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ORIGINAL PAPER

Characterization of extra-hard cheese produced from donkeys' and caprine milk mixture

Ljubiša Ć. Šarić[1] · Bojana M. Šarić[1] · Anamarija I. Mandić[1] · Miroslav S. Hadnađev[1] · Jasmina M. Gubić[1] · Ivan Lj. Milovanović[1] · Jelena M. Tomić[1]

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Abstract Cheese cannot be produced solely from equine or donkeys' milk, because of the unique physico-chemical properties of these milks. The purpose of this study was to characterize a novel dairy product, cheese produced from donkeys' and caprine milk mixture (60:40% *v*/*v*), regarding its chemical, microbiological, textural and sensory properties. Fully ripened cheese was classified as a high-fat, extra-hard cheese, with high sodium (29.97 $g.kg^{-1}$), magnesium (3.07 $g.kg^{-1}$) and potassium (4.70 $g.kg^{-1}$) content. The characterization by lab-on-a-chip electrophoresis revealed lysozyme, α-lactalbumin, immunoglobulins and casein fractions. Palmitic ($C_{16:0}$) and oleic fatty acids ($C_{18:2\ n9\text{-}cis}$) with 25.11 and 24.70%, respectively, were found at the highest concentrations. The medium-chain fatty acids account 18.21% of the total fatty acid content in analysed cheese samples. *Escherichia coli*, *Salmonella* spp., Enterobacteriaceae, coagulase-positive *staphylococci*, *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium perfringens* as well as moulds were under the limit of detection in all analysed samples. After 6 months of cheese ripening (0.94 a_w, pH 4.71), total bacterial count, the counts of lactic acid bacteria and yeasts were 6.34±0.03, 4.80±0.10 and 5.81±0.11 log $CFU.g^{-1}$, respectively. The texture of mature cheese was moderately hard and crumbly. The cheese was described as very salty with strong pronounced creamy, fatty and acidic taste. The characterized donkey/caprine cheese could position this type of cheese as a high-quality functional product, thus having a potential impact on the market.

✉ Ljubiša Ć. Šarić
ljubisa.saric@fins.uns.ac.rs

[1] Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

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1 Introduction

Cheese has been produced for more than 4000 years. Throughout the world, there are more than 400 varieties of cheese produced from milk of different dairy animal species, applying various fermentations and aging conditions to meet the specific consumers' needs (O'Brien and O'Connor 2004; Kindstedt 2012). Cheese consumption varies worldwide, from 15 kg per capita per year in Western Europe and USA, to 2 kg or less in Japan, Ukraine and South Africa (Wielicka and Gorynska-Goldmann 2005). Although the majority of commercially available cheeses on the market originate from bovine milk, in some regions, there is a tradition of cheese production from other milk sources, such as buffalo, caprine and sheep milk (Raynal-Ljutovac et al. 2008; Soliman 2005). On the other hand, cheese cannot be produced solely from equine or donkeys' milk, because of their unique physico-chemical properties (Uniacke-Lowe 2011).

Donkeys' milk has been a subject of a research in a past few years, although its therapeutic properties are well known since ancient times. This milk is characterized by low casein content, a high level of essential amino acids, as well as a lipid and protein profile similar to human milk (Salimei et al. 2004). Regarding the content of nutritional compounds, donkeys' milk has a potential to become a dietetic product and a good alternative for the nourishment of infants and children that have developed an allergy to bovine milk proteins (Tidona et al. 2011). Donkeys' milk shows beneficial effects in the prevention of atherosclerosis (Tidona et al. 2011) and possesses antibacterial properties (Šarić et al. 2012).

In cheese making, caseins are transformed by starter culture into curds, forming the basis of cheese. One of the major problems in the use of donkeys' milk in cheese production is its low casein content (Salimei et al. 2004; Guo et al. 2007). Casein content in donkeys' and equine milk (47.3 and 55% *w*/*w* of total protein, respectively) is significantly lower than in bovine milk (80% *w*/*w* of total protein) (Salimei et al. 2004; Uniacke-Lowe 2011). The composition of donkeys' and equine milk is similar, but the casein content in donkeys' milk is lower compared to that in equine milk. Also, the casein micelles of donkeys' milk are smaller than equine milk micelles (Uniacke-Lowe 2011). However, Uniacke-Lowe (2011) reported that donkeys' milk formed a gel at 30 °C induced by calf chymosin, although it was very weak compared to the gel formed from bovine milk.

Limitations in the production of cheese from donkeys' milk could be overcome by mixing with caprine milk. Caprine milk was chosen owing to its favourable nutritional quality and well-documented health-promoting effects (Barlowska et al. 2011), since the ultimate goal was to make a unique, highly nutritious functional product of extra quality.

The aim of this study was to determine the chemical composition, microbiological, textural and sensory properties of cheese produced from the mixture of donkeys' and caprine milk.

2 Materials and methods

2.1 Cheese making

Donkeys' milk was obtained from an indigenous Serbian breed, Domestic Balkan Donkey, from "Zasavica" Special Nature Reserve, Serbia. Caprine milk was collected from Sanska breed. Cheese was produced according to unique manufacturers' specification at the local goat dairy. Preliminary work with different milk ratios was done in the local goat dairy. The ratio used in this experiment was chosen as the best and further used in cheese production. Only the optimized product specification was available for the authors of the manuscript, while all other details and documentation is confidential and kept in the local goat dairy documentation.

Donkeys' and caprine milk were mixed in the ratio 60:40% *v*/*v* and pasteurized at 65 °C for 30 min. After cooling to 38 °C, commercial starter cultures as 1% inoculation (Clerici-Sacco, Cadorago, Italy) and calcium chloride (food quality grade) (20 g per 100 L) were added. Natural rennet (chymosin/pepsin, 96:4% *w*/*w*) (Clerici-Sacco, Cadorago, Italy) was added after cooling the milk to 32 °C; the pH of the milk was 7.2. After milk renneting process which lasted for 45 min, the curd was cut, followed by draining off the whey. The obtained soft curds were left overnight at 14 °C for additional draining, and consequently, they were poured into the moulds. Cheese samples were dry salted with coarse-grained sea salt, which contributes to the removal of moisture from the curd. Ripening lasted for 6 months at 14 °C. In order to produce 1 kg of cheese, 25 L of donkeys' and caprine milk mixture was used.

2.2 Collection of cheese samples

Eight cheese samples from the same manufacturing batch were analysed after 6 months of ripening. The samples were immediately chilled to 4 °C and transported to the laboratory where they were kept refrigerated prior the analysis. Chemical, microbiological, textural and sensory analyses as well as fatty acid composition determination were performed on each of the eight samples in three replicates, except for the protein profile that was performed on three cheese samples and from each in three replicates.

2.3 Chemical analyses

The contents of total solids, non-casein nitrogen (NCN), total fat, ash, salt, non-protein nitrogen (NPN), casein, and lactose were determined in donkey/caprine cheese according to standard methods (AOAC 2000a, b; FIL-IDF 1981; IDF 1964, 1988, 2001; ISO 2004c; GEA Niro 1978). The total nitrogen content was determined using the Kjeldahl method and was multiplied by a factor (6.38) to determine the crude protein content (AOAC 1975). Moisture content on a fat-free basis (MFFB) and percentage of fat on the dry basis (FDB) were calculated using Codex Standard 283 (1978). Calculation of the energy value of cheese samples was made according to Boikhutso (2010). Water activity (a_w) of samples was measured according to standard method (ISO 2004a) using Testo 650 measuring instrument (Testo AG, USA) with a pressure-tight precision humidity probe. The pH value was measured using the portable pH meter Testo 205 (Testo AG, USA) equipped with a combined penetration tip with temperature probe.

2.4 Quantitative determination of mineral content

Cheese samples were prepared by using a laboratory microwave oven (ETHOS 1, Milestone, Italy). Each sample was digested in 7 mL of HNO_3 and 7 mL of H_2O_2. After digestion, the samples were transferred to 50-mL test tubes and used for determination of Na, K, Ca, Mg, Fe and Zn by AAS (SpectrAA 10, Varian, Australia). Single-element hollow cathode lamps were used for all elements. La and Cs modifiers were employed for Na, K, Ca and Mg determination, while D2 correction was used for Fe and Zn analysis.

2.5 Determination of protein profile

A modified method proposed for donkeys' milk sample preparation was used for the determination of the cheese protein profile (Tidona et al. 2011). Twenty-five milligrams of cheese sample was homogenized with 150 μL of extraction buffer (0.125 mol.L^{-1} Tris HCl, 4% *w/v* SDS, 2% *w/v* glycerol, 2% *w/v* β-mercaptoethanol, pH 6.8) vortexed and heated at 100 °C for 5 min. The precipitate was removed by centrifugation at 14,000×*g* for 10 min at room temperature. Supernatant was used for lab-on-a-chip electrophoresis (LoaC method) which was carried out on the Agilent 2100 bioanalyser (Agilent Technologies, Santa Clara, CA, USA) in combination with the Protein 80 Plus LabChip kit. Sample preparation and sample deposition on chip were performed according to the instructions of the manufacturer.

2.6 Determination of fatty acid composition

Fatty acid methyl esters were prepared from the extracted lipids by transesterification using 14% boron(III)-fluoride in methanol (Karlović Đ and Andrić 1996). The obtained samples were analysed by a GC Agilent 7890A system with flame ionization detector (FID) (Agilent Technologies, Santa Clara, California), auto injection module for liquid samples (CTC Analysis, Switzerland), equipped with fused silica capillary column (Supelco SP-2560, 100 m, 0.25 mm, 0.20 μm). Helium was used as a carrier gas (purity> 99.9997%, flow rate=1.00 mL.min^{-1}). A total of 2 μL of sample was injected in split mode (25:1), and oven temperature was as follows: 140 °C for 5 min, followed by temperature ramp of 3 °C.min^{-1} to 240 °C and held for 10 min. The fatty acid peaks were identified by comparison of retention times with retention times of standards from Supelco 37 component fatty acid methyl ester mix (Sigma-Aldrich, EU) and with data from internal data library, based on previous experiments. Results were expressed as mass of fatty acid or fatty acid group (g) in 100 g of fatty acids.

2.7 Microbiological analysis

The microbial profile of cheese was investigated by enumeration of microorganisms (ISO 2003a), yeasts and moulds (ISO 2008), coagulase-positive staphylococci (ISO 2003b), beta-glucuronidase-positive *Escherichia coli* (ISO 2001),

sulfite-reducing bacteria (ISO 2003c), Enterobacteriaceae (ISO 2004b) and *Bacillus cereus* (EN ISO 2004a). Enumeration of bacterial endospores was performed by incubation of previously heated (100 °C, 5 min) initial suspensions of cheese samples on nutrient agar (HiMedia, India) at 30 °C for 72 h. The lactic acid bacteria count was determined by incubation (30 °C, 72 h) of inoculated Man, Rogosa and Sharpe (MRS) agar (LabM, UK). *Listeria monocytogenes* and *Salmonella* spp. were determined according to international standards (EN ISO 2004b; ISO 2006). Preparation of cheese samples, initial suspension and decimal dilutions for microbiological examination was performed according to international standard (ISO 2010). The number of viable bacteria was expressed as log $CFU.g^{-1}$.

2.8 Textural measurements

Textural properties of cheese samples made from donkeys' and goat milk were investigated using TA.XTplus Texture Analyser (Stable Micro Systems, Godalming, UK) with a 5 kg load cell at 20 °C. Prior to the analysis, the cheese samples were carefully removed from their packaging. Two tests for cheese texture measurements were employed. Cheese penetration test was performed using a 5 mm spherical probe (P/5S), and the obtained parameters were hardness and stickiness. A cheese fracture test was employed using a fracture wedge set in order to assess fracture/"bite force" of hard cheeses, and resulted parameters were hardness and brittleness. Test parameters for both measurements were as follows: 1 $mm.s^{-1}$ pre-test speed, 2 $mm.s^{-1}$ test speed and 10 $mm.s^{-1}$ post-test speed at 10.0% of strain. The obtained results were expressed as the mean values±standard deviation.

2.9 Sensory analysis

Sensory evaluation was carried out in the sensory laboratory which fulfils requirements of international standard (ISO 2007). A panel of seven assessors, with an average age of 40 years, experienced in the sensory assessment of dairy products, participated in the sensory analysis. In four training sessions, panelists were trained on the different types of hard cheese and each training session lasted approximately 1.5 h. During training, panelists were introduced with terms for some of the descriptors reported in literature (Lawlor et al. 2002). Consequently, panelists were asked to make modifications to this terminology and to adapt it to sensory description of the donkey/caprine cheese which was studied. The vocabulary used is in accordance with ISO (2000). Cheese samples were tempered and tested at room temperature. Prior to assessment, samples were cut into 5 g cubes and labelled with 3-digit codes and were served to panelists in individual booths. Odour (aroma), taste and texture were scored. Concerning odour property, the following attributes were examined: animal, strength/intensity and acid odour. Texture characteristics were characterized by greasy, smooth, tender, crumbly and hardness attributes and the taste properties by animal, creamy, fatty, salty and acid attributes. Sensory attributes were scored using a scale from 1-very intense to 9-no intensity (Stone and Sidel 2004), and

quantitative descriptive analysis (QDA) was employed for assessing sensory attributes of tested cheese samples. Panelists had access to water and apple throughout the evaluation.

3 Results

Chemical composition of the cheese is summarized in Table 1. The cheese in this study was classified as a high-fat extra-hard cheese, since its fat content on the dry matter basis (FDB) and moisture content on a fat-free basis (MFFB) were 65.83 and 46.43%, respectively (15). The cheese in this study had an energy value of 1321±46 kJ. 100 g^{-1}.

The Protein 80 assay exhibited clear protein patterns in the range of molecular weight between 5 and 60 kDa (Fig. 1). By comparing protein patterns with those previously published for donkeys' milk (Guo et al. 2007), it was possible to characterize the following proteins: lysozyme (approx. Mr 15 kDa), α-lactalbumin (approx. Mr 12 kDa) and immunoglobulins (approx. Mr 38 kDa). The protein fractions which were in the range of molecular weight corresponding to the casein fractions (23 to 33 kDa) were also visible.

Table 1 Chemical composition of donkey/caprine cheese

Parameter	Mean values (SD)
pH	4.71 (0.03)
Total solids (%)	61.47 (1.17)
Protein (%)	20.72 (1.29)
Total fat (%)	23.41 (0.82)
MFFB (%)	46.43 (2.21)
FDB (%)	65.83 (3.98)
Ash (%)	2.50 (0.15)
Lactose (%)	3.17 (0.09)
Salt (%)	9.55 (0.23)
Na ($g.kg^{-1}$)	29.97 (0.17)
K ($g.kg^{-1}$)	4.70 (0.19)
Ca ($g.kg^{-1}$)	5.77 (0.02)
Mg ($g.kg^{-1}$)	3.07 (0.50)
P ($g.kg^{-1}$)	4.24 (0.05)
Fe ($mg.kg^{-1}$)	1.62 (0.04)
Zn ($mg.kg^{-1}$)	12.75 (0.18)
Total nitrogen (%)	3.25 (0.23)
NCN (%)	0.50 (0.02)
NPN (%)	0.07 (0.00)
Casein (%)	17.54 (1.31)
Whey protein (%)	2.73 (0.11)

Each value is the mean of eight cheeses from the same batch. Standard deviation values are given in parentheses
MFFB moisture content on a fat-free basis, *FDB* fat on the dry basis content, *NCN* non-casein nitrogen, *NPN* non-protein nitrogen

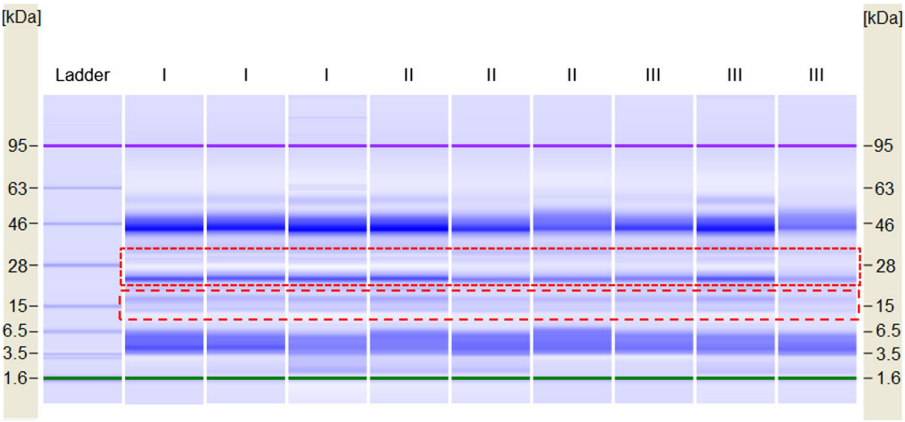

Fig. 1 Gel image of cheese protein fractions obtained by Protein 80 LabChip kit. Identified protein fractions are framed with *red lines*

Main determined fatty acids (>10%) were palmitic ($C_{16:0}$) and oleic acid ($C_{18:2\ n9\text{-}cis}$) with 25.11 and 24.70%, respectively, which accounted for almost half of the total fatty acid content in analysed cheese (Table 2). Other important fatty acids (>1%) were caproic ($C_{6:0}$), caprylic ($C_{8:0}$), capric ($C_{10:0}$), lauric ($C_{12:0}$), myristoleic ($C_{14:0}$), palmitoleic ($C_{16:1}$), stearic ($C_{18:0}$), linoleic ($C_{18:2\ n6\text{-}cis}$), α-linoleic ($C_{18:3\ n3}$) and heneicosanoic acid ($C_{21:0}$).

E. coli, *Salmonella* spp., Enterobacteriaceae, coagulase-positive *staphylococci*, *L. monocytogenes*, *B. cereus* and *C. perfringens* were not detected in any of the tested samples. Total bacterial count, counts of lactic acid bacteria and yeasts were 6.34±0.03, 4.80±0.10 and 5.81±0.11 log CFU.g^{-1}, respectively.

According to performed measurements, the hardness of the cheese samples was 19.25±2.56 N for cheese penetration test and 40.78±1.42 N for cheese fracture test. According to the cheese fracture test, the brittleness value was relatively high (9.45± 0.82 mm) implying that the measuring probe penetrated longer in the sample before cheese rupturing. The stickiness of the samples was −4.135±1.630 N. Cheese texture was moderately hard and crumbly since the samples crumbled in several parts.

Assessments of the sensory properties of the cheese in this study are shown in Fig. 2. The cheese samples had flavour characteristic of goat cheese. The odour of the cheese in this study could be described as moderately acidic. Cheese is rated as very salty with strong, pronounced creamy, fatty and acidic taste. Results of sensory analysis of tested samples were in agreement with data from textural measurements since the cheese in this study was moderately hard and crumbly.

4 Discussion

4.1 Chemical analyses

The results for total solids (61.47%) and fat (23.41%) were comparable with results reported by other authors for hard caprine cheeses (Zeng et al. 2007), which can be

Table 2 Fatty acid composition (% *m*/*m*) of donkey/caprine cheese

Fatty acid	Mean values (SD)
6:0	1.57 (0.05)
8:0	2.84 (0.05)
10:0	8.49 (0.08)
11:0	0.70 (0.01)
12:0	5.31 (0.02)
13:0	0.23 (0.00)
14:0	9.41 (0.01)
14:1	0.32 (0.00)
15:0	0.82 (0.00)
16:0	25.11 (0.04)
16:1	1.98 (0.00)
17:0	0.76 (0.01)
17:1	0.31 (0.05)
18:0	6.92 (0.05)
18:1n9c	24.70 (0.16)
18:2n9c	7.23 (0.04)
20:0	0.20 (0.01)
18:3n3	1.41 (0.00)
20:3n6	0.04 (0.01)
20:3n3	0.05 (0.00)
20:4n6	0.05 (0.00)
21:0	1.09 (0.00)
22:0	0.10 (0.01)
23:0	0.36 (0.02)
SFA	63.91 (0.14)
MUFA	27.32 (0.11)
PUFA	8.77 (0.04)
UFA	36.09 (0.05)

Each value is the mean of eight cheeses from the same batch. Standard deviation values are given in parentheses. *SD* standard deviation, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *UFA* unsaturated fatty acids (total)

explained by the similar amounts of total solids in donkeys' (9.53%) and caprine milk (10.2%) (Guo et al. 2007; Zeng et al. 2007). In contrast, the fat content (23.41%) of the cheese in this study was lower in comparison to data reported for hard caprine cheeses (26.2–28.0%) (Zeng et al. 2007). This could be attributed to low fat level in donkeys' milk (0.3–1.8%) (Guo et al. 2007), which contributed to 60% of milk used in cheese production.

4.2 Mineral composition

The quantity of milk minerals including trace elements depends on a number of factors such as stage of lactation, feeding programs, genetic characteristics and environmental conditions (Gambelli et al. 1999; Fantuz et al. 2012). Moreover, mineral content in dairy products is also influenced by applied technological

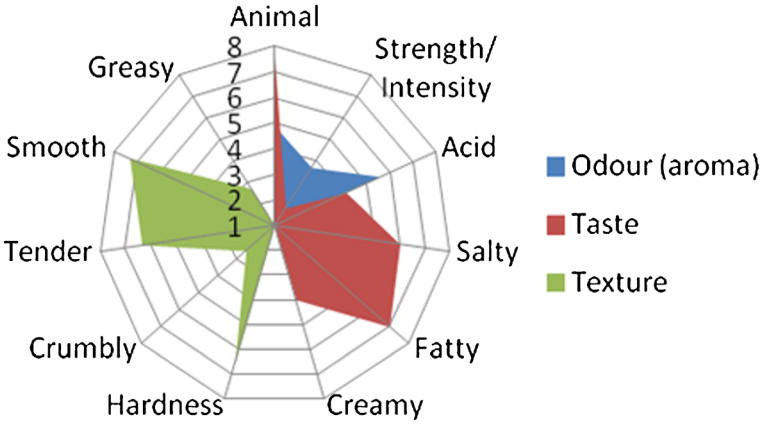

Fig. 2 Sensory properties of the donkey/caprine cheese

treatments. The high sodium values might be attributed to multiple additions of sodium chloride during the cheese production and to the long ripening time. Furthermore, the sodium content in donkeys' milk is about two times higher in comparison to sodium content found in caprine or bovine milk (Soliman 2005; Fantuz et al. 2012). The cheese in this study could be characterized as rich in magnesium and potassium, since determinated values of these minerals (Table 1) were significantly higher than maximum values reported for other types of cheese including hard cheeses made from bovine or caprine milk (Gambelli et al. 1999). Beside technological treatments, high levels of magnesium in the cheese may be a consequence of the high amount of this mineral in donkeys' milk (Fantuz et al. 2012). On the other hand, caprine milk is rich in potassium (Soliman 2005). Magnesium plays an important role in over 300 enzyme reactions in the human body, while potassium participates in maintaining of water and acid–base balance (Soliman 2005).

4.3 Protein profile

High molecular weight fractions, lactoferrin (approx. 75 kDa) and serum albumin (approx. 68 kDa) were not detected (Fig. 1). Since these proteins are present in low concentrations in the donkeys' and caprine milk, their absence in cheese is probably the result of the separation of whey during cheese production. The obtained results showed high number of low molecular mass fraction, probably due to a certain degree of casein proteolysis that occurred during maturation of cheese (Irigoyen et al. 2000). Quantitative determination of protein fractions should be performed using HPLC with mass spectrometry and urea-PAGE methods and will be included in future work.

4.4 Fatty acid profile

The total content of medium-chain fatty acids (MCFAs; $C_{6:0}$, $C_{8:0}$, $C_{10:0}$ and $C_{12:0}$) in the tested cheese samples was determined to be 18.21% of the total fatty acid

content, which is significantly higher than MCFAs reported in bovine milk (Raynal-Ljutovac et al. 2008). MCFAs in milk are considered to have beneficial health effects in human nutrition. These acids are easily absorbed when consumed, passing through the intestine cell wall without esterification and undergoing oxidation in the liver to yield rapid source of energy instead of being deposited in adipose tissues (Raynal-Ljutovac et al. 2008). Owing to this favoured pathway, they may contribute to the lowering of total circulating cholesterol and especially LDL cholesterol, thus having potential cardiovascular benefits. Capric, caprylic acids and medium-chain triglycerides (MCT) are now used in the treatment of medical conditions such as malabsorption syndrome, infant malnutrition, gallstones, etc. (Haenlein 2004); therefore, their content in milk and dairy products is considered to be of great health significance.

4.5 Microbiological profile

Since Enterobacteriaceae including *E. coli* and *Salmonella* spp. cannot grow under a_w 0.95, (Anthony and Fontana 2007), the low water activity of the cheese (0.94) was probably the main reason for absence of these bacteria. Also, the presence of lysozyme from donkeys' milk (Fig. 2) could be an additional antimicrobial factor towards *E. coli* and *Salmonella* spp. (Šarić et al. 2012; Tidona et al. 2011). A reduction in a_w during cheese ripening is the result of water loss by evaporation, salt and the hydrolysis of proteins and amino acids (Beresford et al. 2001). Due to a high NaCl content in cheese samples (Table 1), it is obvious that the salt addition represents the major factor for a_w depression. *Clostridium perfringens* as mild halophile is not capable to grow in environments containing more than 5% of NaCl, while *Bacillus* spp. can tolerate up to 8% of NaCl. The minimum water activity required for *L. monocytogenes* is 0.92 (Anthony and Fontana 2007). Although *L. monocytogenes* is halotolerant and can survive for a long period in the presence of NaCl when the concentration is higher than 10%, environmental conditions in the cheese in this study including pH value (4.71) are not optimal for *L. monocytogenes* propagation. *Staphylococcus aureus* is a member of extreme halophiles, and a_w minimum necessary for its survival is 0.86 (Anthony and Fontana 2007). In order to prevent the contamination of product with these pathogens, good manufacturing practices have to be adhered to. Detected bacteria and yeasts (moulds were under the limit of detection) mainly represented a part of the secondary flora in the cheese in this study. Microorganisms in all cheese varieties could be classified into starters and secondary flora originating from the ingredients or the environment (Beresford et al. 2001). Some part of secondary flora may originate from goat and donkey milk since pasteurization cannot completely eliminate initial microflora. Pasteurization of goat milk reduced the total bacteria count from 5.9 to 3.6 log CFU.mL^{-1} (Buffa et al. 2001). The bacterial community from tested samples probably belonged to highly salt-tolerant groups of bacteria. The other authors reported that bacterial population from Beaufort and Gruyère hard cheeses was mostly composed of highly salt-tolerant (15–20%) coryneform bacteria which managed the concentration of 10^{11} CFU.g^{-1} during ripening (Schubert et al. 1996). Number of yeasts in the same hard cheeses decreased from 10^9 CFU.g^{-1} to the 10^5 CFU.g^{-1} at the end of ripening period.

4.6 Textural properties

One of the crucial criteria for the creation of novel food products and improvements of existing products are determining and predicting the food texture. Cheese texture measurements are very important since they contribute to the classification of the varieties of cheese where categories range from soft to very hard (Noel and Lefier 1991). The initial process that takes place in the development of structure and texture of hard cheese is the formation of different bonds and cohesive forces within and between the curd grains during and after the hard cheese manufacturing process (Pesenti and Luginbühl 1999). Higher hardness values obtained by fracture testing could be attributed to the larger surface area of the measuring probe lowering onto the cheese surface. Cheese texture was moderately hard, probably due to the high salt content (9.55%) and relatively high total solids content (61.47%). Rheological properties, especially hardness values, are generally affected by cheese composition. Cheese hardness values could be also influenced by fatty acid composition of tested cheese sample as well as by the milk fat globule size (Wiking et al. 2004).

According to data shown in Table 2, the content of saturated fatty acids is approximately 64%. It is well known that fats containing higher amount of saturated fatty acids express higher hardness values in comparison to fats containing higher content of unsaturated fatty acids. Moreover, according to Küçüköner and Haque (2006), as the fat content in cheese decreases, the hardness increases. During the ripening period, the cheese in this study became more crumbly due to low moisture content. Relatively high values of brittleness could be attributed to the creaminess of tested samples. These results are in agreement with the reports of other authors (Lawrence et al. 1987) who concluded that during the ripening period, the intact casein network is weakened as the casein fractions are hydrolysed by residual rennet. During this period, the texture of cheese curd rapidly converted into a smoother product (Küçüköner and Haque 2006). Textural age-related changes of cheese properties could be also presented as a compromise between cheese hardening caused by dehydratation and softening affected by proteolysis.

4.7 Sensory attributes

Despite the fact that milk used for cheese production was 60% donkey and 40% caprine milk, the cheese in this study had flavour characteristic of goat cheese. According to literature, several aroma compounds are responsible for the flavour characteristic of goat cheese: 3-methylbutanoic acid, octanoic acid, 4-methyloctanoic acid, 4-ethyloctanoic acid and nonanoic acid (Gaborit et al. 2001). Among them, 4-methyloctanoic acid and 4-ethyloctanoic acid contributed mostly to the specific flavour, since they are perceived even at very low concentrations. Beside octanoic acid (2.89%), caprylic acid methyl ester (1.62%) and capric acid methyl ester (8.57%) were detected in tested samples (Table 2). Previous investigations designated mineral salts and lactic acid as the main taste active compounds in the caprine cheese (Engel et al. 2000). The

distinctly salty taste was the result of salting during cheese production. Regarding its high fat content (Table 1), this cheese was slightly creamy in texture. Fat is very important in cheese production especially for taste and odour creation as well as for softness and ability to melt. According to Delgado et al. (2011), the flavour of cheese depends on several reactions, especially the metabolism of lactose and lactate, lipolysis and proteolysis in the cheese. High lactose content in donkey milk (Guo et al. 2007) allows intensive metabolism of lactose and lactate.

5 Conclusions

The high-fat extra-hard cheese produced from donkeys' and caprine milk mixture had acceptable sensory and textural properties, which were influenced by its unique chemical composition including fatty acid composition, fat and moisture content. Octanoic acid, caprylic acid methyl ester and capric acid methyl ester probably played an important role in the formation of flavour characteristic of goat cheese of tested samples even though their content was lower in comparison to dominant palmitic and oleic fatty acids. The cheese in this study had a smooth and soft texture and can be described as very salty with an intensive creamy, fatty and acidic taste. The moderately hard and crumbly cheese texture can be attributed to the low moisture content. Stickiness and relatively high brittleness of the samples can be attributed to sample creaminess and proteolysis during the 6-month cheese ripening. If the cheese is produced in accordance with good manufacturing practice, it can be considered as microbiologically safe due to its low water activity and high salt concentration. The high amount of medium-chain fatty acids, as well as high levels of magnesium and potassium determined in donkey/caprine cheese, could position this type of cheese as a high-quality functional product.

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Conflict of interest Authors declare that they have no conflict of interest.

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