

Original Article

Microbiological aspects of the traditional Travnik/Vlašić cheese manufactured in Bosnia and Herzegovina

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Abstract

The microbiological aspects of traditional Travnik/Vlašić cheese was investigated. The cheese was made traditionally, from raw sheep milk at three small farms (A, B, C) on Mountain Vlašić. The microbiological quality of the cheese was examined during three stages of ripening (5, 30, 60 days) and followed during three seasons (3 years). Twenty-seven samples of cheese were collected and analyzed for the aerobic mesophilic count, yeasts and molds, coliforms and microorganisms from the group *Staphylococcus* spp. Average values determined for the number of investigated groups of microorganisms of all cheese samples throughout three different stages, seasons, and small farms were: aerobic mesophilic bacteria $8.03 \log_{10} \text{cfu} \cdot \text{g}^{-1}$, yeasts and molds $3.63 \log_{10} \text{cfu} \cdot \text{g}^{-1}$, coliforms $5.16 \log_{10} \text{cfu} \cdot \text{g}^{-1}$, and microorganisms from the group *Staphylococcus* spp. $4.49 \log_{10} \text{cfu} \cdot \text{g}^{-1}$. ANOVA showed that experimental factor ripening stage (days) had a significant effect on all testing parameters. Results obtained with this study indicate that hygiene during the production of traditional products must be increased in order to assure high quality of the final products.

Key words: traditional; Travnik/Vlašić cheese; sheep milk cheese; microbiology; quality.

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Introduction

Traditional Travnik/Vlašić cheese is a very popular cheese in Bosnia and Herzegovina. It belongs to the group of white-brined cheeses. The origin of white-brined cheeses is from the Mediterranean region and the Middle East where ovine, caprine or cow milk was used traditionally [1,2]. Production of traditional Travnik/Vlašić cheese is located on the mountain Vlašić and surrounding mountains (near the town Travnik), situated in the middle of the country. It is produced from raw sheep milk (autochthonous sheep Pramenka) on small farms. Production of traditional Travnik/Vlašić cheese is seasonal, usually starts in mid-May and lasts until early October. During this period, sheep are fed only on the pastures [3].

Cheese is produced in low-grade hygienic conditions where a high quantity of salt is added to prevent the rapid development of cheese microflora. Adding a high amount of salt is aimed to control indigenous microflora, especially the growth of pathogens [4]. Autochthonous production continues to

this day, and causes non-standardized production and very high variability in cheese quality [5].

Traditional cheeses are part of the cultural and historical heritage. They are usually made from raw milk without the addition of starter cultures. In modern milk processing, cheese produced from raw milk can contain a pool of wild microorganisms. Today, artisanal raw-milk cheese is considered to be an important source of lactic acid bacteria genetic diversity [6]. At the same time, the traditional way of cheese making is often associated with poorer hygienic production conditions, and sometimes these cheeses do not comply with the food safety regulations. The safety of food products, including cheese, for the health of consumers, is one of the most important aspects of the production, storage, and preservation of products. The quality of the raw material in production and maintaining standards of hygiene are the main factors on which microbiological quality and safety of traditional cheeses depends [7]. Traditional cheeses are commonly produced from raw milk because of its enhanced organoleptic properties, as well as because of tradition. However, pathogenic

microorganisms present in raw milk, can remain viable in cheese even when they are ripened for several weeks [8].

Traditional production of this cheese is still not standardized. The safety of the food products, including traditional food products, is extremely important for public health. Very few studies have been carried out on the microbiological aspects of traditional Travnik/Vlašić cheese.

This study aimed to examine the microbiological aspects of traditional Travnik/Vlašić cheese produced from raw sheep milk on Vlašić mountain (Bosnia and Herzegovina).

Methodology

Samples collection

The traditional Travnik/Vlašić cheese used in this study was made on three small farms (A, B, C) from raw sheep milk of autochthonous race Pramenka, using traditional cheese-making technology on Mountain Vlašić (near the town Travnik in the central part of Bosnia and Herzegovina). After production and placement of the cheeses in wooden wats that are filed with brine, the cheeses were transported in the wooden wats to the maturation room in the premises of the Faculty of Agriculture and Food Sciences University of Sarajevo for a ripening period of up to 60 days under optimal conditions. Cheese samples were taken on the 5th, 30th, and 60th day of ripening from every wooden wat. The same procedure was repeated for every stage over 3 years and a total of 27 cheese samples were taken. Microbiological analyses of these samples were performed immediately after the samples were taken from the wats.

Microbiological analysis

The following groups of microorganisms were examined to analyze the hygienic quality of autochthonous Travnik/Vlašić cheese: (i) the total number of aerobic mesophilic bacteria on Milk Plate Count Agar (Liofilchem, Roseto degli Abruzzi, Italy) at 30 °C for 72 h; (ii) yeasts and molds on Rose Bengal Agar with Chloramphenicol Supplement (Liofilchem, Roseto degli Abruzzi, Italy) at 25 °C for 5 days; (iii) coliforms on Violet Red Bile Agar (Liofilchem, Roseto

degli Abruzzi, Italy) at 37 °C for 24 h; (iv) number of *Staphylococcus* spp. on Baird Parker Agar supplemented with aseptically added 50 ml Egg Yolk Telurit at 37 °C for 48h.

Statistical analysis

All statistical analyses, including ANOVA, were performed by using SPSS software Statistics 20 (IBM, 2013). Tukey test was performed in a case when the experimental factor had an impact on the observed feature. A *p* value of < 0.05 was considered statistically significant. Results of the analysis are given in a form of means and standard deviations (SD) for cheeses from all three small farms.

Results

Microbiological aspects during ripening of traditional Travnik/Vlašić cheese at the three small farms throughout the three stages are shown in Table 1. The results represent means and standard deviations.

Maximum counts for aerobic mesophilic bacteria (8.98-9.65 log₁₀ cfu.g⁻¹) were observed on day 5 of ripening and after that they decreased till the end of ripening (60th day) to 6.41-6.72 log₁₀ cfu.g⁻¹. Yeast and moulds count decreased slightly from day 5 (3.83-4.29 log₁₀ cfu.g⁻¹) to day 30 (3.94-4.07 log₁₀ cfu.g⁻¹), and afterwards gradually decreased till day 60 (2.71-3.01 log₁₀ cfu.g⁻¹).

Coliforms had maximum counts (7.59-5.70 log₁₀ cfu.g⁻¹) on day 5 and then decreased till day 30 (5.19-5.60 log₁₀ cfu.g⁻¹) and finally decreased gradually to a count of 2.59-3.82 log₁₀ cfu.g⁻¹ on day 60.

Counts for *Staphylococcus* spp. were the highest on day 5 (6.01-6.44 log₁₀ cfu.g⁻¹) and rapidly decreased till day 30 (3.72-4.11 log₁₀ cfu.g⁻¹), having slightly declined till the end of ripening (3.02-3.60 log₁₀ cfu.g⁻¹).

The average value determined for the number of certain groups of microorganisms in all cheese samples at the three farms during the three seasons were: aerobic mesophilic bacteria 8.03 log₁₀ cfu.g⁻¹, yeasts and moulds 3.63 log₁₀ cfu.g⁻¹, coliforms 5.16 log₁₀ cfu.g⁻¹, and microorganisms from the group *Staphylococcus* spp. 4.49 log₁₀ cfu.g⁻¹.

Table 1. Average value of microbiological parameters of traditional Travnik/Vlašić cheese from three small farms throughout three stages of ripening and seasons.

Ripening period (days)	Aerobic mesophilic bacteria	Yeast and molds	Coliforms	<i>Staphylococcus</i> spp.
5	9.29 ± 0.7	4.09 ± 0.5	6.79 ± 0.4	6.26 ± 0.3
30	8.21 ± 0.8	3.99 ± 0.4	5.37 ± 0.7	3.86 ± 0.4
60	6.60 ± 1.0	2.82 ± 1.1	3.32 ± 1.1	3.36 ± 0.1

ANOVA analysis indicated that the cheese producer (farm) had no significant effect ($p > 0.05$) on any of examined groups of microorganisms, while the ripening stage (days) had a significant effect ($p < 0.05$) on all. The count for aerobic mesophilic bacteria, as well as count for yeasts and moulds, was significantly lower on day 60 of ripening compared to all other ripening stages. Counts of coliforms and *Staphylococcus* spp. were significantly higher on day 5 compared to day 30, and the count on day 30 was significantly higher compared to the count on day 60 of ripening. Based on these results, it is obvious that the count of examined groups of microorganisms changed during the ripening time and was in decline from day 5 till day 60 of ripening. Despite all the investigated groups of microorganisms decreasing from the beginning till the end of ripening, all the groups are detected even after 60 days.

A strong negative correlation ($A = -0.98$; $B = -0.99$; $C = -0.97$), between the count of aerobic mesophilic

bacteria and ripening days, was determined while a correlation between the count of yeasts and moulds and ripening days was strong negative in the cheese samples from the farms A (-0.88) and B (-0.86) and medium in cheese samples from farm C (-0.65). Count of coliforms and days of ripening had high negative correlation coefficients in cheeses from farms B and C (-0.99) and A (-0.91). Correlation between the count of *Staphylococcus* spp. and days of ripening was very strong negative in A (-0.91) and strong negative in B (-0.89) and C (-0.80) farm cheese samples (Figures 1-4).

Discussion

Traditional cheeses represent a special group of food products and unique microbial ecosystems. Since indigenous microflora from raw milk is included in the process of production of traditional cheeses, safety of the product is important to safeguard and protect health of the consumers. Safe food improves population health and saves lives, while strengthening economic growth

Figure 1. Total count of aerobic mesophilic bacteria during the ripening period of traditional Travnik/Vlašić cheese throughout three different stages, seasons, and farms.

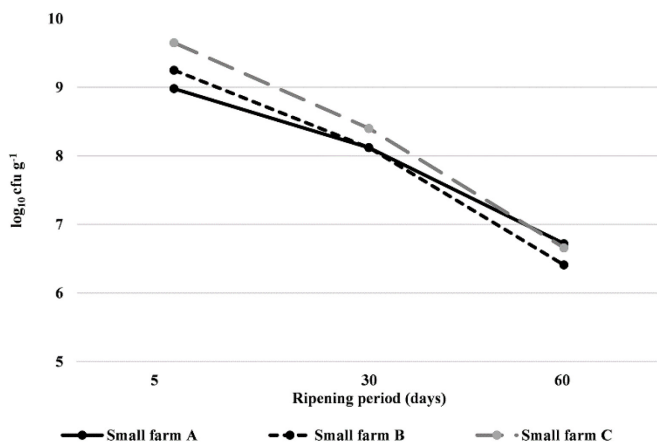


Figure 3. Total coliforms count during the ripening period of traditional Travnik/Vlašić cheese throughout three different stages, seasons, and farms.

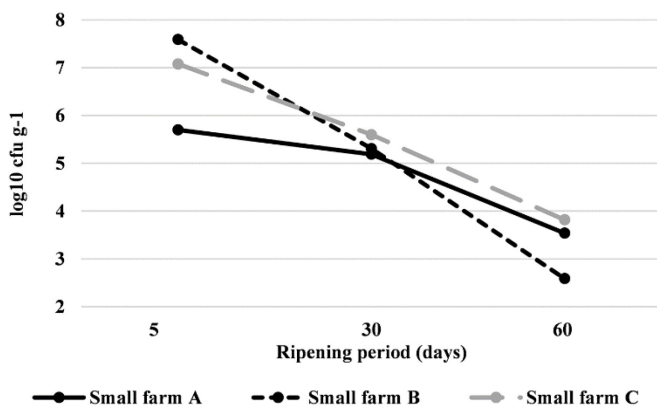


Figure 2. Total yeast and molds during the ripening period of traditional Travnik/Vlašić cheese throughout three different stages, seasons, and farms.

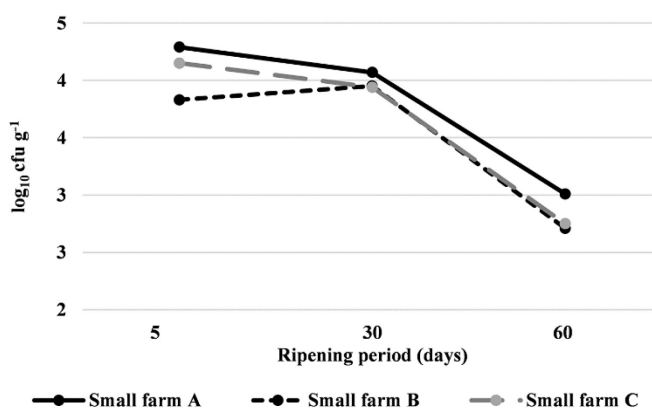
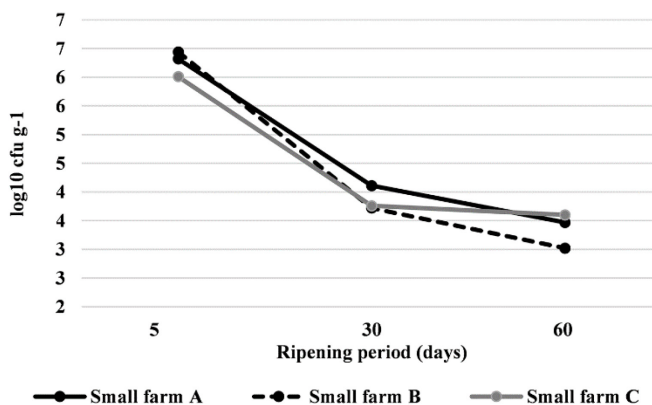


Figure 4. Total *Staphylococcus* count during the ripening period of traditional Travnik/Vlašić cheese throughout three different stages, seasons, and farms.



in the region [9]. Poor hygiene is the main problem in the production of such products, because natural microbial flora is composed of not only lactic acid bacteria, but also pathogens. Due to this, study on the microbiological quality of traditional Travnik/Vlašić cheese from Bosnia and Herzegovina is important not only to show us diversity of pathogens in raw milk cheese, but also to give us a new knowledge on the quality of the cheese.

Based on the results obtained with this study, counts of aerobic mesophilic bacteria were higher in the beginning of ripening period, and similar at the end of ripening when compared to counts in traditional white brined Greek cheese Batzos made from raw goat milk [10], where the count of aerobic mesophilic bacteria was $7.69 \log_{10} \text{ cfu.g}^{-1}$ on day 1 of ripening, $7.56 \log_{10} \text{ cfu.g}^{-1}$ on day 30 and $6.95 \log_{10} \text{ cfu.g}^{-1}$ on day 60. The counts in this research were higher than that in Urfa cheese made in Turkey. In addition, aerobic mesophilic bacteria were also present during all ripening period up to day 60 in Urfa cheese [11]. The results obtained with this study show that aerobic mesophilic bacteria were higher in traditional Travnik/Vlašić cheese in comparison with other white brined cheeses, which can be due to low sanitary conditions during milking and milk handling.

Since yeasts and moulds can cause alterations in sensory parameters of dairy products their presence is undesirable [12]. The appearance of yeasts in some acidified dairy products in high numbers may be caused by their ability to grow in low water activities (a_w) and low temperatures, and ability to tolerate low pH and high salt concentrations [13]. The presence of yeasts can lead to various quality defects, especially when grown in high numbers $5\text{-}6 \log \text{ CFU/g}$, and this can result in softening of the cheese texture, discoloration, off-flavours, and swelling of the cans [14]. The mean and minimum counts for yeasts and moulds, obtained in our study, were slightly higher than those for artisanal Turkish White Pickled cheese [15], while the maximum count was similar. The reason for this may be poor hygienic conditions and non-standardized production, and the ability of yeasts to resist high salt concentration as well as low pH.

As coliforms are highly sensitive to chemical sanitisers, they are useful to indicate inadequate sanitation of equipment and if they are present in high numbers that is a sign for inappropriate cleaning and sanitation [16]. The coliform counts in this study were higher than that presented for Greek Feta cheese made of sheep pasteurized milk [17], but the decline in counts were similar. Lactic acid bacteria consume sugars for

their growth resulting in low pH and lack of oxygen where the population of coliforms is decreasing, so coliforms can grow only in the beginning stages of cheese making when conditions (e.g. pH and temperature) are favourable [18,19]. It seems that the maximum levels of yeasts and coliforms for most cheeses are at day 4 [20], and this study showed that the highest levels of these microorganisms were in the beginning of the ripening period.

Counts of *Staphylococcus* spp. during the ripening of Feta cheese from raw ewe's milk were $5.91 \pm 1.0 \log_{10} \text{ cfu.g}^{-1}$ on day 4, $3.54 \pm 3.1 \log_{10} \text{ cfu.g}^{-1}$ on day 30, and $4.88 \pm 0.2 \log_{10} \text{ cfu.g}^{-1}$ on day 60 [20]. Traditional Travnik/Vlašić cheese had an initial higher count when compared to results of Feta cheese, and at the end of ripening count of *Staphylococcus* spp. was notably lower in Travnik/Vlašić cheese. The count of *Staphylococcus* spp. was in accordance with the results in Turkish feta cheese [21] where the count of *Staphylococcus aureus* increased on the first day of production, but salting of the cheese, as well as lower pH, resulted in inhibition of this pathogen.

Conclusions

This study on the microbiological aspects of traditional Travnik/Vlašić cheese is important because this cheese is one of the most important traditional cheeses in Bosnia and Herzegovina and the study provided useful knowledge on the diversity of pathogens present in the ecosystem of this cheese. The results obtained from this research indicates that cheese is produced in poor hygienic and sanitary conditions, with low level of cleaning of materials used in production. Although the total count of each of the examined groups of microorganisms was decreasing during ripening, these microorganisms were detected up to the end of the ripening period, which shows that microbiological quality of the cheese must be improved. This study highlights that it is vital to introduce and sustain good hygiene practice in the production of this traditional cheese in order to have product of high quality which is safe for consumers. One way to create safe final products is to organize trainings on good milking practice, cheese production and ripening, and storage practices for small producers. Research on microbiological quality of traditional Travnik/Vlašić cheese will lead to improvement in product safety.

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References

- Geronikou A, Srimahaeak T, Rantsiou K, Triantafyllidis G, Larsen N, Jespersen L (2020) Occurrence of yeasts in white-brined cheeses: methodologies for identification, spoilage potential and good manufacturing practices. *Front Microbiol* 11: 582778.
- Hayaloglu AA (2017) Cheese varieties ripened under brine. In Fox PF, McSweeney PLH, Cogan TM, Guinee TP, editors. *Cheese: chemistry, physics and microbiology*. Fourth Edition. Academic Press. 997-1040.
- Dizdarević T (2009) Determining the extent of ripening and content of volatile components in Travnik cheese. Master's thesis. Faculty of Agriculture and Food Sciences, University of Sarajevo.
- Abdalla OM, Davidson PM, Christen GL (1993) Survival of selected pathogenic bacteria in white pickled cheese made with lactic acid bacteria or antimicrobials. *J Food Prot* 56: 972-976.
- Dozet N, Stanišić M, Bijeljac S, Perović M (1983) Examination of technology of white cheese-Travnik type. *Mljekarstvo* 33: 132-138. [Article in Croatian]
- Kongo JM (2013) Lactic acid bacteria as starter-cultures for cheese processing: past, present and future developments. In Kongo JM, editor. *Lactic acid bacteria-R & D for food, health and livestock purposes*. Intech, open science/open minds. 1-22.
- Pyz-Łukasik R, Knysz P, Gondek M (2018) Hygiene quality and consumer safety of traditional short-and long-ripened cheeses from Poland. *J Food Qual*: 8732412.
- Gonzales-Barron U, Gonçalves-Tenório A, Rodrigues V, Cadavez V (2017) Foodborne pathogens in raw milk and cheese of sheep and goat origin: a meta-analysis approach. *Curr Opin Food* 18: 7-13.
- Fung F, Wang HS, Menon S (2018) Food safety in the 21st century. *Biomed J* 41: 88-95.
- Psoni L, Tzanetakis N, Litopoulou-Tzanetaki E (2003) Microbiological characteristics of Batzos, a traditional Greek cheese from raw goat's milk. *Food Microbiol* 20: 575-582.
- Uraz G, Coskun S, Ozer B (2008) Microflora and pathogen bacteria (*Salmonella*, *Klebsiella*, *Yersinia*, *Pseudomonas*, *Aeromonas*, *Escherichia coli*, *Staphylococcus aureus*) in Urfa cheese (a traditional white-brined Turkish cheese). *Pak J Nutr* 7: 630-635.
- Chipilev N, Daskalov H, Stoyanchev T (2016) Study on the prevalence of lipolytic yeasts and moulds in raw cow milk and white brined cheese. *Bulg J Vet Med* 19: 117-126.
- Fadda ME, Cosentino S, Deplano M, Palmas F (2001) Yeast populations in Sardinian feta cheese. *Int J Food Microbiol* 69: 153-156.
- Geronikou A, Larsen N, Lillevang SK, Jespersen L (2022) Occurrence and identification of yeasts in production of white-brined cheese. *Microorganisms* 10: 1079.
- Dagdemir E, Ozdemir S (2008) Technological characterization of the natural lactic acid bacteria of artisanal Turkish white pickled cheese. *Int J Dairy Technol* 61: 133-140.
- Donnelly CW (2007) What factors should be considered to reduce coliform counts? In McSweeney PLH, editor. *Cheese problems solved*. Woodhead Publishing, UK. 143.
- Sarantinopoulos P, Kalantzopoulos G, Tsakalidou E (2002) Effect of *Enterococcus faecium* on microbiological, physicochemical and sensory characteristics of Greek feta cheese. *Int J Food Microbiol* 76: 93-105.
- Walstra P, Noomen A, Geurts TJ (1993) Dutch-type varieties. In Fox PF, editor. *Cheese: chemistry, physics and microbiology, major cheese groups*. 2nd edn. Chapman & Hall, London, UK. 74– 76.
- Gripon JC (1993) Mould-ripened cheeses. In Fox PF, editor. *Cheese: chemistry, physics and microbiology*. Springer, Boston, MA. 111-136.
- Litopoulou-Tzanetaki E, Tzanetakis N (2011) Microbiological characteristics of Greek traditional cheeses. *Small Rumin Res* 101: 17-32.
- Erkmen O (1995). Behavior of *Staphylococcus aureus* in Turkish feta cheese during manufacture and ripening. *J Food Prot* 58: 1201-1205.

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