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Textural and physico-chemical characteristics of white brined goat cheeses made from frozen milk and curd. The use of square I - distance statistics

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Abstract

Objective of this study was to investigate the effect of short term frozen storage of milk and curd on textural properties and physico-chemical composition of white brined goat cheese. Raw milk and curds (at various stages of pressing) were frozen and kept for seven days at -27 °C. Following the freezing, all *the experimental cheeses* were manufactured by the standard procedure, the same that was used for *the control cheese* sample which did not undergo freezing at any stage of production. *The Square I - distance* was used in order to rank the cheeses according to their similarity to *the control cheese* in terms of texture attributes and physico-chemical characteristics. The results show that, in terms of all examined variables, the cheese made from frozen milk is the most similar to *the control cheese*.

Key words: texture, white brined cheese, caprine milk, frozen milk, frozen curd

Introduction

The goat dairy industry is in competition with cow, sheep and even buffalo milk products (Dubeuf et al., 2004). The importance of goats as providers of essential meat and dairy products around the world has been discussed in many publications (Boyazoglu and Morand-Fehr, 2001; Haenlein, 2004; Haenlein, 2001; Boyazoglu et al., 2005). Their importance is also demonstrated by the significant increase in the number of goats during the last few decades (Haenlein, 2004; FAOSTAT, 2013). Since ancient times, cheese production has been considered an efficient way of the exploitation of milk from indigenous small ruminant breeds, (Moatsou and Govaris, 2011), but the availability of goat cheeses has been limited due to the seasonal nature of the supply of goat milk.

In general, freezing is the most common method used to extend the shelf life and availability of highly perishable foods, but it has mixed effects on cheeses. Freezing has a detrimental effect on the texture and the flavour of most semi-hard and hard bovine milk cheeses (Luck, 1977), although some exceptions have been reported. For example, semi-hard Mozzarella cheese can be frozen without a significant effect on texture (Cervantes et al., 1983).

Reference data on freezing and on frozen storage of goat milk, curd and cheese is limited. Van Hekken et al. (2005) studied the frozen storage of soft goat cheeses and found that there were only slight changes in texture during the storage. Additionally, according to Park and Drake (2005), the sensory properties of soft goat cheeses after three months of frozen storage were acceptable. Also,

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Monterey Jack goat cheese has acceptable sensory attributes even after five years of frozen storage (Park, 2013).

Significant amounts of frozen goat lactic acid curd are exported from different parts of the world to mainly France and USA (Dubeuf et al., 2004). It is therefore important to investigate the effect of frozen storage of goat curd on different characteristics of cheese. Some research in that area has already been done (Seçkin et al., 2011; Raynal-Ljutovac et al., 2011), but, to our knowledge, there is no available data from instrumental analysis concerning the textural characteristics of cheeses made from frozen goat curd. It is beyond doubt that, for consumers, texture is a significant factor in evaluation of food quality and, particularly in the case for cheese, it is among the most important attributes that determine the identity of the product (Buffa et al., 2001).

This study was set, therefore, to investigate the effect of freezing of goat milk and curd on the physico-chemical composition and the texture attributes of goat white brined cheeses.

In addition, the square I-distance was used in order to rank the experimental cheeses according to their similarity to the control cheese in terms of texture attributes and physico-chemical characteristics. Crucial argument for the usage of I-distance method is its capability to integrate large number of different measures variables into one value (Jeremic, 2012). The construction of the I-distance is iterative. The value of the discriminate effect of the variable X_1 is calculated (the most significant variable, which provides the largest amount of information on the phenomena that are to be ranked). The value of the discriminative effect of X_2 which is not covered by X_1 is added. Then the value of the discriminative effect of X_3 which is not covered by X_1 and X_2 is added. Procedure is repeated for all variables until complete discriminative effect was obtained for each entity that needs to be ranked (Ivanovic, 1977; Jeremic et al., 2011). Considering the characteristics of this statistical tool, it should be used when there is a need to compare experimental entities to some fictional or control entity examining a number of variables, which would result with a single number for each entity. In that way we could rank experimental entities according to similarities (or dissimilarities) to control or fictional entity. To our knowledge, until now, the I-distance has only been used for ranking entities in various socio-economic subjects (Jeremic et al., 2011).

Materials and methods

Cheese production

Three batches of raw milk were collected from a single flock of Saanen goats during three consecutive weeks in the month of September. The quantity of each batch was 30 L of milk. Each batch was divided into 6 lots (5 L each) and cheese-making process of control cheese and five experimental cheeses was conducted on the same day.

Production of control cheese

The raw milk was pasteurized at 65 °C/30 min, cooled to 31 °C, and commercial starter culture MFC-4 (Biochem s.r.l., Italy) and 0.02 % (w/v) $CaCl_2$ were added. Finally, 0,02 g of calf rennet powder (94 % of chymozin, 6 % pepsine) per 1 L of milk was added (Clerici-Sacco Group, Cadorago, Italy). The coagulation lasted 45 minutes at 31 °C, gel was cut in ≈5 cm cubes and left to rest for another 15 min. The cheese mass was then carefully transferred from cheese vats into the mould. The curd was drained for 20 minutes and then pressed in two stages: 1) 2 kg per 1 kg of curd; 2) 4 kg per 1 kg of curd. Each stage lasted one hour at room temperature (~25 °C). After pressing, the cheese was cut into blocks, the surface was dry-salted and it was left over night at room temperature. Brine was prepared with 6 % of NaCl, and pH was adjusted to 4.6 with 80 % lactic acid (Sigma Aldrich, Steinheim, Germany). The ripening in brine, at 12-14 °C, lasted for seven days, which is a minimum ripening time for soft cheeses according to the Serbian Regulation on the quality of dairy products and starter cultures.

Cheese produced from frozen curd

Curd has been frozen in two stages: (1) after 20 minutes of draining - drained curd, and (2) after draining and the first stage of pressing - pressed curd.

Frozen storage of curds lasted for seven days at -27 °C. At this temperature ice crystals are smaller, so the protein structure and fat globule membrane are less damaged during the forming of ice crystals (Koschak et al., 1981; Wendorff, 2001). The thawing of the drained curd and of the pressed curd was done in a refrigerator at 4°C and lasted for 36-48 hours. After thawing, one part of each curd (drained and pressed) was heated to room temperature (~25 °C) in direct contact with whey, and the other part was heated indirectly, inside plastic bags, with water as the heating medium. The heating was followed by pressing and the rest of the production was identical to the procedure used for the control cheese.

Cheese produced from frozen milk

Raw milk was frozen in thin layers (~1 cm) inside plastic bags and stored at -27 °C for seven days. After thawing in a refrigerator at 4 °C for 36-48 hours, the milk was processed in the way described for the control cheese.

In summary, five experimental cheeses were produced:

- **FM** cheese made from frozen milk;
- **DCPB** cheese made from drained frozen curd heated in plastic bag after thawing;
- **PCPB** cheese made from pressed frozen curd heated in plastic bag after thawing;
- **DCW** cheese made from drained frozen curd heated in whey after thawing;
- **PCW** cheese made from pressed frozen curd heated in whey after thawing.

The control cheese was coded **CC**.

Composition of raw milk and physico-chemical characteristics of cheeses

The composition of raw milk was determined by the following methods: total solids - by standard drying method at 102±2 °C (FIL-IDF, 1987); fat content - according to Gerber method (FIL-IDF, 1981); nitrogen content - by Kjeldahl method (AOAC, 1990). Protein content was calculated as nitrogen content multiplied by 6.38. The pH was measured using a digital pH-meter (Consort, Turnhout, Belgium).

The physico-chemical composition of cheeses was determined by the following methods: dry matter - by standard drying method (FIL-IDF, 1982); fat content - (FIL-IDF, 1986); nitrogen content - by Kjeldahl method (AOAC, 1990). Protein content was calculated as nitrogen content multiplied by 6.38. Based on the cheese composition, the following parameters were calculated: fat in dry matter (FDM), moisture in nonfat substance (MNFS), protein in dry matter (PDM). The pH was measured using a digital pH-meter (Consort, Turnhout, Belgium) in cheese slurry (Ardö and Polychroniadou, 1999).

Texture analysis

Texture analysis was performed by the TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., Vienna Court, Lammas Road, Godalming, Surrey GU7 1YL, UK) using a 5 kg load cell. Cheeses were tested after 7 days in brine and samples were equilibrated to room temperature (~25 °C) before testing.

The first test was done using a wire cutter tool (Gunasekaran and Mehmet Ak, 2003). Testing parameters were: 2 mm/s test speed, 15 mm penetration, trigger force 50 gf (gram-force). The dimensions of cheese samples were 15x7x2 cm. Probe penetration direction was normal to 15x7 area of the sample. The textural attribute obtained from the test was the cutting force (CF), measured in gram-force.

The second test was done using a fracture wedge tool (Vincent et al., 1991). Testing parameters were: 2 mm/s test speed, 10 mm penetration, trigger force 5 gf. The dimensions of cheese samples were 3x2x2 cm. Probe penetration direction was normal to 3x2 area of the sample. The textural attributes obtained from the test were: the fracture force - (FF), measured in gram-force, and the brittleness - (B), expressed as the distance needed for the fracture wedge tool to penetrate through cheese up to rupture (maximum penetration was up to 10 mm).

The third test was done using a spherical probe tool (diameter - 2.54 cm) (Buňka et al., 2013). The testing parameters were: 2 mm/s test speed, 5 mm penetration, trigger force 2.5 gf. The dimensions of cheese samples were 15x7x2 cm. Probe penetration direction was normal to 15x7 area of the sample. The textural attribute obtained from the test was hardness in compression (HC), measured in gram-force.

All the tests for each sample were repeated at least 5 times.

Statistical analysis

The ANOVA was carried out using the freezing procedure as the main sources of variation, including five experimental and one control cheese sample. Mathematical model was:

$$X_{ij}=\mu + \alpha_i + \varepsilon_{ij}$$

The means of different variables for the six cheese samples were compared using the Duncan's test.

Finally, the square I-distance was used to rank all the experimental cheeses in order to find out which one is the most similar to the control cheese regarding the four physico-chemical characteristics (MNFS, FDM, PDM and pH), and the four textural atributes (CF, FF, B and HC).

In this paper, distance between entities (cheeses) was calculated using the Square I-distance (Jeremic et al., 2011) where d_i is the distance between the control and the experimental cheese for variable X_i, σ_i is the standard deviation for variable X_i, and $r_{ji.12...j-1}$ is a partial coefficient of the correlation between X_i and X_j, ($j < i$).

Partial coefficients of correlatons between variables and ANOVA were calculated using the SPSS 21.0 (Chicago, Illinois, USA).

Results and discussion

Composition of raw milk and physico-chemical characteristics of cheeses

The composition of raw milk used in this experiment was as follows: fat (%) - 3.45±0.07, proteins (%) - 2.72±0.18, total solids (%) - 11.31±0.86, titrable acidity (°SH) - 6.15±0.21, pH value - 6.55±0.03. The values for fat and protein content of raw milk samples are somewhat lower than average values reported in literature, but the composition of milk can vary with diet, breed, parity, season, environmental conditions, stage of lactation (Park et al., 2007). Results are comparable to (Miloradovic et al., 2015).

Physico-chemical characteristics of cheese are presented in Table 1.

It could be seen from Table 1 that all examined cheeses could be classified as full-fat (>45 % FDM). Regarding MNFS, the two experimental cheeses, DCW and DCPB, could be classified as semi-hard, and the rest as soft cheeses (CAC, 2013). The control cheese (CC) does not differ significantly from PCW and PCPB regarding the fat and the protein in dry matter, and from PCW regarding the pH value. As for the moisture in nonfat substance (MNFS), all the experimental cheeses show a statistically significant difference to the control cheese ($P<0.05$).

MNFS is a parameter that is used to classify cheeses regarding their textural attributes, because variations in MNFS, which are basically a ratio of water to protein, can lead to differences in textural quality (Tunick et al., 1993). Regarding MNFS, all the experimental cheeses had lower values compared to CC (Table 1). It has been reported that cheese body and texture become more crumbly and mealy after freezing and thawing (Goff and Sahagian, 1996). Such effect could be explained by an increase in the unordered structure of the protein in frozen cheese - and particularly in slowly frozen samples - consistent with greater damage to the microstructure (Fontecha et al., 1993). Also, Van Hekken et al. (2005) concluded that cheeses that had been frozen and then immediately thawed showed the largest change in rheological properties, and they suggested that a minimum holding time may be required to allow the curd to adjust to the ice crystals within the cheese matrix and to possible freeze concentration effects. In the present study, we hypothesize that the increase in unordered structure of proteins and the damage to the microstructure, eventually contributed to a release of a greater amount of water

Table 1. Physico-chemical characteristics of cheeses

	pH	FDM (%)	PDM (%)	MNFS (%)
CC	4.70±0.04^a	54.25±2.87^a	30.77±1.81^a	75.17±4.17^a
FM	4.54±0.03^b	50.88±1.93^{b,c}	34.05±1.07^b	71.97±3.79^b
DCW	4.83±0.03^c	51.00±1.83^c	33.37±2.01^b	63.55±3.52^c
DCPB	4.87±0.02^c	49.44±2.04^b	35.06±1.71^c	64.07±2.91^c
PCW	4.76±0.05^a	55.22±1.56^a	31.26±1.32^a	70.00±3.13^d
PCPB	4.85±0.05^c	54.38±3.20^a	31.34±2.63^a	68.57±3.71^e

The values presented are the means of three replicates ± standard deviation
Values in columns with the same letter in superscript are in homogeneous subsets
FDM - fat in dry matter; PDM - protein in dry matter; MNFS - moisture in non fat solids

after thawing of curds and, as a consequence, lower values of MNFS were obtained. Heating the curds in whey was an attempt to rehydrate proteins after thawing. It had a significant effect only in case of the curd frozen in later stage of pressing. PCW had a significantly higher MNFS from PCPB, but still significantly lower than CC. Alichanidis et al. (1981) proposed that lower moisture content of brined cheese made from frozen curd could be partly compensated by the addition of enough brine in the can in which the cheese is packaged.

Cheese samples FM, DCW and DCPB have significantly lower FDM and significantly higher PDM than CC. A possible explanation could be that proteins, damaged during frozen storage, release more fat into whey if the curd were not pressed before freezing, because a significant whey separation occurs after thawing.

A considerable precipitation of calcium phosphate might take place during the freezing of milk (Goff and Sahagian, 1996). The formation of insoluble calcium phosphate salts shifts buffering capacity to a lower pH (Salaün et al., 2005), hence the FM cheese has the lowest pH value.

Texture analysis

Textural attributes of all cheeses are presented in Table 2.

Cheeses DCW and DCPB had the highest values of textural attributes. Considering variable B, CC sample did not differ significantly ($P<0.05$) from FM sample, while for all the other textural attributes, control sample (CC) was the single sample in the subset and differed significantly ($P<0.05$) from all experimental samples.

During the freezing process, the formation of ice crystals gives rise to a local dehydration of proteins, and, as already mentioned, inducing breaking of the protein structure. This allows contact between small fat globules and the formation of fat granules. The proteins become more compact or they interact to form disulphide bridges around new fat granules. Upon thawing, the proteins are unable to fully rebind water. This leads to a harder and more elastic cheese structure with less free oil. During the period of frozen storage, the diameter of the crystal increases due to the ice re-crystallization. It makes the relocation of water at the lipid-casein interface after thawing more difficult and diminishes its lubricant effect, all leading to a harder cheese structure (Alvarenga et al., 2011; Diefes et al., 1993). The same mechanism might also be the explanation why frozen curds lose water upon thawing and have higher values of all textural attributes, as seen in Table 2.

The highest values for B (10 mm) indicate that there was no rupture of samples DCW and DCPB during the fracture wedge test. PCW and PCPB also have high values for B (8.62 and 9.78 respectively). These results imply that the structure of protein matrix of thawed curds causes cheese to become less brittle/more elastic. Some authors examined the texture attributes of ewes' frozen curds and cheese using the sensory analysis (Tejada et al., 2000; Alichanidis et al., 1981; Sendra et al., 1999) and reported a crumbly, mealy or a softer texture of their experimental cheeses. Our results show that all the textural attributes of our experimental cheeses were significantly higher ($p<0.05$) than the control cheese, which indicates that, in comparison, their texture is harder and more elastic.

Table 2. Textural attributes of cheeses

	CF (gf)	FF (gf)	B (mm)	HC (gf)
CC	232.95 ± 6.20^{a}	96.79 ± 10.48^{a}	5.62 ± 1.57^{a}	1644.26 ± 30.21^{a}
FM	360.47 ± 24.90^{b}	202.69 ± 19.03^{b}	5.18 ± 1.09^{a}	2011.76 ± 120.72^{b}
DCW	644.07 ± 19.26^{c}	434.24 ± 26.46^{c}	10.00 ± 0.00^{b}	4028.63 ± 314.18^{c}
DCPB	695.54 ± 9.91^{c}	598.91 ± 50.36^{d}	10.00 ± 0.00^{b}	5530.89 ± 138.08^{d}
PCW	547.55 ± 50.49^{d}	295.17 ± 35.06^{e}	8.62 ± 0.70^{c}	3229.27 ± 253.88^{e}
PCPB	469.45 ± 27.31^{e}	359.83 ± 43.33^{f}	$9.78\pm0.49^{b,c}$	2803.65 ± 112.68^{f}

The values presented are the means of three replicates ± standard deviation
Values in columns with the same letter in superscript are in homogeneous subsets
CF - cutting force; FF - fracture force; B - brittleness; HC - hardness in compression; gf - gram-force

Square I - distance analysis

Ranks of experimental cheeses, along with the calculated values of Square I-distance were as follows: I - FM (D^2 = 3.03), II - PCW (D^2 = 5.52), III - PCPB (D^2 = 8,35), IV - DCW (D^2 = 17,72), V - DCPB (D^2 = 23,46)

It could be seen from the calculated distances and ranks that, regarding all examined variables, FM is the most similar to CC. The cheeses made from pressed curds (PCW and PCPB) are more similar to CC than the ones made from drained curd (DCW and DCPB). Also, curds that were heated directly in whey (DCW and PCW) are more similar to CC than their counterparts heated inside plastic bag, with water used as the heating medium (DCPB and PCPB).

Statistical tools that compare means of various variables often do not tell a lot about similarities between entities (in terms of ranking), especially when the entities are observed through a number of variables. The Square I - distance can be used if there is a need, for example, to develop a new production procedure and optimize product characteristics in terms of an overall similarity with the referent one. In our study, the Square I - distance clearly showed which experimental cheese was the most similar to the control cheese, in addition to all the data and analysis we already discussed. Although CC was not statistically different from PCW and PCPB regarding some physico-chemical parameters (PDM and FDM), and although it was different from FM regarding all physico-chemical parameters, the Square I - distance showed that, in fact, FM was the most similar to CC when all examined parameters were included.

Conclusions

In summary, freezing of milk and curd had a significant effect on all the analyzed variables of cheeses. Values of the square I - distance statistical tool gives more comprehensive view of the matter, because it shows that the FM experimental cheese is the most similar to CC, even though there is a statistically significant difference regarding various attributes that were examined.

More research is needed to investigate the possibility of long term frozen-storage of milk and curd; and also to find whether the effects of frozen storage are more pronounced after longer periods of ripening.

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Teksturalne i fizikalno-kemijske karakteristike bijelih kozjih sireva u salamuri od smrznutog mlijeka i gruša. Primjena kvadratnog I - odstupanja

Sažetak

Cilj ovog istraživanja bio je istpitivanje utjecaja kratkotrajnog skladištenja smrznutog mlijeka i gruša na teksturalne i fizikalno-kemijske karakteristike kozjeg bijelog sira u salamuri. Sirovo mlijeko i gruš (u različitim fazama prešanja) smrznuti su i skladišteni sedam dana na temperaturi od -27 °C. Po odmrzavanju, svi eksperimentalni sirevi proizvedeni su prema standardnoj proceduri, identičnoj kao i za kontrolni sir, koji nije bio podvrgnut smrzavanju ni u jednoj fazi proizvodnje. Kvadratno I - odstupanje primijenjeno je pri rangiranju eksperimentalnih sireva prema sličnosti s kontrolnim sirom u smislu teksture i fizikalno-kemijskih karakteristika. Rezultati pokazuju da je sir od smrznutog mlijeka najsličniji kontrolnom siru prema svim ispitivanim parametrima teksture i fizikalno-kemijskih karakteristika.

Ključne riječi: tekstura, bijeli sir u salamuri, kozje mlijeko, smrznuto mlijeko, zamrznut gruš

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