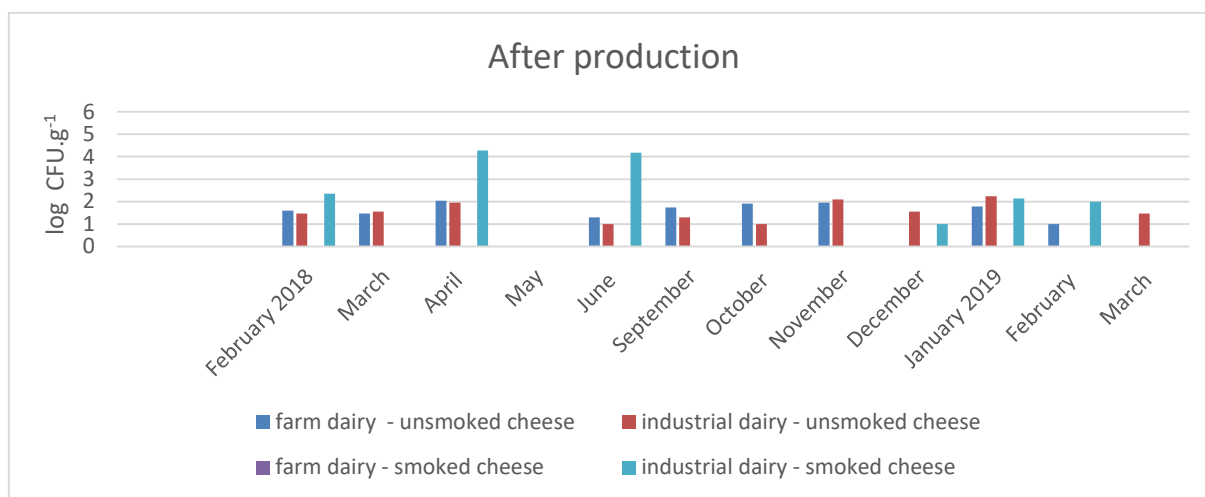


Figure 1 Counts of yeasts in smoked and unsmoked parenica cheeses from farm and industrial dairy one day after production

The counts of yeasts in the samples of unsmoked cheeses after production were comparable and reached average value 1.65 log CFU.g⁻¹ in cheeses from farm dairy (yeasts detected in 75% of samples) and 1.57 log CFU.g⁻¹ from industrial dairy (yeasts detected in 83.3% of samples). There was no statistically significant difference (P>0.05) between the samples of cheeses from farm and industrial dairy. Comparable counts of yeasts were determined by Drončovský et al. (2017) in steamed cheeses made from lumps from pasteurized cow milk, where the yeasts counts ranged from <10 CFU.g⁻¹ to 6.1x10² CFU.g⁻¹. Higher counts of yeasts from 5.7x10² to 6.9x10⁵ CFU.g⁻¹ they found in steamed cheeses made from lumps from raw ewe's milk. Berthold-Pluta et al. (2011) reported that the presence of yeasts and molds was established in 100 % and 63 % of steamed

unsmoked Oscypek cheeses. Yeasts and molds were present in the amounts of 10³ - 10⁵ CFU.g⁻¹ and 10¹ - 10⁴ CFU.g⁻¹ respectively.

After cheeses production, yeasts weren't detected in samples of smoked parenica cheeses from the farm dairy, however their presence was found in 50% of smoked parenica cheeses from industrial dairy with average value 2.67 log CFU.g⁻¹. It can be assumed that the smoking process in small farm dairies is not controlled and higher temperatures of smoke are used opposite to cold smoke (28-30 °C) used in industrial dairy. Thus, higher temperatures of smoke in the farm dairy may inhibit the surface microbiota of steamed cheeses. There was a statistically significant difference (P<0.01) between the counts of yeasts in unsmoked and smoked parenica cheeses from farm dairy. No statistically

significant difference ($P>0.05$) was found by comparison of unsmoked and smoked parenica cheeses from an industrial dairy. As mentioned above, the smoking (temperature as well as the smoke components) may inhibit the microbiota, which has also been confirmed in smoked cheese from the farm dairy. On the contrary to this, higher yeast counts were found in some samples of smoked cheeses than unsmoked cheeses from an industrial dairy. It can be assumed that the reason was probably the contamination of these smoked parenica cheeses during the drying process, which takes place after the smoking process.

Alegria et al. (2012) analyzed samples of smoked Oscypek cheeses from 4 producers. The average counts of yeasts and molds ranging from 4.69 log CFU.g⁻¹ to 5.79 log CFU.g⁻¹ depending on the cheese manufacturers.

Berthold-Pluta et al. (2011) based on their results stated, that in the case of Oscypek cheeses, the process of smoking may lower total bacterial count and the

count of molds and yeasts by approximately 1 log row compared with unsmoked Oscypek cheeses. However, cheese smoking does not reduce the counts of coliforms, *Escherichia coli* and *Clostridium perfringens*. These authors determined values of yeasts in the range from 6.5x10² to 2.0x10⁵ CFU.g⁻¹ and molds from 1.5x10¹ to 4.0x10³ CFU.g⁻¹ in smoked Oscypek cheeses.

Mařová et al. (2017) compared quality of a steamed unsmoked and smoked cheeses from Slovak, Polish and Ukrainian producers. Yeasts and molds determined on DRBC agar reached the values 9.09x10¹ - 1.81x10² CFU.g⁻¹ in Ukrainian cheeses, not detected - 3.36x10³ CFU.g⁻¹ in Slovak cheeses and 5.09x10³ CFU.g⁻¹ in Polish steamed cheeses.

The results of yeasts counts in parenica cheeses samples after 7 days of storage at 6°C are shown in Fig. 2.

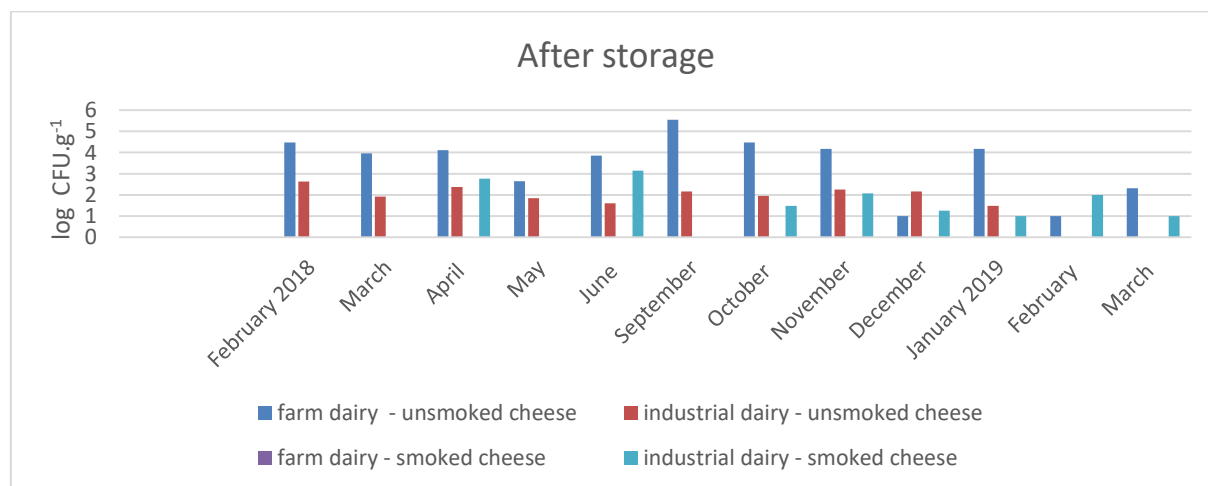


Figure 2 Counts of yeasts in smoked and unsmoked parenica cheeses from farm and industrial dairy after 7 days of storage at 6 °C

The molds appeared in parenica cheeses samples only sporadically after storage. The presence of yeasts was found in all analyzed samples of unsmoked parenica cheeses from the farm dairy after storage and their average count was 3.48 log CFU.g⁻¹. In unsmoked parenica cheeses from industrial dairy, yeasts were found in 83.3% of samples with average value 2.04 log CFU.g⁻¹. In both types of unsmoked parenica cheeses, the increase of yeasts counts occurred during storage, but it was not statistically significant ($P>0.05$). A higher increase of yeasts counts during storage in samples of unsmoked parenica cheeses from the farm dairy in comparing to the samples from the industrial dairy, is probably related to the way the cheeses packaging. Cheeses from farm dairy were in vacuum packaging while cheeses from industrial dairy were in modified atmosphere packaging. The modified atmosphere consisted of nitrogen and carbon dioxide, which have antimicrobial effect.

Regarding to the smoked parenica cheeses after storage, yeasts were not detected in the samples from farm dairy again but were present in 66.7% of samples from industrial dairy and their average count reached the value 1.84 log CFU.g⁻¹. In the case of smoked cheeses from an industrial dairy, during 7 days of cheeses storage at 6 °C, the counts of yeasts decreased but it was not statistically significant ($P>0.05$). The decrease of yeasts counts in smoked parenica cheeses during

storage appears to be related to the action of the modified atmosphere as well as the action of the antimicrobial agents of the smoke.

Fuentes et al. (2015) evaluated Oaxaca cheeses (a Mexican steamed cheese) from 3 dairy plants, vacuum-packaged and stored at 8°C up to 24 days. Molds and yeasts in the Oaxaca cheeses reached the following average values: 5.05 CFU.g⁻¹ (1st day), 5.52 CFU.g⁻¹ (8th day), 5.20 CFU.g⁻¹ (16th day) and 4.35 CFU.g⁻¹ (24th day). The counts of molds and yeasts did not show significant changes during cheese storage. Onipchenko (2012) determined changes of yeasts and molds in steamed cheeses after production and after storage. She reported that yeasts and molds have reached the level 3.93 CFU.g⁻¹ in final product and after first and third month 3.80 CFU.g⁻¹ and 4.70 CFU.g⁻¹ respectively.

Tripaldi et al. (2018) reported that yeasts were lower than detection limit of the method at day of steamed cheese production and grew during cheese storage (3.3 CFU.g⁻¹ at 20th day and 2.9 CFU.g⁻¹ at 40th day).

On the basis of our results and the results of other authors, it can be stated that in terms of the yeast counts after production and after one week of storage, analyzed parenica cheeses can be considered to be of good quality.

Comparison of chemical parameters of unsmoked and smoked parenica cheeses from farm and industrial dairy is in Table 1.

Table 1 Selected parameters of unsmoked and smoked parenica cheeses from farm and industrial dairy

parameters	dairy	cheese sample	min	max	average±SD	cv
dry matter (g.100g ⁻¹)	farm	unsmoked	42.27	49.69	45.41 ±1.92	4.29
		smoked	44.79	51.37	47.46±2.19	4.62
	industrial	unsmoked	47.50	53.35	49.47±1.63	3.29
		smoked	47.98	54.21	51.05±1.79	3.51
pH after production	farm	unsmoked	5.20	5.53	5.35±0.09	1.74
	industrial	unsmoked	5.16	5.51	5.34±0.12	2.20
		smoked	5.32	5.55	5.42±0.08	1.54
pH after storage	farm	unsmoked	5.28	5.62	5.44±0.11	1.96
		smoked	5.21	5.51	5.36±0.07	1.37
	industrial	unsmoked	5.17	5.48	5.35±0.09	1.62
		smoked	3.31	5.54	5.43±0.08	1.40
NaCl (g.100g ⁻¹)	farm	unsmoked	5.25	5.67	5.45±0.12	2.23
		smoked	2.25	4.51	3.12±0.73	23.44
	industrial	unsmoked	1.66	4.03	2.62±0.73	27.85
		smoked	1.32	2.30	1.88±0.29	15.40
			1.44	3.03	2.08±0.49	19.79

SD – standard deviation, cv –coefficient of variation

All analysed samples of parenica cheeses from farm dairy had higher content of dry matter than was declared (min value 41%) by producer. The average value of dry matter was in unsmoked cheeses 45.41% and in smoked cheeses 47.46%. Also the samples of cheeses from industrial dairy were in accordance with the requirements of the legislation for semi-soft and semi-fat cheese labelled on package (average value of dry matter was 49.47% in unsmoked cheese and 51.05% in smoked cheeses). In terms of a higher dry matter content, the parenica cheeses from the industrial dairy can be considered to be of higher quality because they offer to the consumers more compounds in the cheeses.

Maľová et al. (2017) determined dry matter from 53% to 68% in Ukrainian steamed cheeses, from 44% to 60% in Slovak steamed cheeses and 52% in Polish steamed cheeses and they stated that the 1 sample from Slovak producer was not in accordance with value declared by the producer.

Semjon et al. (2017) observed statistically significant effect of smoking on values of dry matter ($P < 0.0001$). Similarly, the statistically significant effect ($P < 0.01$) of smoking on the dry matter values was also found in the samples of the parenica cheeses from the farm and industrial dairies.

The results also show that the smoking process had no statistically significant effect ($P > 0.05$) on the pH of the cheeses and these findings are in accordance with results of **Semjon et al. (2017)**.

Declared content of NaCl in parenica cheeses from farm dairy was 2%, no samples of unsmoked parenica cheese and only 33.3% of smoked parenica cheeses were in accordance with declared content. Better results were found after determination of salt content in the parenica cheeses from an industrial dairy, where 33% of the unsmoked cheeses and 25% of smoked cheeses were in accordance with data (1.8%) declared on the package. The high coefficients of variation for the NaCl content as well as the dry matter content indicate that it is necessary to hold on the relevant parameters of the technological process, especially in the production of steamed parenica cheeses in the farm dairy, but also in the industrial dairy.

On the contrary, **Maľová et al. (2017)** stated that the Slovak samples of smoked and unsmoked steamed cheeses were in accordance with content of salt declared by the producer on the package and their contents ranged from 1.5% to 2.5%. In Ukrainian smoked and unsmoked cheeses, the salt contents ranged from 7.2 to 9.2% and exceeded the declared salt content by two to three times.

Sodium chloride is traditionally added to cheeses as a preservative and to improve flavour. However, a positive correlation between high level of sodium and osteoporosis, kidney stones and hypertension has been found (**Shah and Ayyash, 1994**). Hence, there is an increased interest to reduce salt.

CONCLUSION

The presence of yeasts and molds in steamed cheeses can be considered as an indicator of the hygiene level during cheese production.

In the analysed samples of unsmoked and smoked steamed cheeses from the farm and industrial dairy after production and storage, the presence of yeasts was found and the presence of molds was only sporadically after storage. Therefore, it is necessary to improve the level of hygiene in cheese production establishments. In term of yeasts presence, the quality of analyzed parenica cheeses can be considered as good in generally. The results also indicate that the growth of yeasts during storage is more inhibited in samples of smoked cheeses and in samples of cheeses packaged in a modified atmosphere. The dry matter content of the cheese samples was in accordance with the declared values. However, from the point of view the higher dry matter content, parenica cheeses from the industrial dairy are of more interest for the consumers. Both producers of parenica cheeses have exceeded the declared salt content of steamed cheeses. It is necessary to hold on the relevant parameters of the technological process, especially in the production of steamed parenica cheeses in the farm dairy, but also in the industrial dairy.

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