

Characterization of Preveli cheese, traditional Croatian dried acid-coagulated cheese

Karakterizacija prevelog sira, tradicionalnog hrvatskog sušenog kiselinskog sira

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Abstract

The aim of this paper was to study physicochemical composition and microbiological characteristics of Preveli cheese, Croatian artisanal dried acid-coagulated cheese, as well as physicochemical composition and hygienic quality of cheese milk. The production of 35 batches of Preveli cheese was observed at 18 small scale dairy plants. Research showed that hygienic quality of milk was partially satisfying, which means that 51.42% and 17.14% of milk samples had total bacterial count and somatic cell counts above the threshold value ($> 300,000$ and $> 400,000 \cdot \text{mL}^{-1}$, respectively). According to the content of total solids, Preveli cheese belongs to the group of soft cheeses, while according to the content of fat in total solids it can be categorized as a fat cheese. Due to the non-standardized technological processes, mainly during skimming the milk fat, salting and drying, results of analysis showed notable variability in the physicochemical composition of the Preveli cheese and sour cream. The presence of yeast and moulds as well as *Staphylococcus aureus* were detected in 22.86% and 11.43% of cheese samples, respectively, which indicated the need of improving the hygiene of the manufacturing processes.

Keywords: dried acid-coagulated cheeses, microbiological quality, physicochemical composition, Preveli cheese

Sažetak

Cilj ovog rada bio je utvrditi fizikalno-kemijski sastav i mikrobiološke karakteristike prevelog sira, hrvatskog sušenog kiselinskog sira, te fizikalno-kemijski sastav i higijensku kvalitetu mlijeka za sirenje. Za potrebe istraživanja proizvedeno je 35 uzoraka prevelog sira prikupljenih na 18 obiteljskih gospodarstava. Istraživanja su pokazala da je higijenska kvaliteta mlijeka djelomično zadovoljavajuća, odnosno da 51,42% i 17,14% uzoraka mlijeka imaju ukupan broj bakterija i broj somatskih stanica iznad dopuštenih vrijednosti ($> 300,00$ i $> 400,000 \cdot \text{mL}^{-1}$). Prema ukupnom sadržaju suhe tvari, preveli sir pripada skupini mekih sireva, a prema sadržaju masti u suhoj

tvori može se klasificirati kao masni sir. Zbog nestandardiziranih tehnoloških postupaka, uglavnom tijekom obiranja mliječne masti (vrhnja), soljenja i sušenja, rezultati analize pokazali su značajnu varijabilnost u fizikalno-kemijskom sastavu prevelog sira i vrhnja. Prisutnost kvasaca i plijesni te bakterije *Staphylococcus aureus* utvrđena je u 22,86% i 11,43% uzoraka sira, što ukazuje na potrebu poboljšanja i održavanja higijenskih uvjeta tijekom procesa proizvodnje.

Ključne riječi: fizikalno-kemijski sastav, mikrobiološka kvaliteta, preveli sir, sušeni svježi sir

Introduction

Fermented milk products such as fresh acid-coagulated cheese varieties and yogurt are popular food in the human diet due to the many health benefits and mild and pleasant flavour and odour. A beginning of production of fermented milks and cheese dates back deep into the history. Lactic acid fermentation, together with salting and drying, is considered as one of the oldest methods of preservation and extending the shelf life of food in general. Because of the high content of moisture (whey), acid-coagulated cheeses for example Quark and Cottage are quite perishable and shortened durability, especially during the summer months. Due to that fact, drying as a method of preservation of acid-coagulated cheeses was developed.

In Croatia, the main characteristic of acid-coagulated cheeses production is spontaneous clotting of fresh raw cow's milk, as opposed to the cheese production in karst areas, where cheese is produced from ewe's, goat's milk or their mixture with addition of rennet (Andrić, 2003; Kirin, 2004; Kirin, 2009). Drying of acid induced coagulum is common characteristic in production of Croatian traditional cheeses such as Prgica, Kvargl, Turoš (cone-shaped cheeses), Sušeni cheese and Preveli cheese.

Production of Preveli cheese is characteristic for Moslavina and Croatian Posavina area, mostly in the Nature park of Lonjsko polje (Moslavac, 2003). Preveli cheese is cake-shaped cheese, with a diameter of around 15 cm and to 5 cm height (Figure 1). Drying of cheeses is occurred in sirnica, cone-shaped net made of thread or string with diameter of 40 cm (Figure 2), and it can be made on one or two levels. During the summer months, cheese in sirnica is dried in the wind, while during winter time sirnica is placed above the stove. Besides salt, in Preveli cheese can be added spices like pepper, sweet or chilli pepper or garlic, and after drying cheese can be smoked. The best colour, flavour and aroma is achieved by smoking the cheese on the wood of beech and hornbeam, while other types of wood contain too much tannin and are not suitable for smoking of the cheese (Dolenčić, 2004). It is white to light yellow colour while the color of smoked variety depends on the duration of smoking and can be light to dark yellow. At the cut surface it is visible layering texture. As it is produced from skimmed milk, a by-product of Preveli cheeses production is sour-cream.

In some countries, dried acid-coagulated cheeses are very important part of traditional cheese making, such as Gaperon (France), Bryndza and Oštepka (Slovakia), Cokerek and Keş (Turkey), as well as Genestoso (Spain). In spite of that,

there are limited researches regarding manufacturing procedures, structure and physicochemical properties of dried acid-coagulated cheeses, contrary to the fresh acid-coagulated cheeses which were the issue of several studies (Ahmed et al., 2013; Lobacz et al., 2016; Farkye, 2017). Therefore, the aim of this paper was to study physicochemical composition and microbiological characteristics of Preveli cheese, Croatian artisanal dried acid-coagulated cheese, as well as physicochemical composition and hygienic quality of cheese milk used for production of Preveli cheese.



Figure 1. Preveli cheese



Figure 2. Drying process of Preveli cheese in simnica

Materials and methods

Technology of production of Preveli cheese

The production of 35 batches of Preveli cheese was observed at 18 small scale dairy plants (family farms) in the area of Sisak city. Cheese was made from raw (mixture of evening and morning milk), filtered cow's milk, which was fermented at room temperature by the activity of natural Lactic Acid Bacteria (LAB). After acid coagulation (2 - 3 days), separated cream was removed from the surface of curd and curd was heated up to a temperature of 55 °C during period of two hours. The curd

was placed to drain in perforated moulds. After draining process cheese was removed from the mould, salted and dried 2 - 7 days in sirnica.

Sampling

The physicochemical analysis of milk and cheese were conducted at the Dairy Science Department, Reference Laboratory for Milk and Milk Products, University of Zagreb, Faculty of Agriculture, as well as microbiological analysis of milk. The content of milk fat, lactose, and non-fat total solids were measured on the instrument MilkoScan FT 120 using infra-red spectrometry (ISO 9622:2001). Proportion of casein in milk was determined by precipitation of casein at pH=4.6 by direct reference method (ISO 17997-2:2004).

The concentration of urea in milk was determined by enzymatic spectrophotometric method. Somatic cell count was measured on the instrument Fossomatic opto-electron method (ISO 13366-2:2007) and total bacteria count was measured on the instrument BactoScan FC according to manufacturer's instructions.

Total solids content of Preveli cheese were determined by drying at 102 °C (ISO 5534:2008). Protein content was determined on the instrument Kjelttec 2300 according to Kjeldahl method (ISO 8968-2:2003), fat content was determined by the method of Van Gulik (ISO 3433:2009), salt content was determined by Mohr method (AOAC, 2000) and the pH value of milk and cheese were determined by pH-meter (SevenMulti, Mettler Toledo, Switzerland).

Microbiological analyses of Preveli cheese were carried out at the Institute for Hygiene and Technology of foods at the University of Zagreb, Veterinary Faculty. Microbiological quality of cheese was determined by counting colonies on nutrient media for colonies of: *Salmonella* spp. (ISO 6785:2001) and *Listeria monocytogenes* in 25 g of cheese (ISO 11290-1:1999), *Escherichia coli* (ISO 11866-1:2001), *Staphylococcus aureus* (ISO 6888-1:2003), sulphite-reducing clostridia (ISO 15213:2003) and the yeasts and moulds in 1 g of cheese (ISO 6611:2004).

Statistical analysis

Statistical analysis was done using a SAS statistical software (v. 9.2; SAS Institute, 2011). The values of TBC and SCC were transformed into logarithmic values (\log_{10}).

Results and discussion

Physicochemical composition and hygienic quality of cheese milk

The quality of raw milk has extremely large influence on the efficacy of milk processing, as well as on physicochemical and sensory properties of produced cheese. The total bacterial count (TBC) and the somatic cell count (SCC) are commonly used parameters for the evaluation of the hygienic quality of raw milk. According to the EU Regulation (European Parliament and Council regulation 2004/853/EC) the threshold value of the TBC and SCC in raw cow's milk used for dairy products manufacturing is 300,000 and 400,000·mL⁻¹, respectively. Table 1

shows hygienic quality of milk for Preveli cheese production. The geometric mean of total bacteria count in milk was above the permitted limit of $300,000 \cdot \text{mL}^{-1}$. Moreover, results of this research showed that microbiological requirements fulfil only 49.57% of the milk samples. Poor microbiological quality of raw milk could be a result of microorganisms' contamination during milking or improper temperature during milk storage before processing. Despite that, the measured acidity of the milk showed that milk was suitable for coagulation, as indicated by parameters such as content of milk fat, protein, lactose, casein and casein number (Table 2). The casein number is a good method for detection of mastitis and is defined as the ratio of the content of casein and total nitrogen. Table 2 shows that the average value of casein number is 79.78 which is in accordance with recommended value of 80 (Boland, 2003). As well as, absence of mastitis is visible from the average number of somatic cell count (Table 1) which is below the threshold value. There were 82.86% of milk samples which satisfied the requirements for SCC. It is common knowledge that increased number of somatic cells has a negative effect on technological properties of milk: increasing the coagulation time, decreasing the curd firmness (Caballero Villalobos et al., 2015), and the cheese yield (Summer et al., 2015), as well as the sensory attributes of cheese (Vianna et al., 2008; Chen et al., 2010).

Table 1. Hygienic quality of cheese milk (n = 35)

Parameter	$\bar{x} \pm \text{s.d.}$	Incorrect
Somatic cells count·mL ⁻¹	5.18 ± 0.47	6
Total bacteria count·mL ⁻¹	5.44 ± 0.95	18

Results are expressed as a geometrical mean value \pm standard deviation (s.d.). Incorrect: $> 400\,000 \text{ SCC} \cdot \text{mL}^{-1}$ and $> 300\,000 \text{ TBC} \cdot \text{mL}^{-1}$.

Urea is the ingredient of milk that indicates the diet regime. This means that if the protein content is within normal ranges (3.2 - 3.8%), and urea concentration is between $15 - 30 \text{ mg} \cdot 100 \text{ mL}^{-1}$, it is considered that the energy and crude protein supply are at optimum level (Bendelja et al., 2011). Although, the average content of urea in milk for Preveli cheese production was within the range mentioned above (Table 2), it concerns its high variability (s.d. = ± 10.72), which indicates the need of changing the diet regime on some farms, due to the fact that 4 out of 35 milk samples had urea content above $30 \text{ mg} \cdot 100 \text{ mL}^{-1}$. High urea concentrations in cheese milk are prolonged coagulation time, formation of weaker sections of the curd, high percentage of separated whey, premature development of irregular fermentations, and a more intense proteolysis (Karapetkovska Hristova et al., 2014).

Table 2. Physicochemical composition of cheese milk (n = 35)

Parameter	$\bar{x} \pm s.d$
Milk fat (g·100 g ⁻¹)	4.05 ± 0.68
Protein (g·100 g ⁻¹)	3.29 ± 0.36
Casein (g·100 g ⁻¹)	2.62 ± 0.31
Casein number (%)	79.78 ± 2.96
Lactose (g·100 g ⁻¹)	4.54 ± 0.15
Total solids (g·100 g ⁻¹)	12.72 ± 0.84
Total solids non-fat (g·100 g ⁻¹)	8.68 ± 0.35
Urea (mg·100 mL ⁻¹)	16.39 ± 10.72
pH	6.6 ± 0.12

Results are expressed as means ± standard deviation (s.d.).

Physicochemical composition of Preveli cheese and sour cream

Table 3 shows physicochemical composition of Preveli cheese and sour cream. The variability in the physicochemical composition of the Preveli cheese and sour cream is the most visible in the content of fat and total solids due to the non-standardized technological processes, mainly during skimming the milk fat, salting and drying, which were carried out manually.

According to the content of total solids (36.54 g·100 g⁻¹ cheese) Preveli cheese can be categorized as soft cheese (Regulation, 2009). Similar type of traditional Croatian acid-coagulated dried cheese, Prgica cheese, made at family farms also belongs to the group of soft cheeses (39.34 g of total solids·100 g⁻¹ cheese). On contrary, significant difference is observed for Prgica cheese took from supermarkets. With 56.59 g of total solids·100 g⁻¹ cheese, it belongs to the group of hard cheeses. That can be described by the difference in the intensities of the drying procedure applied as well as skimming of milk during cheese production on family farms (Valkaj et al., 2014), similar to this research. As well as, authors stated that Prgica cheese purchased from the supermarket was more intensively dried for extending the shelf life and improving the quality. Genestoso cheese, a Spanish variety of acid-coagulated cheese, has very similar manufacturing procedure to Preveli cheese. Genestoso cheese ripens for 60 days, which is the main difference in comparison to the Preveli cheese production technology. However, ripening and drying process has the same role: removal of the excess moisture from the product. Arenas et al. (2004) reported that Genestoso cheese after 2 and 7 days of ripening belongs to the group of soft cheeses (30.35 and 42.6 g of total solids·100 g⁻¹ cheese, respectively) but after 15 days of ripening it can be classified as hard cheese and extra hard cheese at the end of ripening. The content of total solids in Çökelek cheese (Turkish acid-

coagulated cheese), is 31% (Durlu-Özkaya and Gün, 2014) which is in accordance with the results of this study.

Table 3. Physicochemical composition of Preveli cheese and sour cream (n = 35)

	Parameter	$\bar{x} \pm s.d.$
Preveli cheese	Milk fat (g·100 g ⁻¹)	9.79 ± 4.94
	Protein (g·100 g ⁻¹)	19.51 ± 3.13
	Moisture content (g·100 g ⁻¹)	63.46 ± 6.92
	Water in non-fat matter (g·100 g ⁻¹)	70.23 ± 5.33
	Fat in total solids (g·100 g ⁻¹)	25.89 ± 9.73
	Salt (%)	2.07 ± 1.11
	pH	4.38 ± 0.54
Sour cream	Milk fat (g·100 g ⁻¹)	31.81 ± 8.59
	Protein (g·100 g ⁻¹)	2.66 ± 0.42
	Total solids (g·100 g ⁻¹)	41.51 ± 10.32
	pH	4.46 ± 0.15

Results are expressed as a means ± standard deviation (s.d.).

Although the part of milk fat was removed by skimming, according to Regulation (2009), Preveli cheese with the 25.89 g of milk fat in total solids/100 g cheese, belongs to a group of fat cheeses. This result is in accordance with Çökelek cheese research which is with 34% of milk fat in total solids also in the category of fat cheeses (Durlu-Özkaya and Gün, 2014). Valkaj et al. (2013) found that Prgica cheese produced at family farms, due to the separation of cream, belongs to the group of semifat cheeses, while Prgica cheese purchased from supermarket and produced from pasteurized milk has significantly higher fat content and belongs to the group of fat cheese. Contrary to the results of this study, Genestoso cheese belongs to the group of full fat cheeses (46.2 g fat·100 g⁻¹ of total solids) while it is produced from raw full fat cow's milk (Arenas et al., 2004). Preveli cheese is a very good source of proteins of animal origin (19.51 g·100 g⁻¹ cheese) which are rich in essential amino acids. A dietary reference intake of proteins for adult man is 56 g and 46 g for adult woman (DRI, 2005). The quantity of 100 g of Preveli cheese meets 34.84% of protein requirements for men and 42.41% for women. This result is in accordance with result of Valkaj et al. (2014) who reported 22.09 g of proteins·100 g⁻¹

cheese in family farm made Prgica cheese. Industrially produced Prgica cheese, as it was case with the content of milk fat, has the higher content of proteins due to the different method of drying. Contrary to this research, Arenas et al. (2004) found that Genestoso cheese is poor source of proteins. After 2 and 7 days of ripening it contains 11.93 and 16.66 g of proteins·100 g⁻¹ of cheese, respectively. Preveli cheese as acid-coagulated cheese, besides low pH value (pH=4.38) has high moisture content (Table 3) and is a favourable medium for the growth and development of yeasts and moulds. The removal of excess moisture from the finished product by drying process is the specificity of Preveli cheese production. The intensity of yeasts and moulds growing depends on duration of drying process, which also determines shelf life of cheese. The growth of most common bacteria is in neutral pH and acid sensitive flora does not survive at low pH (< 5). It is known that undissociated form of the organic acids such as lactic acid, acetic acid and propionic acid are the real inhibitors. Due to the higher proportion in acid-coagulated cheeses lactic acid is the major inhibitor to the acid sensitive microorganisms (Berefsord et al., 2001; Arenas et al., 2004). Similar results for pH found Valkaj et al. (2014) for Prgica cheese (pH=4.4). In Genestoso cheese the pH underwent a rapid fall during ripening of the cheeses, reaching values of about 4 in the 2-day old cheese (Arenas et al., 2004).

Salting in the Preveli cheese production plays an important role since it is an acid-coagulated cheese which has high moisture content. Salt has a positive effect on the development of cheese flavor. As well as it has a preservative role and reduces the content of active water, and thus the possibility of growth and development of undesirable and pathogenic microorganisms in the cheese. The content of salt in Preveli cheese (Table 3) is significantly higher compared to various fresh cheeses. The salt concentration in fresh cheese, which was usually made from milk by acid and/or rennet-coagulation, ranges from approximately 0.7 to 4 g·100 g⁻¹ (Aly and Galal, 2002; Abdalla and El-Zubeir, 2006; El-Bakry, 2012). In addition to salt, in acid-coagulated cheeses can be added spices like garlic, pepper and ginger which have preservative properties. In the production of the West African soft cheese ginger extract has an anti-fungal, anti-histamine and antibacterial effect, while garlic extract can inhibit the growth of some bacterial strains like *E. coli*, *S. aureus* and *Salmonella* spp. (Schulick, 1993; Mercola, 2003; Belewu et al., 2005).

Microbiological characteristics of Preveli cheese

Predominant microorganisms in Preveli cheese which determine its microbiological quality were yeasts and moulds (Table 4). The increased number of moulds and yeasts, especially during drying process and storage could be explained by the possibility of yeasts and moulds to metabolize lactic acid at lower pH (Turkoglu et al., 2003; Amran and Abbas, 2011). *Staphylococcus aureus*, an ubiquitous bacteria which has the ability to produce enterotoxins and is a major cause of food poisoning (Argudín et al., 2010) was found in four samples of Preveli cheese (Table 4). Presence of high concentration of lactic acid and consequently lower acidity of cheese (pH<5), inhibit *S. aureus* growth in acid-coagulated cheeses (Samaržija et al., 2007). However, this is not the case in the production of Preveli cheese where *S. aureus* were present in four cheese samples, so it can be concluded that *S. aureus*

probably originates from milk of animals suffering from mastitis (Table 4). *E. coli*, enteric pathogen like *Salmonella* that are present in the intestinal tract of ruminants, including domestic animals used in milk production, most frequently cows, sheep and goats (Baylis, 2009), was present in one cheese sample. In general, the effective cleaning procedures, including removing faecal material from udders prior to milking and good manufacturing practices during cheese making process can reduce the risk (Pacheco and Galindo, 2010) and to ensure the production of hygienically correct and safe products for the market.

Table 4. Microbiological characteristics of Preveli cheese (n = 35)

Microorganism	Incorrect	Correct
<i>Escherichia coli</i> (cfu·g ⁻¹)	1	34
<i>Staphylococcus aureus</i> (cfu·g ⁻¹)	4	31
<i>Salmonella</i> sp. (cfu·25 g ⁻¹)	0	35
Sulphite Reducing Clostridia (cfu·g ⁻¹)	0	35
Yeasts and moulds (cfu·g ⁻¹)	8	27
<i>Listeria monocytogenes</i> (cfu·g ⁻¹)	0	35

Conclusion

Acid-coagulated cheeses are very frequent type of cheese which is produce in small scale dairy plants in Croatia due to its simplicity of production and very good value for money (good cheese yield and production of valuable by-product – sour cream). It is important to take into account the need of hygienic milk production, milk processing and storage conditions, while this kind of cheeses do not undergo ripening process and are consumed fresh, soon after production. Hygienic quality of milk is the key quality issue in Preveli cheese production as physicochemical composition of cheese (high moisture and low pH value), is favorable condition for growth and development of yeast and moulds and *Staphylococcus aureus*.

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