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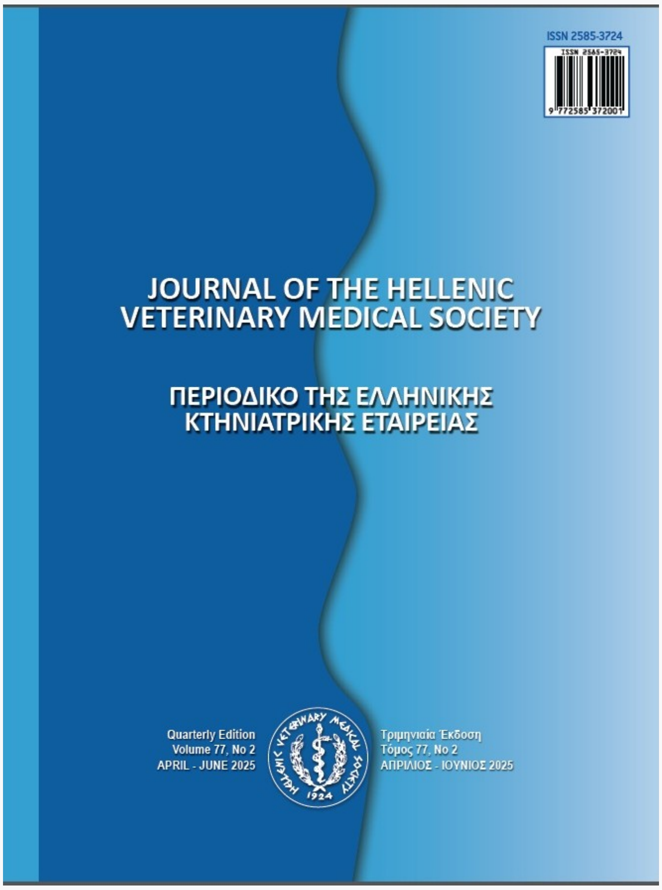

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Research article
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Characteristics of a hard cheese manufactured using high heat-treated sheep or mixed sheep-goat milk

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ABSTRACT: The cheese-making conditions of hard cheeses produced from high heat treatment of sheep (S) milk or mixture of sheep-goat (SG) milk (50:50) from a mountainous area were studied. The cheeses were assessed for their composition, biochemical, textural and organoleptic characteristics during ripening and storage. The manufacturing technology included heating milk at high temperature (90°C for 15 min), addition of starter culture at 45°C, clotting the milk with rennet, cutting and scalding-up at 47-48°C, pressing, salting in brine, ripening at 16-18°C for 30 days and storage up to 180 days. The cheese had a yellow crust, a compact texture and a pleasant flavour. The moisture of the cheeses (30-180 days), regardless of the type of milk used, ranged from 34.89% to 30.24% and the fat-in-dry matter (FDM) content was from 54.28% to 50.04%. The above results indicate that cheeses of the present study were classified as hard cheeses (referred as those with maximum moisture content 38%) of excellent quality (maximum moisture 35% and minimum FDM 47%), according to the Greek legislation. Generally, nitrogenous fractions and total free fatty acids content increased during ripening and storage, regardless of the type of milk used. In general, no differences were observed in the composition and the biochemical characteristics of S and SG (50:50). The cheeses manufactured using SG milk were harder ($P<0.05$) than S cheeses at 60 and 90 days of ripening and storage. The antioxidant activity of the cheeses ranged from 49.65 to 58.82 2,2-diphenyl-1 picrylhydrazyl % percentage of radical scavenging activity regardless of the type of milk used and the different sampling dates. High scores were obtained during the organoleptic evaluation, for both cheeses. In conclusion, using high heat treated milk and the present manufacturing technology an excellent quality hard cheese can be produced.

Keyword: hard; cheese; sheep; goat; high heat temperature.

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INTRODUCTION

Non-bovine milk cheeses are very much appreciated all over the world as they have unique flavor and nutritional value while some of them can be associated with the cultural heritage of the place where they are produced. Many non-bovine milk cheeses are manufactured using only one type of milk such as sheep or goat milk. It is known that sheep milk has high protein and fat content and is preferred for cheesemaking. Goat milk has a poorer cheesemaking ability compared to cow due to the lower casein content as well as other properties of casein micelles such as composition, hydration and size. Also, cheeses made from goat milk, generally, are whiter in color (Park *et al.,* 2007). Therefore, differences between sheep and goat milk when compared to cow milk with regards to their chemical composition, among other factors, affect cheesemaking behavior and are responsible for the unique organoleptic characteristics of the cheeses produced. In many cases, the use of mixtures of different types of milk can result in the manufacture of cheeses with distinct biochemical, organoleptic and textural properties as well as with better acceptance by consumers and could be used by cheesemakers to expand their production. The chemical, physicochemical and sensory characteristics of cheeses manufactured by mixing milk of different species were reviewed by Boukria *et al.* (2020). The great majority of sheep and goat milk cheeses originate from countries such as Greece, Italy, France, Spain and Portugal. Greece produces mainly ovine and caprine cheeses, as 155,897 tons of sheep, 36,886 tons of goat and 23,782 tons of cow cheeses were produced in the year 2021 (FAOSTAT, 2021). Mainly goat milk is mixed up to 30% with sheep milk for producing Feta Protected Denomination of Origin cheese. Goat milk is also added to sheep or cow milk during the cheesemaking of other Greek cheeses except of a minor quantity that is used for drinking, yoghurt production or manufacture of cheeses only from goat milk.

During cheese manufacture, generally milk is pasteurized, however, cheesemilk can be heated at temperatures higher than those used in conventional pasteurization. During high heat treatment of milk, generally whey proteins are denatured and associated with κ-casein; especially β-lactoglobulin is interacted with κ-casein. As the denatured whey proteins are incorporated in the cheese curd and not lost in the whey, cheeses made from high heat treatment of milk tend to have an increased nutritional value, higher protein and moisture content and therefore yield (Guinee, 2021; Singh and Waungana, 2001). The increased cheese yield from this heat-induced association between caseins and whey proteins with the minimal undesirable changes of the cheese quality has been cheesemakers' principal interest (Kelly *et al.,* 2008). The retention of the whey proteins can be beneficial only after adaptation of the manufacturing conditions to produce a cheese of high quality (Hinrichs, 2001). Usually, the heating of milk at high temperatures is more suitable in the production of fresh acid curd cheeses (Masotii *et al.,* 2017; Guinee 2021) or is used to improve the texture and sensory properties of reduced fat rennet-curd cheeses (Guinee, 2021; Guyomarch, 2006). However, high temperature heating of cheesemilk is considered to be a challenge when producing rennet-curd cheeses because many technological problems can occur such as long coagulation times, reduced syneresis and weak structure of the curd (Guinee, 2021; Singh and Waungana, 2001; Guyomarch, 2006), affecting the composition (changes in mineral equilibrium, pH reduction), texture (forming a crumbly and soft texture with poor meltability) and flavor (development of off-flavors such as bitterness) of cheeses. High heat treatment of milk has been used to produce different types of cheeses such as Cheddar cheese (Banks, 1988; Rynne *et al.,* 2004; Guinee *et al.,* 1998; Banks *et al.,* 1995; Calvo *et al.,* 1992), white brined from goat milk (Miloradovic *et al.,* 2017; Miloradovic *et al.,* 2021), Quark-type cheese from cow and/or goat milk (Miloradovic *et al.,* 2018; Lepesioti *et al.,* 2021; Vaziri *et al.,* 2010), soft cheese from goat milk supplemented with *Penicillium candidum* (Kaminarides *et al.,* 2019), Mozzarella from goat milk (Faccia *et al.,* 2021), semi-hard cheese from sheep milk (Moatsou *et al.,* 2019), Dambo cheese (Benfeldt *et al.,* 1997), Camembert-type cheese (Ghosh *et al.,* 1999), Cheshire cheese (Marshall, 1986).

A hard cheese is manufactured using high heat treated sheep milk, in the mountains of North West part of Greece, near the Albanian borders (height ≈ 800m). It is inoculated with a thermophilic starter culture, clotted with rennet and ripened for a long time (sometimes up to a year). It has a crust, yellow color, compact texture without holes generally, pleasant taste and locally is called mountainous cheese from boiled milk. Its shape is cylindrical and each cheese weighs about 600-700g. The aim of the present work was to study the cheesemaking technology, the biochemical, textural and organoleptic characteristics of this cheese produced by overheated sheep milk and mixed sheep-goat milk (50:50) during ripening and storage. This ratio (50:50) is typically used by the cheesemakers of the region. Since it is very popular regionally, the information of this study could be used for the industrial scale production of the cheese, making it more available to the consumers.

MATERIALS AND METHODS

Cheesemaking procedure

Two cheeses were produced using either 100% sheep milk (S) or mixture of 50% sheep and 50% goat milk (SG) at a creamery in Dolo Pogoniou, during summer 2023. The sheep animals were of Boutsiko breed whereas goats were of the native breed *Capra Prisca.* Three cheesemaking trials took place.

Starter yoghurt inoculum was prepared by heating sheep milk at 90°C for 10 min, cooling it at 45°C and adding yoghurt from the market (without the crust) at 1%. After 2-2.5 h the pH was approximately 4.90 and it was transferred to the refrigerator.

Raw sheep milk (62 kg) with pH values 6.73±0.03 or mixed sheep- goat milk 50:50 (66 kg) with pH 6.64±0.02 was heated at 90°C for 15 min under stirring and cooled at 45°C in double wall vat under agitation by using steam and/or tap water, respectively. Starter yoghurt culture (0.25%), prepared the previous day, was added in the milk. Ten minutes later, $CaCl_2$ solution (40%, w/v) was added at a quantity of 10 ml for 100 kg milk to assist curdling of milk and rennet powder (diluted in water) from a local producer (1.100 International Milk Clotting Units /g), at a quantity of 2.25 g for 60 kg milk was used to obtain a fixed coagulation time of 30 min at 38°C. After coagulation the sheep curd had pH 6.48±0.02 and the mixed sheep-goat had 6.38±0.02 pH values. The curd was cut into cubes of 6–8 cm, left to rest for 10 min and then scalded up to 47-48°C in 20 min under continuous stirring. At that temperature the curd particles were stirred again for 10 min. The temperature was constantly monitored throughout the whole cheese-making process. At the end of scalding the sheep curd had pH 6.38±0.02 while the mixed sheep-goat had 6.23±0.05. Then the curd was carefully transferred to cylindrical plastic perforated moulds with 8.5 cm height and 16 cm diameter. During transferring to the moulds, the sheep curd had pH 6.26±0.09, while that of mixed sheep-goat had 6.12±0.08 pH values. To assist drainage, they were pressed with a weight approximately equal to cheese-weight (1:1) for 20 min and then with a weight almost doubled the cheese-weight (2:1) for another 20 min. Then the weight was removed and the cheese curd was turned over into moulds. This was repeated twice, every 3 hours. During drainage, whey from sheep and sheep-goat cheese manufacture was collected following the IDF (2008). At the end of pressing, the pH of sheep cheese was 5.65±0.04 and that of mixed sheep-goat was 5.83±0.22. Then cheeses were left overnight in the moulds at room temperature (16-18 °C) for draining. The following day, the pH of sheep cheeses was 5.43±0.04 and of mixed sheep-goat cheeses 5.20±0.03 and both cheeses were salted by immersion in brine (22%) for 6 h and left for 30 days maturation (16-18 °C and relative humidity 85-87%). Then they were vacuum packaged in polyethylene bags (Valko Favola 415/20, Italy) and were transferred to the cold store (2-5 °C) until the age of 180 days.

Assessment of physicochemical analyses

The composition of milk and whey samples was determined by the MilkoScan, model 6000 (Foss Electric, Hillerød, Denmark). Cheese samples were analyzed for fat content according to the Gerber Van Gulik method (Schneider, 1954), moisture content by drying to constant weight at 105 °C (IDF, 1982). Ash content was determined gravimetrically by oven-drying at 500 °C for 5 h (IDF, 1964) and salt content according to the modified Volhard method (Kosikowski, 1982). The pH of the cheese was determined by inserting the pH probe directly into the cheese mass (pH meter Micro pH 2002; Crison, Barcelona, Spain). Yield was expressed in kg of cheese per 100 kg of milk. The fat in dry matter and moisture-in-non-fat substance contents were calculated (Pappa, Kandarakis, Anifantakis, & Zerfiridis, 2006).

Assessment of proteolysis

Water-soluble nitrogen (WSN) and nitrogen soluble in 12% trichloroacetic acid (TCA) nitrogen, were determined as described by Kuchroo and Fox (1982) except that for the homogenization the Sorval Omni-Mixer (Dupont Company, Newton, CT, USA) was used and a No 42 filter paper was used for the filtration of the supernatant. Nitrogen soluble in 5% phosphotungstic acid (PTA) was prepared according to Stadhouders (1960), except that the extract was prepared as above mentioned. Total Nitrogen (TN) and all soluble nitrogen (SN) fractions were determined using the Kjeldahl method (IDF, 1993). The nitrogen content of the extracts was expressed as a percentage of TN. Protein was calculated using the following equation:

$$\text{Protein} = TN \times 6.38.$$

Assessment of lipolysis

Lipolysis was determined using the method of Deeth and Fitz-Gerald (1976) by adding cheese (3 g) and water (5 mL) to the test tubes, and a paste was formed. An extraction mixture (10 mL) of isopropanol: petroleum ether: 4 N sulfuric acid (40:10:1) was initially added to the paste produced and then petroleum ether (6 mL). After vigorous shaking of the tubes, two layers were created and allowed to settle; then the upper layer (5 mL) was titrated with methanolic potassium hydroxide solution (0.02 N) using 1% methanolic phenolphthalein (6 drops). The total free fatty acid (TFFA) content was calculated from this titration and results were expressed as milliequivalents (meq) potassium methoxide (CH_3OK) per g of cheese.

Assessment of antioxidant activity

The radical scavenging activity of 2,2-diphenyl-1 picrylhydrazyl (DPPH) of the water-soluble cheese extracts was used to determine the antioxidant activity of the cheeses, using established methods (Gupta *et al.,* 2009; Meira *et al.,* 2012). The water-soluble extract was prepared as described previously. Results were expressed as % percentage of radical scavenging activity (RSA).

Assessment of textural characteristics

A texture profile analyser (EZTest, EZ-X Series, Shimadzu Europa Gmbh, Germany) equipped with a plunger with a diameter of 40 mm and a 50 N load cell was used to assess the textural characteristics using a double-bite compression test from which the textural parameters were calculated (O'Callaghan and Guinee, 2004). The dimensions of the cheeses were 20 mm x 20 mm x20 mm and the samples were compressed to 60% of their original height. The textural characteristics that were calculated were springiness (ratio), brittleness (kg), hardness (kg), adhesiveness (kg × mm), (d) cohesiveness (ratio). The software TRAPEZIUM X (Shimadzu Autograph, Software, Shimadzu Europa Gmbh, Germany) was used for data collection. From each cheese sample, five replicate measurements took place, at ambient temperature (18 ± 2 °C).

Organoleptic assessment

During grading organoleptic evaluation, cheeses were coded and assessed by a panel of seven laboratory staff members well experienced in cheese quality judging at 30, 60, 90 and 180 days of ripening and storage. The cheeses were cut in small cubes with dimensions approximately 2 cm and had ambient temperature (18 ± 2 °C). The attributes of appearance, body-texture and flavor were evaluated (IDF, 2023) and graded on a scale of 0 (lowest quality) to 10 (best quality). The scores of appearance were multiplied by a factor 1 (therefore 10 was the maximum value), of body-texture by 4 (therefore 40 was the maximum) and of flavor by 5 (therefore 50 was the maximum), according to the importance that was given to each attribute. The sum of the scores of the three attributes gave the total values of the organoleptic evaluation, so an excellent cheese can receive 100 as a total score. Panelists were also asked to report any defects using the quality terms of International Dairy Federation (2023).

Statistical analysis

Two-way analysis of variance (ANOVA) with a subsequent least significant difference (LSD) test was applied between the ripening time and type of milk used. Differences were considered statistically significant at 95% confidence level ($P < 0.05$). The software package Statgraphics Plus for Windows v. 5.2 (Manugistics Inc., Rockville, MD, USA) was used. The same software was used to find a correlation between parameters.

RESULTS AND DISCUSSION

Physicochemical characteristics

The mean physicochemical and microbiological characteristics (mean values ± standard error) of sheep milk used for the manufacture of the cheese of the present study were 6.77±0.04 pH, 7.39±0.21 fat %, 5.58±0.10 protein%, 4.35±0.05 lactose%, 18.06±0.36 total solids% and 218±87 x 10^3cfu/ml total viable counts. The respective values for the mixed sheep-goat milk (50:50) were 6.64±0.03, 5.45±0.12, 4.23±0.08, 4.16±0.06, 14.59±0.24 and 635±88 x 10^3cfu/ml. The above results showed that the milk used for the cheese-making was of good quality.

The composition (mean values ± standard error) of sheep whey was fat 1.13% ±0.02, lactose 4.71% ±0.04, protein 0.89%±0.07, total solids 7.45%±0.12 and of mixed sheep-goat whey was 0.38%±0.04, 4.54%±0.01, 0.69%±0.05, 6.36±0.07, respectively. The physicochemical characteristics of the whey depend on the composition of milk used, the type of cheese produced and the specific manufacturing conditions. Sheep whey obtained from cheeses such as Graviera, Kefalotyri and Halloumi is reported to have 0.39-1.26% fat, 1.41-1.74% proteins, lactose 3.93-5.35% and total solids from 6.73% to 8.74% (Kaminarides *et al.,* 2015; Kandarakis, 1986, Kaminarides *et al.,* 2018; Kaminarides *et al.,* 2020). The fat, lactose and total solid content of the sheep whey of the present study was in accordance with the above values. However, the protein content of the whey was lower because proteins were incorporated into the curd rather than transferred to the whey.

It is known that the appropriate acidification rate and time is very important for the manufacture of good quality cheese (Fox and McSweeney, 2017). For this reason, the pH changes of the sheep and mixed sheep-goat cheeses were monitored during manufacture as well as at different ripening and storage days. There is a sharp pH decrease, from the beginning of the cheesemaking process (6.73±0.03 in sheep and 6.64±0.02 in mixed sheep-goat milk) until next day (5.43±0.04 in sheep and 5.20±0.03 in mixed sheep-goat cheese). The rate of pH decrease was similar ($P>0.05$) to both cheeses (S, SG) until the end of pressing (5.65±0.04 in sheep and 5.83±0.22 in mixed sheep-goat cheese), however the sheep cheese had higher ($P<0.05$) values than mixed sheep-goat cheese after 24 h (5.43±0.04 in sheep and 5.20±0.03 in mixed sheep-goat cheese). The higher pH values of sheep cheese comparing to those of mixed sheep-goat cheeses were possibly because of the higher buffering capacity of sheep's milk compared to that of mixed milk due to its higher protein content (5.58±0.10 and 4.23±0.08 respectively). Possibly LAB and NSLAB microorganisms might have played an important role, but this needs to be further investigated. The present results agree with those of Mallatou and Pappa (2005) for Teleme cheese and of Mallatou *et al.* (1994) for Feta cheese. However, these differences were not observed at the 5th day (Table 1).

The physicochemical characteristics of S and SG cheeses ripened until day-30 and then stored under vacuum until 180 days are presented in Table 2. The moisture of the cheeses (30-180 days), regardless of the type of milk used, ranged from 34.89% to 30.24% and the fat-in-dry matter (FDM) content was from 54.28% to 50.04%. The above results indicate that cheeses of the present study are classified as hard cheeses (referred as those with maximum moisture content 38%) of excellent quality (maximum moisture 35% and minimum FDM 47%), according to the Greek legislation (Greek Codex Alimentarius, 2009).

Generally, no statistical differences were observed between the sheep and mixed sheep-goat cheeses (Table 1). However, the S cheese had higher (P<0.05) pH values than SG cheese at day-90 and day-180 (Table 1). Also, S cheeses had lower salt at day-90, fat at day-30 and day-60 and FDM content at day-60 than SG cheese. The ripening and storage time did not also affect ($P>0.05$) the pH values, except that at 180th day the pH of sheep cheese increased ($P<0.05$). The moisture content remained stable during storage (30-180 days old) due to the vacuum packing of cheeses. The yield of young cheeses (5-day old) was high due to the high moisture content, but then no statistical differences were found as cheeses were vacuum packed after day-30. However, the S cheese had higher ($P<0.05$) yield than that of SG cheese, at all ages as expected. Picante cheese (Freitas *et al.,* 1997) made from sheep milk had lower fat and similar moisture content than that of mixed sheep-goat milk (50:50). General no differences were observed in the physicochemical characteristics of Teleme cheese by Mallatou and Pappa (2005) and of Feta cheese by Mallatou *et al.* (1994) made from sheep and mixed sheep-goat milk (50:50). In accordance with the results of the present study, Sakkas *et al.* (2023) also found pH values around 5 in semi hard cheese made from sheep milk. The different types of cheeses, heat-treated conditions of milks and manufacturing protocols may explain the above differences.

Proteolysis

Proteolysis is one of the three biochemical events (together with lipolysis and glycolysis) that take place during the ripening of a cheese and the quantities of nitrogen compounds soluble in water or various precipitants such as thrichloroacetic acid, phosphotungstic acid represent well established measures of it (Christensen *et al.,* 1991). The WSN and TCA- soluble N result mainly from the action of enzymes of rennet, plasmin and starter culture and NSLAB on casein, whereas PTA-soluble N originates from the hydrolysis of larger peptides to low molecular weight peptides and free amino acids due to enzymes from starter bacteria and other microorganisms such as NSLAB etc (McSweeney and Fox, 1997).

The different nitrogenous fractions of the S or SG cheeses of the present study are shown in Table

Table 1. Physicochemical characteristics of cheeses produced from sheep (S) milk or mixed sheep-goat milk (SC) (Means of three cheese-making trials ± standard error)

Age (days)	Type of milk	pH	Salt (%)	Fat (%)	Moisture (%)	Proteins (%)	Ash (%)	Fat-in-dry-matter (%)	Moisture-in-non-fat substance (%)	Yield (%)
5	S	5.29±0.01^{A}	1.61±0.12^{A}	27.75±0.25^{A}	46.48±1.01^{A}	20.93±0.19^{aA}	3.69±0.11^{A}	51.87±1.44^{A}	64.33±1.61^{A}	19.98±0.11^{aA}
	SG	5.18±0.06^{A}	1.62±0.19^{A}	27.16±0.44^{A}	45.28±0.25^{A}	19.62±0.03^{b1}	3.47±0.22	49.65±0.97	62.17±0.66^{A}	17.47±0.33^{bA}
30	S	5.43±0.02^{A}	2.45±0.27^{AB}	32.93±0.97^{aB}	34.89±2.16^{B}	23.86±0.64^{B}	4.74±0.30^{aAB}	50.60±0.61^{A}	51.95±2.52^{B}	16.57±0.91^{aB}
	SG	5.27±0.06^{A}	3.03±0.16^{B}	36.26±0.62^{bBC}	32.64±0.35^{B}	24.82±0.06^{B}	5.01±0.14^{B}	53.85±1.11^{C}	51.24±0.94^{B}	12.82±0.24^{bB}
60	S	5.41±0.06^{A}	2.45±0.32^{AB}	33.70±0.87^{aB}	33.98±1.73^{B}	25.04±0.54^{B}	4.79±0.09^{AB}	51.04±0.02^{aA}	51.20±1.94^{B}	15.77±0.49^{aB}
	SG	5.28±0.02^{A}	3.09±0.20^{B}	37.33±0.60^{bC}	32.30±0.26^{B}	25.45±0.25^{B}	5.24±0.19^{B}	54.28±0.16^{bC}	51.07±0.21^{BC}	12.54±0.15^{bB}
90	S	5.39±0.04^{aA}	2.68±0.18^{aB}	34.86±1.37^{B}	34.34±1.61^{B}	24.50±0.64^{B}	4.99±0.22^{B}	51.45±1.02^{A}	51.85±1.25^{B}	15.65±0.98^{aB}
	SG	5.21±0.03^{bA}	3.42±0.16^{bB}	36.93±0.87^{C}	30.24±1.38^{B}	25.48±0.60^{B}	5.35±0.13^{B}	52.95±0.52^{BC}	47.92±0.92^{B}	12.45±0.36^{bB}
180	S	5.59±0.06^{aB}	2.63±0.36^{B}	33.03±0.73^{B}	33.98±1.09^{B}	26.03±0.45^{C}	4.91±0.37^{B}	50.04±0.86^{A}	50.73±1.35^{B}	16.16±1.07^{aB}
	SG	5.33±0.04^{bA}	3.16±0.18^{B}	34.96±0.33^{B}	31.93±0.41^{B}	25.74±0.04^{B}	5.11±0.19^{B}	51.36±0.21^{B}	49.09±0.4^{BC}	12.57±0.13^{bB}

[a, b] Means in each column, at the same age, with a small letter differ significantly (P<0.05)

[A,B,C] Means in each column, at the same type of milk, with different capital letters differ significantly (P<0.05)

Table 2. Proteolysis of cheeses produced from sheep (S) milk or mixed sheep-goat milk (SG) (Means of three cheese-making trials ± standard error)

Age (days)	Type of milk	WSN %TN*	TCA % TN*	PTA % TN*
5	S	13.43±1.14^{A}	7.79±0.03^{A}	3.31±0.57^{A}
	SG	14.12±0.20^{A}	7.51±0.44^{A}	4.21±0.33^{A}
30	S	20.07±1.29^{B}	14.97±0.34^{B}	6.36±0.39^{aAB}
	SG	17.11±1.12^{A}	11.90±3.09^{B}	8.79±0.17^{bAB}
60	S	24.49±0.41^{aB}	16.8±1.99^{BC}	6.65±0.81^{AB}
	SG	16.35±1.92^{bA}	13.01±1.18^{B}	6.60±1.73^{AB}
90	S	21.57±2.20^{B}	18.47±0.7^{BC}	9.5±1.16^{BC}
	SG	19.00±2.31^{A}	16.05±1.41^{B}	7.43±1.61^{AB}
180	S	19.93±0.33^{B}	20.57±2.22^{C}	11.87±1.73^{C}
	SG	17.98±0.59^{A}	16.24±0.46^{B}	10.27±1.77^{B}

*TN, Total nitrogen; WSN, Water soluble nitrogen; TCA, nitrogen soluble in 12% trichloroacetic acid; PTA, nitrogen soluble in 5% phosphotungstic acid

$^{a, b}$ Means in each column, at the same age, with a small letter differ significantly (P<0.05)

^{A,B,C} Means in each column, at the same type of milk, with different capital letters differ significantly (P<0.05)

2. Generally, nitrogenous fractions increased during ripening and storage and values of WSN ranged from 13.43 to 24.49 %TN, of TCA from 7.51%TN to 20.57%TN and of PTA from 3.31% to 11.87% TN. In general, no differences were observed in the different fractions between S and SG (50:50) cheeses in agreement with the results of Mallatou *et al.* (2004) for Teleme cheese. However, Picante cheese made from sheep milk showed lower WSN %TN values than cheese made from 50% sheep – 50% goat milk (Freitas *et al.,* 1997).

Textural characteristics

Texture is an important cheese characteristic that affects the consumers' acceptability. The textural profile analysis (TPA) results of the different cheeses are presented in Table 3. The parameters adhesiveness which is the necessary energy to overcome the attractive force between the surface of a cheese and materials the cheese comes into contact, cohesiveness which is the maximum extent at which the cheese can deform before breaking, springiness which is the ability that a cheese has, so as to return after the first compression, to its original height, brittleness i.e., the ease with which the cheese breaks under load and hardness, the maximum peak force which is observed during the first compression cycle (Iruoayaraj *et al.,* 1999; Maldonado *et al.,* 2013) were determined.

As cheese ripens various physicochemical changes alter its structure, while several factors such as milk composition and quality, acidification rate by the starter culture bacteria, the biochemical changes during maturation can influence its textural properties (da Silva *et al,* 2020). An important factor which affects the cheese's texture characteristics is proteolysis, as during ripening the caseins are hydrolyzed, the water activity of the curd is decreased due to changes in the water binding of the new ionic groups (carboxylic and amino groups) formed during hydrolysis and peptides which are cleaved do not contribute to the cheese structure anymore. Therefore, changes in textural properties are affected by modifications in the protein matrix, mainly α_{s1}-and β-casein degradation (Creamer and Olson, 1982; Lucey *et al.,* 2003; McSweeney, 2004).

The cheeses manufactured using SG milk were harder (*P*<0.05) than S cheeses at 60 and 90 days of ripening and storage (Table 3). Hardness of S cheese was correlated with its TCA level (correlation coefficient=0.84, P<0.05) and of SG cheese with its moisture content (correlation coefficient=0.55, P<0.1). Further study of the protein profile is necessary to explain possible textural differences of the cheeses. Similar results to the present study were found by Bertola *et al.,* (1992). In accordance with our re-

Table 3. Textural characteristics of cheeses produced from sheep (S) milk or mixed sheep-goat milk (SG) (Means of three cheese-making trials ± standard error)

Age (days)	Type of milk	Springiness	Brittleness (kg)	Hardness (kg)	Adhesiveness (kg x mm)	Cohesiveness
30	S	1.40 ± 0.06^{A}	0.34 ± 0.08^{aA}	7.31 ± 0.49^{A}	0.11 ± 0.01^{A}	0.21 ± 0.01^{A}
	SG	1.30 ± 0.07^{AB}	1.15 ± 0.36^{bA}	7.91 ± 0.26^{A}	0.08 ± 0.04^{A}	0.18 ± 0.02^{A}
60	S	1.30 ± 0.08^{A}	0.64 ± 0.41^{aAB}	7.96 ± 0.57^{aA}	0.08 ± 0.02^{A}	0.18 ± 0.03^{A}
	SG	1.20 ± 0.12^{A}	1.76 ± 0.20^{bA}	11.97 ± 0.58^{bB}	0.12 ± 0.01^{A}	0.15 ± 0.00^{A}
90	S	1.36 ± 0.11^{A}	1.34 ± 0.34^{aB}	10.72 ± 0.43^{aB}	0.12 ± 0.05^{A}	0.18 ± 0.00^{A}
	SG	1.15 ± 0.15^{A}	2.15 ± 0.47^{bA}	14.55 ± 0.69^{bBC}	0.15 ± 0.05^{A}	0.19 ± 0.05^{A}
180	S	1.36 ± 0.02^{aA}	1.02 ± 0.11^{aAB}	15.19 ± 1.38^{C}	0.19 ± 0.08^{A}	0.23 ± 0.08^{A}
	SG	1.69 ± 0.0^{bB}	2.26 ± 0.26^{bA}	15.23 ± 2.0^{C}	0.10 ± 0.04^{A}	0.14 ± 0.01^{A}

[a, b] Means in each column, at the same age, with a small letter differ significantly ($P<0.05$)

[A,B,C] Means in each column, at the same type of milk, with different capital letters differ significantly ($P<0.05$)

sults, hardness of Feta cheese (Mallatou *et al.,* 1994; Tsigkros *et al.,* 2003) produced from mixture of sheep and goat milk increased as the percentage of goat milk increased. In the present study adhesiveness, cohesiveness and springiness were not affected by the type of milk used and age of the cheeses generally, whereas brittleness was higher in mixed sheep-goat cheese compared to sheep cheese (Table 3). Also, Mallatou *et al.* (1994) observed that Feta cheeses made with mixed sheep-goat milk (50:50) were more brittle than those manufactures using only sheep milk. It is known (Maldonado *et al.,* 2013) that values near 1 indicate high cohesiveness, 0.5 intermediate cohesiveness, while 0 indicates no cohesiveness, therefore cheeses of the present study showed low cohesiveness (values from 0.14 to 0.23, Table 3).

Lipolysis

Lipolysis is essential for flavour development especially when is properly balanced with proteolysis. Lipolysis was the most important biochemical event occurred at Cacioricotta cheese (Faccia *et al.,* 2007) manufactured using overheated (90°C) milk. The increase of lipolysis level during aging can be a useful index of maturity (McSweeney and Fox, 1997). The lipolysis level of cheeses of the present study is presented in Table 4. The TFFA content of cheeses increased ($P<0.05$) during ripening and storage and its value ranged from 0.81 to 1.83 meq CH_3OK/ g cheese. In the present study, no statistical differences were found between S and SG cheeses. Similarly, no differences were found in lipolysis levels of Teleme cheese made from sheep and mixed 50%sheep-50% goat milk (Mallatou *et al.,* 2003).

The optimum pH for the activity of milk lipase is 8.9-9.0, and the optimum temperature 35-40°C, while its action is inhibited by salt (Vlaemynck, 1992). The values of the pH and the temperature of the cheeses of the present study were not close to the optimum requirements, therefore a moderate lipolysis level occurred. The results of the present study agree with those observed by Atasoy *et al.* (2008) for Urfa cheese manufactured with different heat-treated milk and by Faccia *et al.* (2007) who produced Cacioricotta cheese by heating sheep or goat milk at 90°C without the addition of starter culture.

Antioxidant properties

The antioxidant properties of cheeses are related to the presence of carotenoids, sulfur containing amino acids, and vitamins (A, D) (Stobiecka *et al.,* 2022). Specifically, the antioxidant activity of whey proteins is well established and is due to the scavenging of free radicals from sulfur amino acids and chelation of transition metals from lactoferrin, therefore food containing whey proteins have better antioxidant properties (Khan *et al.,* 2019). The antioxidant activity has a significant role in maintaining the antioxidant defense system by preventing the formation of active oxygen species and of free radicals which can result in oxidative damage to biomolecules causing cancer, stroke etc. (Gupta *et al.,* 2009) therefore its

Table 4. Textural characteristics of cheeses produced from sheep (S) milk or mixed sheep-goat milk (SG) (Means of three cheese-making trials ± standard error)

Age (days)	Type of milk	Springiness	Brittleness (kg)	Hardness (kg)	Adhesiveness (kg x mm)	Cohesiveness
30	S	1.40±0.06^{A}	0.34±0.08^{aA}	7.31±0.49^{A}	0.11±0.01^{A}	0.21±0.01^{A}
	SG	1.30±0.07^{AB}	1.15±0.36^{bA}	7.91±0.26^{A}	0.08±0.04^{A}	0.18±0.02^{A}
60	S	1.30±0.08^{A}	0.64±0.41^{aAB}	7.96±0.57^{aA}	0.08±0.02^{A}	0.18±0.03^{A}
	SG	1.20±0.12^{A}	1.76±0.20^{bA}	11.97±0.58^{bB}	0.12±0.01^{A}	0.15±0.00^{A}
90	S	1.36±0.11^{A}	1.34±0.34^{aB}	10.72±0.43^{aB}	0.12±0.05^{A}	0.18±0.00^{A}
	SG	1.15±0.15^{A}	2.15±0.47^{bA}	14.55±0.69^{bBC}	0.15±0.05^{A}	0.19±0.05^{A}
180	S	1.36±0.02^{aA}	1.02±0.11^{aAB}	15.19±1.38^{C}	0.19±0.08^{A}	0.23±0.08^{A}
	SG	1.69±0.0^{bB}	2.26±0.26^{bA}	15.23±2.0^{C}	0.10±0.04^{A}	0.14±0.01^{A}

$^{a, b}$ Means in each column, at the same age, with a small letter differ significantly (P<0.05)

^{A,B,C} Means in each column, at the same type of milk, with different capital letters differ significantly (P<0.05)

evaluation in the cheeses of the present study can provide useful information. The determination of scavenging of stable organic radical DPPH has been widely used as an index of antioxidant capacity of cheeses. The antioxidant activity of cheeses is due to the ability to transfer hydrogen or electron to DPPH, resulting in neutralizing the free radical character. It is reported that the antioxidant activity of a cheese was not generally affected by thermal treatments (Rinaldi *et al.,* 2023; Fardet and Rock, 2018).

In this study, the antioxidant activity of the cheeses ranged from 49.65 to 58.82 DPPH %RSA regardless of the type of milk used and the different sampling dates (Table 4). The S cheeses showed lower (P<0.05) values of antioxidant activity than SG cheeses at day-180. No differences were observed in the antioxidant activities of cheeses produced with different types of milk used by Revilla *et al.* (2016). Also, the radical scavenging activity of the S cheeses of the present study was not affected (P>0.05) by storage time while that of SG cheeses increased (P<0.05) until day-90. The antioxidant activity was correlated with WSN%TN in S and TCA%TN in SG cheese (correlation coefficient 0.51 and 0.54, respectively). A correlation between antioxidant activity and proteolysis has been observed in other cheeses too (Meira *et al.,* 2012; Gupta *et al.,* 2009).

Organoleptic characteristics

The results regarding the organoleptic characteristics of the cheeses of the present study are shown in Table 5. The cheeses had a light yellow colour, compact texture and no holes generally. All cheeses had pleasant taste, piquant aroma and received high score values.

Generally, no statistical differences were found between S and SG cheeses for all sensory attributes (Table 6). However, at day-180, S cheeses received higher (P<0.05) scores than SG cheeses for taste and total organoleptic characteristics. Generally, the SG cheeses were characterized as more brittle than S cheeses in agreement with the textural results (Table 3).

Singh and Waungana (2001) reported that cheeses produced from heated milk can develop increased levels of off-flavor, mainly bitterness. No such defects were observed by the panelists for the cheeses of the present study. On the contrary, cheeses were very much appreciated, receiving high scores during the organoleptic evaluation. Also, the panelists did not report any notable 'goaty' flavor.

CONCLUSIONS

In the present work, a hard cheese manufactured from high heat treated sheep or mixed sheep-goat (50:50) milk in a creamery in the mountains of North West part of Greece was studied, during ripening and storage. Using the present manufacturing procedure no technological problems occurred during cheesemaking, both cheeses were of excellent quality according to the Greek legislation (maximum moisture 35% and minimum FDM 47%) and received high organoleptic scores. General cheeses

Table 5. Lipolysis and antioxidant activity of cheeses produced from sheep (S) milk or mixed sheep-goat milk (SG) (Means of three cheese-making trials ± standard error)

Age (days)	Type of milk	TFFA (meq CH_3OK/ g cheese) *	DPPH %RSA*
5	S	0.81±0.05^{A}	51.79±0.04^{A}
	SG	0.83±0.01	50.31±1.26^{A}
30	S	1.07±0.06^{AB}	55.02±0.54^{A}
	SG	1.32±0.01^{B}	49.65±3.04^{A}
60	S	1.26±0.18^{AB}	53.43±1.37^{A}
	SG	1.51±0.09^{BC}	58.82±1.01^{B}
90	S	1.33±0.18^{AB}	53.96±2.37^{A}
	SG	1.66±0.15^{CD}	57.12±0.98^{B}
180	S	1.46±0.23^{B}	52.02±0.04^{aA}
	SG	1.83±0.01^{D}	55.58±0.22^{bAB}

*TFFA, Total Free Fatty Acids; DPPH % RSA, 2,2-diphenyl-2-picrylhydrazyl radical % radical scavenging activity

$^{a, b}$ Means in each column, at the same age, with a small letter differ significantly (P<0.05)

^{A,B,C,D} Means in each column, at the same type of milk, with different capital letters differ significantly (P<0.05)

Table 6. Organoleptic characteristics of cheeses produced from sheep (S) milk or mixed sheep-goat milk (SG) (Means of three cheese-making trials ± standard error)

Age (days)	Type of milk	Appearance (10) *	Texture (40)*	Taste (50) *	Total (100) *
30	S	8.9±0.08^{A}	36.4±0.23^{A}	44.83±0.44^{A}	90.2±0.4^{A}
	SG	8.93±0.06^{A}	34.93±0.93^{A}	44.16±0.16^{AB}	88.03±1.03^{A}
60	S	9.26±0.08^{A}	36.66±0.26^{A}	46.0±0.28^{AB}	91.93±0.58^{A}
	SG	9.13±0.12^{A}	36.26±0.35^{A}	45.16±0.60^{AB}	90.56±1.03^{A}
90	S	9.23±0.17^{A}	36.66±0.48^{A}	46.16±0.17^{B}	92.06±0.72^{A}
	SG	9.26±0.18^{A}	36.66±0.70^{A}	45.83±0.88^{A}	91.76±1.74^{A}
180	S	9.27±0.03^{A}	36.66±0.35^{A}	46.0±0.5^{aAB}	91.93±0.87^{aA}
	SG	9.03±0.08^{A}	35.20±0.46^{A}	43.5±0.57^{bB}	87.73±1.09^{bA}

*The values in brackets show the maximum scores.

$^{a, b}$ Means in each column, at the same age, with a small letter differ significantly (P<0.05)

^{A,B} Means in each column, at the same type of milk, with different capital letters differ significantly (P<0.05)

had similar physicochemical, biochemical, textural and organoleptic characteristics which could provide information regarding their identity.

DECLARATION OF COMPETING INTEREST

We have no conflict of interest to declare.

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