Chemical and Microbiological Characterization of Turoš Cheese

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Summary

Turoš cheese belongs to the group of fresh, acidic, dried cheeses, flavoured with dried red pepper and cone shaped, produced in Međumurje region of Croatia and Hungary by Croats from Pomurje. The goal of this paper was to investigate the production procedure, physico-chemical properties and microbiological qulity of Turoš cheese, whose production takes place on the family farms and in small dairy plants. Due to skimming the sour cream during the production at family farms a significant part of milk fat was removed that resulted in a lower content of milk fat in traditional Turoš cheese ($P<0.01$) in comparison to the Turoš cheese produced at small scale dairy plant. A significantly higher salt content ($P<0.001$) and pH value ($P<0.05$) was found in Turoš cheese produced at family farms. The investigations have shown a significant difference in height ($P<0.0001$) and in weight ($P<0.05$) in favor of the cheeses produced in small scale dairy plants. Microbiological analysis showed that all the analyzed cheeses met the requirement of hygienic conditions. Further analyses have shown the presence of yeasts and molds in the cheeses.

Key words

Turoš, cream, fresh cheese, yeasts and mold

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Introduction

Turoš cheese belongs to the group of fresh, acidic, dried and cone shaped cheeses, flavored with dried red pepper and salt, which is traditionally produced on family farms in northwest part of Croatia. Turoš cheese is produced in Međimurje region of Croatia nearby Hungarian border and according to Kerecsényi (1982) in Hungary by Croats from Pomurje. Similar cheeses to Turoš cheese from Croatia are Prgica from Podravina region (Valkaj et al., 2013a), Suhi sir (Dry cheese) from Moslavina and Posavina regions (Andrić et al., 2003), Kvargl from Bjelovar region (Kirin, 2004). Moreover, Slovenian Suhi sirek (Dried small cheese) from Prlekije and Slovenian Gorice regions also belongs to this group of cheeses (Čuček et al., 2007). In spite of similarities of Turoš cheese to the above mentioned cheeses, consumers from the Međimurije region recognise and distinguish the autochthonous cheese called Turoš from similar cheeses like Prgica and Kvargl originating from the neighbouring regions of Međimurje (Valkaj et al., 2013b). In the context of globalised markets, consumers prefer, recognise, and distinguish traditional products, especially those with a known origin (Mesić et al., 2010). Pillonel et al. (2005) stated that the food authenticity and traceability of its origin has become a subject of a great interest during the last decade. A growing tendency towards the quality rather than the quantity of food products has created a growing market for products with a strong designation from this geographic region.

Turoš cheese is produced from raw cow's milk without added starter cultures. Clay or glass jar of volume of 2.5 to 5 L filed with milk is left on warm place for acidification. When the milk becomes sour, the cream that is separated at surface is skimmed. Skimmed sour milk was poured in the pot that was heated (42°C) up to three hours (without stirring) until the cheese curd appears at the surface. Cheese curd is poured in cheese cloth and it is left to drain for one day. Obtained fresh, drained cheese is flavored with salt and dried red pepper, mixed and shaped into cones, which are sun dried or dried above the oven for about seven days. According to Tišlarić (1992) to the 1000 g of fresh cheese is added up to 20 g of salt and 10 g of dried red pepper.

Many cheeses, including Turoš cheese, are produced on family farms as well as in small scale dairy plants. The manufacturing procedure of these cheeses could vary in different conditions (Valkaj et al., 2014), which could influence their chemical and microbiological characteristics. Moreover, in literature just two authors (Kirin, 2004; Andrić et al., 2003) mentioned Turoš cheese, but there are not any investigation focused on Turoš cheese and its characteristics depending on the condition where it is produced (family farms versus small scale dairy plants). Therefore, the goal of this paper was to survey the chemical and the microbiological characteristics of Turoš cheese, whose production takes place on the family farms and in small dairy plants.

Materials and methods

Traditional production of Turoš cheese on family farms

Traditional Turoš cheese is made from fresh cow's milk with addition of salt and, dried sweet red pepper in the cheese curd. Spicy red pepper is often added (in 9 out of 15 cases). From that curd small cone shape cheeses are hand shaped and dried on the air.

During the monitoring of cheese making the following parameters were measured: acidity (pH) and weight of fresh cheese that is obtained from 10 L of milk, total and individual weight of fresh and dried Turoš cheeses, number of produced cones, then the content of salt, sweet and hot peppers that were used for cheesemaking, height and width of fresh and dried cheeses and humidity and temperature during drying cheeses. All parameters that were measured are presented in Table 1. The average weight of fresh cheese obtained from 10 L of raw milk was 1,845 g and cream weight was 812 g (Table 3). In the production of Turoš cheese cream is not used for cheese making.

In average 40 g of salt, 20 g of sweet red pepper and 2 g of spicy red pepper (9 out of 15 cases) were mixed with fresh cheese (1,845 g). Cones with an average weight of 165 g were formed from that mixture (in average 12 cones per batch). The average height of fresh cone cheese was 8.6 cm and the diameter was

Table 1. Parameters related to the production of Turoš cheese (n = 15)

Value	Mean	Min	Max	Standard deviation	Variation coefficient
pH of fresh cheese	4.27	4.05	4.50	0.12	2.73
Weight of fresh cheese produced from 10 L of milk (g)	1845	1574	2520	240.05	13.01
Weight of fresh *Turoš* (g)	1838	1548	2560	262.05	14.26
Weight of fresh cones (g)	165	115	198	28.65	17.38
Hight of fresh cone (cm)	8.4	6.5	10.5	1.12	13.23
Width of fresh cone (cm)	6.8	5.0	8.0	0.73	10.77
Weight of dried *Turoš* (g)	915	556	1221	203.29	22.21
Weight of dried cone (g)	81	46	106	18.00	22.17
Hight of dried cone (cm)	6.5	5.0	8.0	0.78	11.99
Width of dried cone (cm)	5.6	4.0	6.5	0.73	13.09
Total number of cones*	12	8	18	3.17	26.59
Salt used per batch (g)**	40	16	85	16.85	41.86
Sweet peppers used per batch (g)**	20	8	49	9.60	47.53
Hot peppers used per batch (g) (n=9)**	2	1	4	1.00	42.11
Relative air humidity during drying of Turoš cheese (%)	55	38	75	8.86	16.09
Drying temperature (°C)	19.4	11.2	25.0	3.53	18.25

* Produced from 10 L of milk; ** Batch = 10 L processed milk

6.8 cm. Fresh cheeses are dried for a week. Dried Turoš cheese was 6.5 cm high with a diameter of 5.6 cm and a weight of 81 g.

Sampling of cream and Turoš cheese

Cream samples (obtained during the production of Turoš cheese) and Turoš cheese samples were collected from 15 family farms that were located in Međimurje region. Experimental Turoš cheese was produced from 10 L of raw milk. Weights of cream and Turoš cheese obtained during cheesemaking were determined. After manufacturing three randomly selected Turoš cheese and cream samples were frozen at -18°C for further analysis. For chemical and microbiological analysis samples were taken from the same batch of Turoš cheese for each farm. Frozen samples of cheese and cream were analyzed in the Reference laboratory of Dairy Science Department at Faculty of Agriculture, University of Zagreb. For microbiological analysis, samples were analyzed in the Department of Hygiene and Technology of Animal Products at the Veterinary Faculty, University of Zagreb.

For investigation of Turoš cheese produced in small scale dairy plants, 15 cones from stores were purchased. Three cones were taken from the same batch (the same date of production). Five batches were purchased for analysis. Samples were originally vacuum packed with a valid duration.

Cream analysis

The fat content was measured according to the butyrometric method (Köhler) – (Sabadoš, 1996). The content of proteins was measured according to the Kjeldahl method (ISO 8968-2:2003). The dry matter content was measured by drying at 102±2°C (ISO 6731:1999). The pH value was measured with a pH meter (Mettler Toledo Seven Multi, according to manufacturer's instructions). Titratable acidity, Soxhlet-Henkel degrees (°SH), was measured by using Soxhlet-Henkel (Sabadoš, 1996) method. Weight of the cream was determined on a digital balance FA-6406 (with the accuracy of 1 g).

Chemical analysis of Turoš cheese

The fat content was measured according to the Van Gulik method (HRN EN ISO 3433:1999). The protein content was measured by using Kjeldahl method (HRN ISO 8968-2:2003). The dry matter content was measured by drying at 102 ± 2 ° C (HRN EN ISO 5534:2008). The salt content was measured by Mohr method (AOAC 935.43:2000). The pH value was measured with a pH meter (Mettler Toledo Seven Multi). Weight of cheese was determined on a family farm by digital balance FA-6406 (with the accuracy of 1 g).

Microbiological analyses of Turoš cheese

Cheeses were analyzed for presence and number of *Salmonella spp* (Method: EN ISO 6785:2001), *E. coli* (Method: ISO 11866-1:2005), *S. aureus* (Method: ISO 6888-1:2004) *sulphur-reducing Clostridium* (Method: ISO 15213:2004), *L. monocytogenes* (ISO 11290-1:1999), yeasts and molds (ISO 6611:2001).

Statistical analysis

The statistical analysis was performed using the statistical software SAS Version 9.2 (Institute Inc., Cary, USA) and the GLM procedure.

Results and discussion

Cream composition

Sour cream was produced from milk that has been used in the production of Turoš cheese. Homemade cream was produced by spontaneous acidification of full fat raw milk by spontaneous separation of fat to the surface in the form of sour cream. Sour cream was removed from the surface as a separate product (Kirin, 2009). From 10 L of raw milk in average 812 g of sour cream was produced. Evidently, higher fat content of cream was obtained in our study comparing it with the studies of previous authors (Kirin, 2009; Lukač & Samaržija, 1990; Lukač-Skelin & Sabadoš, 1978) (Table 2). Studies had shown a wide variability of composition of homemade cream. Amount of sour cream and its composition were associated with the chemical composition of fresh cheese used for Turoš cheese production. Sour cream obtained during the production of fresh cheese is not used in the production of Turoš cheese, but it was a separate product.

Physical and chemical composition of Turoš cheese

According to the Regulations of the cheeses and cheese products (Official Gazette, 20/2009), Turoš cheese produced on family farms and in small scale dairy plants belong to semi-hard cheeses whose moisture in total nonfat solids was between 54% and 69%. With regard to the fat content in dry matter of cheese, this cheese can be classified as fat cheese, whose content of fat in dry matter of cheese was between ≥25% and <45% (Table 4).

During the skimming of sour cream in traditional production of Turoš cheese on family farms a significant portion of milk fat was removed (Table 3), which resulted in significantly lower content of fat ($P<0.01$) and lower content of fat in dry

Table 2. The content of fat and pH value of on-farm produced sour cream obtained during Turoš cheese production compared with similar cheeses production

N	Fat	°SH	pH	Authors
15	32.18	28.4	4.62	Our investigation
12	26.50	33.9	4.32	Kirin (2009)
12	25.90	29.9	-	Lukač and Samaržija (1990)
157	23.02	-	-	Lukač-Skelin and Sabadoš (1978)

Table 3. Quantity and composition of sour cream obtained during Turoš cheese production

Value	Mean	Min.	Max.	Standard deviation	Variation coefficient
Weight (g) (from 10 L of milk)	812.00	318.00	1281.00	251.55	30.97
pH	4.62	4.12	5.31	0.40	9.18
°SH	28.40	12.8	45.30	6.97	15.38
Fat (g/100g)	32.18	23.00	44.00	6.38	23.64
Proteins (g/100g)	2.83	2.08	4.42	0.59	19.32
Dry matter (g/100g)	40.58	30.98	54.03	7.83	22.95

Table 4. The characteristics, physical properties and chemical composition of Turoš cheese produced on family farms (n = 15) and in small scale dairy plant (n = 5)

Parameter	Family farm cheese (mean ± standard deviation)	Cheese produced in small scale dairy plant (mean ± standard deviation)	Level of significance
Dry matter (g/100g)	57.26±9.52	64.12±2.10	NS (P=0.133)
Proteins (g/100g)	28.23±5.16	31.10±1.23	NS (P=0.239)
Fat (g/100g)	15.60±4.99	24.50±2.09	P<0.01
Salt (g/100g)	3.98±0.86	2.09±0.43	P<0.001
pH	4.46±0.13	4.32±0.02	P<0.05
Moisture in total solids-nonfet (%)	58.34±7.12	60.38±2.37	NS (P=0.543)
Fat in dry matter (%)	26.98±6.13	38.21±3.12	P<0.001
Weight (g)	81.21±18.00	98.80±4.82	P<0.05
Height (cm)	6.50±0.78	9.00±0.00	P<0.0001
Width (cm)	5.57±0.73	6.00±0.00	NS (P=0.208)

matter (P<0.001) of Turoš cheese produced on family farms in comparison to Turoš cheese produced in small scale dairy plant (Table 4). Significantly higher salt content (P<0.001) and slightly higher pH value (P<0.05) in Turoš cheese produced on family farms compared to the Turoš cheese produced in small scale dairy plant were determined (Table 4). Traditionally, Turoš cheese is consumed with local wine, therefore a more salty cheese is preferred by consumers. But, Turoš cheese produced in a small scale dairy plant contained an average salt content compared to similar cheeses that appear on the market that fulfill the average consumers preference (Havranek et al., 2014). But, this small scale produced Turoš cheese did not strictly follow the traditional way of manufacturing. On the other hand, salt plays a preservative role in cheese and inhibits the growth of some undesirable microorganisms (Choisy et. al., 2000). This is important if cheese is produced in poor hygienic conditions that are common on family farms. We presume that the differences in the pH value between the two groups of cheese could be due to the fact that Turoš cheese is produced from fresh cheese before shelf life expending that was not the case for the Turoš cheese produced on family farms (cheeses produced for experimental purposes). A difference in height (P<0.0001) and in weight (P<0.05) was determined in favor of cheese produced in small scale dairy plant. Therefore, adjusting the shape of Turoš cheese in the small scale dairy plant to a traditional manufacturing procedure is recommended.

Microbiological quality of cheese

According to the Guide for the Microbiological Criteria for Food (2010) for semi-hard cheeses the following bacteriological parameters were analyzed: *Salmonella, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes and sulphur-reducing Clostridium*. All 15 Turoš cheese produced on family farms and five Turoš cheese produced on small scale dairy plant satisfied the required criteria. Survey of cheeses on yeasts and molds showed their presence in all of the analyzed cheese (Table 5). All samples contained yeasts and molds with an average value of 5.46 $\log^{10}$ cfu/g. Our results are similar to those obtained by others. For instance, in Croatian Kvargl cheese that belongs to the same group the number of yeasts and molds ranged from 4 to 6.2 $\log^{10}$ cfu/g (Kirin, 2004). For Prgica cheese produced on family farm the rang of yeasts and moulds vary between 2.7 to 4.45 $\log^{10}$ cfu/g, while Prgica cheese produced from pasteurized milk contained between 2.3 and 4.48 $\log^{10}$ cfu/g (Valkaj et al., 2013a). The average number of yeasts and molds in the Turkish Kufflu cheese was 6.36 $\log^{10}$ cfu/g (Hayaloglu & Kirbag, 2006). The average value of yeasts and molds in Beyaz cheese was 4.43 $\log^{10}$ cfu/g while the Civil cheese contained number of yeast and mould similar to Beyaz cheese (4.54 $\log^{10}$ cfu/g) (Ozdemir et al., 2010). The Ethiopian Ayib cheese had 7 $\log^{10}$ cfu/g yeasts in 70% of samples, while the remaining 30% consisted of 8 $\log^{10}$ cfu/g or more. Number of molds in the most of the samples of Ayib cheese was 5 $\log^{10}$ cfu/g (Ashenafi, 1989). According to the Slovenian study (Godič et al., 2007) conduced on semi-hard cheese, the average number of yeasts and molds was 2.8 $\log^{10}$ cfu/g. Seen from this, it can be concluded that a large number of yeasts can often be found in cheese produced with the lack of hygiene, but it is also known that a high number of yeasts plays a significant role in contributing to the organoleptic characteristics, especially for native cheeses. Yeasts have a major role in ripening of some cheeses and affect to the final product (Viljoen, 2001). However, they can also be the cause of deterioration, excessive gas production, the appearance of atypical flavors, mucus production or discoloration (Fleet, 1990). Together with molds, they are responsible for creating flavors by proteolysis and lipolysis. It is proved that yeasts are not from excessive importance in the beginning of cheese production, but later they take on an important role, where they are present as natural contaminants during ripening (Welthagen & Viljoen, 1998; 1999). They have the ability to grow under conditions unfavorable to many bacteria in cheese and therefore play an important role in the ripening of some cheeses (Wyder & Puhan, 1999; Viljoen, 2001; Wyder, 2001). Some studies have shown the benefits of yeast as a supplement along with starters such as enhanced flavor and less time spent on cheese ripening. For example, in a study conducted by Ferreira and Viljoen (2003), where there were added *D. hansenii* and *Y. lipolytica*, these yeasts multiplied and competed with other forms of natural population of yeasts in the cheese and with starter bacteria without inhibiting the starter culture. These species have accelerated the development of pronounced flavor of Cheddar cheese, although bitter and fruity flavors appeared when yeasts were inoculated individually. However, when both species were incorporated as part of the starter culture, cheese had good and pronounced flavor after four months of ripening.

Table 5. Microbiological quality of Turoš cheese (n = 20)

Microorganisms	*Salmonella* spp.	*Escherichia coli*	*Staphylococcus aureus*	*Listeria monocytogenes*	*Sulphur-reducing clostridium*	Yeasts and molds	
Method	HRN ISO 6785:2001	HRN ISO 11866-1:2001	HRN EN ISO 6888:2004	HRN EN ISO 11290-2:1999	HRN ISO 15213:2004	HRN ISO 13681:2001	
Criteria*	0 CFU/25g	$<10^3$ CFU/g	$<10^3$ CFU/g	0 CFU/25g	$<10^2$ CFU/g	(CFU/g)	(log^{10} CFU/g)
Family farm 1	0	<10	<10	0	<10	3.8×10^5	5.60
Family farm 2	0	<10	<10	0	<10	1.8×10^6	6.25
Family farm 3	0	<10	<10	0	<10	9×10^5	5.95
Family farm 4	0	<10	<10	0	<10	9×10^2	2.95
Family farm 5	0	<10	<10	0	<10	5×10^4	4.70
Family farm 6	0	<10	<10	0	<10	3×10^6	6.50
Family farm 7	0	<10	<10	0	<10	9.6×10^4	5.00
Family farm 8	0	<10	<10	0	<10	2.2×10^5	5.35
Family farm 9	0	<10	<10	0	<10	4×10^5	5.60
Family farm 10	0	<10	<10	0	<10	3.8×10^5	5.60
Family farm 11	0	<10	<10	0	<10	5.8×10^4	4.75
Family farm 12	0	<10	<10	0	<10	4×10^4	4.60
Family farm 13	0	<10	<10	0	<10	2×10^6	6.30
Family farm 14	0	<10	<10	0	<10	8×10^5	5.90
Family farm 15	0	<10	<10	0	<10	2.4×10^6	6.40
T[5] 1.	0	<10	<10	0	<10	3×10^6	6.50
T[5] 2.	0	<10	<10	0	<10	2.4×10^5	5.40
T[5] 3.	0	<10	<10	0	<10	1.7×10^5	5.25
T[5] 4.	0	<10	<10	0	<10	2×10^5	5.30
T[5] 5.	0	<10	<10	0	<10	2.3×10^5	5.35

[5] Turoš cheese produced in small scale dairy plants; * Guide for the Microbiological Criteria for Food (2010).

It had a clean, slightly sweet, pleasant taste that was kept after nine months, compared to the control cheese (used only starter cultures) developed a bitter and slightly unclean taste.

Yeasts have the ability to grow at low temperatures, ferment lactose, assimilate organic acids produced by the lactic acid bacteria such as succinic, lactic and citric acids; they have proteolytic and lipolytic activities, they are resistant to high salt concentrations and to cleaning compounds and sanitizers. They can inhibit undesired microorganisms and create different compounds such as B-vitamins, pantothenic acid, niacin, riboflavin and biotin. They have the ability to tolerate low pH and low water activity values (Wyder & Puhan, 1999; Viljoen, 2001; Ferreira & Viljoen, 2003). The average pH value of Turoš cheese produced on family farms was 4.46 and 4.32 of Turoš cheese produced in small scale dairy plants (Table 3). Acid environment in the cheese favors growth of yeasts and molds (Fleet, 1990; Jordano et al., 1991; Robinson et al., 2002) especially during drying period. As presence of yeasts and moulds in all analyzed Turoš cheese was evident, it could be concluded that they are natural microflora due to difficulty to avoid their presence in the cheese because of its specific way of production (drying). It would be useful to find out if dominant species of yeast and moulds are harmful for consumers' health, if not, they could be considered as native microflora which contributes to the desirable and "traditional" sensory properties of Turoš cheese.

Conclusions

Turoš cheese belongs to the group of fresh, acidic, dried, semi-hard, fat cheese, flavored with salt, dried sweet red pepper and often with addition of spicy, red pepper and is produced in Međimurje region and by river Mura in Hungary. Taking into consideration that Turoš cheese produced in small scale dairy plants differed from the ones produced on family farms in the content of fat, salt, pH, weight and height it was obvious that the manufacturing procedure conducted in the small scale dairy plants did not strictly follow the traditional way of Turoš cheese production recorded by this research. Therefore, some adjustments of the manufacturing procedures in small scale dairy plants are recommended.

The traditional way of the production of Turoš cheese provides a safe product, free from any unhealthy and harmful microorganisms, regardless of the production conditions (farms or small scale dairy plants). As yeasts and molds were regularly found in all analysed Turoš cheese it could be considered as a native microflora which contributes to the desirable and "traditional" sensory properties of Turoš cheese.

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