Barbaresca sheep milk: lipid and mineral composition under the influence of grazing in the Nebrodi mountains

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ABSTRACT

Sheep milk is a crucial food source in many Mediterranean cultures and is a significant economic resource in the hilly and mountainous regions of Sicily. This study evaluated the milk composition of Barbaresca sheep, an indigenous Sicilian breed considered at high risk of extinction, known for its rusticity and adaptability to harsh environments. This research specifically focuses on the fatty acid profile and the presence of essential and toxic mineral elements, linking these characteristics to the Nebrodi pasture where the sheep graze. The experimental design involved collecting milk samples from Barbaresca sheep on a family-run farm located in a non-industrialized region of Sicily surrounded by the Nebrodi mountains. The sheep were managed under a semi-extensive farming system, grazing freely on pastures characterized by diverse flora, including Graminaceae, Asteraceae, Leguminosae, and various tree species like olive and chestnut. The collected milk samples were analyzed for mineral elements using ICP-MS and DMA for mercury, and for fatty acid composition using GC-FID. The main findings highlight the high content of essential minerals in Barbaresca sheep milk. Specifically, it significantly contributes to the recommended daily intake of essential minerals such as calcium and zinc. Other macroelements like potassium, sodium, and magnesium were also found in high concentrations. Essential trace elements like iron, manganese, copper, chromium, and molybdenum were also detected. The study also found limited health risk from toxic elements due to grazing in an environment far from sources of pollution. The milk has a rich and complex lipid profile, with a significant content of beneficial fatty acids such as linoleic and linolenic acids, and a balance between omega-6 and omega-3. The presence of short-chain fatty acids (SCFA) contributes to the typical flavor of the milk and its products. The lipid quality indices were favorable for a product of animal origin and suggest potential positive effects on cardiovascular health. This beneficial fatty acid composition is likely influenced by the inclusion of pasture in the sheep diet, particularly the diverse endemic vegetation of the Nebrodi Mountains.
In conclusion, the milk of the Barbaresca sheep can be considered safe and healthy due to the grazing in the varied vegetation endemic to the Nebrodi Mountains. This study contributes to the valorization of Barbaresca sheep milk as a high-quality product linked to the Nebrodi territory, promoting the sustainability of the grazing system and local biodiversity.

KEY WORDS

Barbaresca sheep milk; Essential minerals elements; Toxic elements; Fatty acid profile; Nutritional value.

INTRODUCTION

Sheep milk is an important food in many cultures, particularly in the Mediterranean area, and is a significant economic in resource in the hilly and mountainous regions of Sicily [1].
Traditional cheeses, including some with the protected designation of origin (PDO) or the protected geographical indication (PGI), are made from the milk of indigenous breeds such as Barbaresca. Sheep milk is an excellent raw material for cheese production due to its fat and protein content., which are significantly higher than cow milk [2]. The lipid fraction of sheep milk is rich in volatile branched chain fatty acids, such as 4-methyl octanoic, 4-ethyl octanoic and 4-methyl nonanoic acids, that contribute to its typical flavor [3]. Sheep milk also has higher concentration of beneficial fatty acids, including butyric acid, trans fatty acids and conjugated linoleic acids (CLA), and omega-3 fatty acids, which are of growing interest to health-conscious consumers [2,4].
Milk from semi-intensive production systems, where sheep graze freely, has a significantly more beneficial fatty acid composition and nutritional value than milk from intensive systems, main due to the inclusion of pasture in the sheep diet [5,6]. Sheep milk contains macro-elements (Ca, K, Mg, P) and trace elements (Zn, Fe, Mn, Cu, Se, Mo, Co) vital for physiological processes and metabolism. Their concentrations vary according to breed, diet, season and state of health of the animal [7,8]. The presence of toxic heavy metals (As, Cd, Cr and Pb) is a food safety concern, originating from the environment. In fact, the concentrations are low in areas undisturbed by anthropogenic activities but increase in polluted areas [9].

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Therefore, the mineral content of milk indicates both the nutritional value of the milk and the nutritional status of the animals, whereas the presence of toxic elements indicates both environmental pollution and animal health [10]. As a result, the monitoring of the mineral elements in milk is of great importance for the quality and safety of the food.
In this context, the Barbaresca sheep emerges as a particularly interesting case: an autochthonous Sicilian breed considered to be at high risk of extinction, known for its rusticity and ability to adapt to harsh environments. It is a dual-purpose breed, reared both for milk and meat, primarily using a semi-extensive farming system in a very limited area of central Sicily, typical of the Nebrodi area [11]. This farming system allows the flora of this region, characterized by a rich variety of species with a notable presence of endemic species, to play a key role in the feeding of the animals. Graminaceae (*Bromus* spp., *Festuca arundinacea*, *Dactylis glomerata*) and Asteraceae (*Helichrysum stoechas*) dominate the herbaceous component of the pastures [12,13]; the endemic species *Genista aristata* and *Trifolium bivonae* further enhance the floristic composition, while the presence of Leguminosae (*Medicago* spp. and *Trifolium* spp.), albeit in smaller amounts, and species of the genus Amaranthus (*cruentus*, *hybridus*, *hypocondriacus*, and *retroflexus*) add to the diversity and complexity of the animals diet. Brassicaceae plants also grow on the Nebrodi pastures and have been shown to have a significant impact on the acidic profile of Nebrodi black pig meat [12]. The Nebrodi woods composition is defined by the variety of oaks (*Quercus gussonei*, *Quercus congesta*, *Quercus suber*, and *Quercus ilex*) and the presence of olive trees (*Olea europaea*), chestnut trees (*Castanea sativa*) and beech trees (*Fagus sylvatica*). The ecosystem richness and complexity are influenced by the presence of other tree and shrub species, including *Erica arborea, Juniperus communis, Arbutus unedo, Myrtus communis, Euphorbia dendroides, Pistacia lentiscus, Carlina nebrodensis*, and *Calicotome spinosa* [13,14].
The aim of this study was to evaluate the influence of the Nebrodi pasture on the composition of the milk of the Barbaresca sheep, focusing on the fatty acid profile and the presence of essential, toxic and potentially toxic mineral elements. This research would contribute to enhancing the value of Barbaresca sheep milk as a high-quality product linked to the Nebrodi area, promoting the sustainability of the grazing system and local biodiversity.

MATERIALS AND METHODS

Sampling

25 milk samples of Sicilian Barbaresca sheep were collected in 2023 from a family-run farm in the non-industrialized Messina province of southern Italy, an area surrounded by the Nebrodi mountains. The sheep were raised using a semi-extensive farming system, grazing freely on pastures featuring diverse flora, including olive and chestnut trees. 25 animals were randomly selected from a population of 300, all of which were between two and three years old, uniform in calving order and age, and in their sixth month of lactation at the time of sampling. Each sample was taken from a single animal during the evening milking, collected in falcon tubes (30 ± 5 mL), transported to the lab in a cooler, and frozen at -20°C until analyses. The water available to the animals was also examined and found to contain no excesses of minerals.

Chemicals and standard solutions

Ultrapure argon and helium were delivered by Rivoira gases (Milan, Italy). Nitric acid (65% v/v), hydrogen peroxide (30% v/v, trace metal analysis grade) and ultrapure water (18.2 MW/cm) were purchased from J.T. Backer (Milan, Italy). Methanol, chloroform, *n*-heptane and 37% concentrated hydrochloric acid were purchased from Merck (Darmstadt, Germany), while potassium hydroxide, sodium chloride and anhydrous sodium sulphate were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).
The whole milk powder NIST RM 8435 (National Institute of Standards and Technology, NIST, Gaithersburg, MD, USA) was acquired as a Certified Reference Material from Nova Chimica S.r.l. (Milan, Italy).
For fatty acid analysis, a standard mixture of 37 FAMEs (FAME mix, Supelco, Bellefonte, PA, USA) was supplied by Sigma Aldrich Chemical Co. (St. Louis, MO, USA), and specific FAME isomers (iso C15:0, anteiso C15:0, iso C16:0, iso C17:0, anteiso C17:0, C22:5 n-3 and CLA cis-9, trans-11) were acquired from Larodan Fine Chemicals (AB, Malmö, Sweden).
For mineral analysis, stock standard solutions (1000 mg/L in 2% nitric acid) of various elements including Na, Ca, Mg, K, Zn, Fe, Mn, Cu, Cr, Ni, As, Cd, Pb, Mo, Sn, Co, Be, Li, Sb, and Ti were purchased from Merck (Darmstadt, Germany), whereas Hg stock standard solution (1000 mg/L in 3% nitric acid) and Re, Sc, Rh and Bi stock standard solutions (1000 mg/L in 2% nitric acid) were purchased from Fluka (Milan, Italy).
To tune the instrument, an ICP-MS tuning solution of ^{7}Li, ^{59}Co, ^{138}Ba, ^{209}Bi, ^{142}Ce, ^{115}In and ^{238}U (1 mg/L in 2% nitric acid and 0.5% hydrochloric acid) was obtained from Thermo Fisher Scientific (Milan, Italy).
Mixed working calibration standard solutions were prepared at concentration ranges appropriate for the analytes under investigation (range from 1 to 50 mg/L for the elements Ca, K, Na and Mg, from 0.05 to 1 mg/L for Zn and Fe, and from 0.5 to 50 mg/L for Mn, Cu, Cr, Ni, As, Cd, Pb, Mo, Sn, Co, Be, Li, Sb, and Ti). Five-point calibration curves were constructed at these intervals and used for measurements in the ICP-MS analysis.
To check the digestion of the sample and to account for volumetric variations, a working calibration solution of Re was prepared at a concentration of 0.5 mg/L. To correct for instrumental drift and matrix variations, a working calibration solution of Sc, Rh and Bi was prepared at a concentration of 1 mg/L.
From Hg stock standard solution seven working calibration standard solutions were prepared at concentration ranges from 0.5 to 50 mg/L and then used for measurements by DMA analysis.
All glassware and laboratory equipment were washed with 10% HNO_3, rinsed with ultra-pure water, and dried before use.

Multielement analysis by ICP-MS

Sample preparation

About 0.5 g of sheep milk was subjected to microwave-assisted acid digestion using a Milestone Ethos One microwave digestion system. This involved adding 1 mL of Re (internal standard, 0.5 mg/L), 7 mL of 65% nitric acid (HNO_3) and 1 mL of 30% hydrogen peroxide (H_2O_2). In the digestion program, the temperature was raised linearly to 200 °C for 10 minutes, and then the temperature was maintained at 200 °C for 20 minutes. After cooling, the digested samples were diluted to 25 mL with

Table 1 - Method performance parameters for the investigated elements.

Element	R^2	LOD (mg·L^{-1})	LOQ (mg·L^{-1})	Accuracy (%)	Repeatability (RSD%)	Intermediate precision (RSD%)
Ca	0.9989	0.0006	0.002	103	5.24	8.12
K	0.9993	0.0006	0.002	102	4.43	6.57
Na	0.9994	0.0003	0.001	105	6.22	8.88
Mg	0.9995	0.0003	0.001	103	5.54	7.84
Zn	0.9992	0.0003	0.001	92	3.24	6.02
Fe	0.9990	0.0015	0.005	84	5.74	7.54
Mn	0.9997	0.0006	0.002	97	4.36	8.44
Cu	0.9998	0.0003	0.001	94	4.22	4.96
Cr	0.9999	0.0003	0.001	91	6.33	7.22
Ni	0.9999	0.0015	0.005	97	5.22	7.46
Mo	0.9995	0.0006	0.002	88	4.74	6.01
As	0.9999	0.0003	0.001	83	3.21	5.88
Pb	0.9997	0.0015	0.005	98	5.41	6.34
Cd	0.9991	0.0003	0.001	90	3.55	5.00
Sn	0.9997	0.0006	0.002	95	4.31	6.58
Co	0.9993	0.0006	0.002	101	4.58	6.47
Be	0.9994	0.0003	0.001	98	4.98	6.13
Li	0.9996	0.0003	0.001	98	5.84	7.88
Sb	0.9995	0.0006	0.002	95	4.27	6.87
Ti	0.9993	0.0006	0.002	96	5.01	7.15
Hg	0.9998	0.0001	0.0003	98	1.1	1.7

R^2, determination coefficient; LOD, limit of detection = 3.3 /S; LOQ, limit of quantification =10 /S; RSD, relative standard deviation.

ultrapure water and filtered through 0.45 m PTFE filters [15]. The Certified Reference Materials, previously reconstituted in deionized water to 83.2% (the average water content of the samples under investigation), were digested under the same conditions [10]. Preparations were done in triplicate.

Instrumental analysis

A Thermo Scientific iCAP-Q ICP-MS (Cetac Technologies Inc., Omaha, NE, USA) was used for elemental analysis. The system included a standard PFA cyclonic spray chamber and an external nebulizer. Nickel sampler and skimmer cones were used. To reduce polyatomic interferences from plasma and matrix, a Qcell system with helium gas (KED mode) was employed. As per Dogan et al. [14], the ICP-MS operating conditions involved a RF generator of 1550 W; a sample depth of 5 mm; and specific gas flows for plasma, auxiliary, and carrier argon, as well as CCT gas (He). Samples were analyzed with 3 replicates and a 1-second dwell time scanning condition. Monitoring was done for the following isotopes: ^{7}Li, ^{9}Be ^{23}Na, ^{24}Mg, ^{39}K, ^{44}Ca, ^{50}Sn, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{98}Mo, ^{111}Cd, ^{121}Sb, ^{205}Ti and ^{208}Pb.

Analytical performances

Eurachem guidelines (2025) were used to evaluate the method linearity, sensibility, accuracy, repeatability, and intermediate precision. The obtained values are shown in Table 1.

By analyzing calibration standard solutions, the linearity of the results was verified. Five-point calibration curves were generated and analyzed six times per concentration level, showing satisfactory coefficient of determination over the investigated ranges.

Limits of detection (LODs) and limits of quantification (LOQs) were 3.3σ/S and 10σ/S, respectively. The slope of the calibration curve is denoted as S, and σ is the standard deviation of the response of ten blanks. The reliability of the concentration determination of various elements is guaranteed as their LOQ values were significantly lower than their natural levels in the analyzed matrix.

The certified reference material of whole milk powder (NIST RM 8435) was analyzed six times to verify the method accuracy. For all elements showed acceptable mean recoveries, indicating a good level of accuracy.

The relative standard deviation (RSD %) of measurements made on the certified reference material analyzed in the same batch (n=6) and in different days (n=12) was used to quantify the repeatability and intermediate precision. The results obtained are acceptable for all elements and indicate a satisfactory level of precision.

Mercury analysis by DMA

The mercury content was determined using a direct mercury analyzer (DMA-80, Milestone S.r.l., Milan, Italy). The instrument allows direct analysis of the sample without pre-treatment. About 100 mL of milk was weighed directly into the nickel cuvettes and then dried at 250 °C for 60 seconds and thermally decomposed at 750 °C for 150 seconds into the furnace. Atomic absorption spectrometry was performed at 253.54 nm. As stated for other elements in section 2.3.3, the method validation was performed according Eurachem guidelines (2025). Table 1 contains the achieved values.

Table 2 - Elements content (mg/L) in Barbaresca sheep milk.

	Ca	K	Na	Mg	Zn	Fe	Mn	Cu	Cr	Ni	Mo
Means** ± S.D.	2692.4±231.0	1398.3±130.5	373.60±23.73	201.05±9.21	22.07±3.29	6.01±1.38	0.197±0.065	0.159±0.037	0.044±0.026	0.028±0.015	0.025±0.014
Ranges	2345.7-3103.2	1125.6-1652.3	329.57-408.11	184.56-221.17	16.93-27.91	3.87-9.21	0.11-0.35	0.10-0.25	0.01-0.11	0.01-0.06	<LOQ -0.05
	As*	Pb*	Cd*	Sn	Co	Be	Li	Sb	Ti	Hg*	
Means ± S.D.	0.017±0.014	0.010±0.008	0.005±0.002	-	-	-	-	-	-	-	
Ranges	<LOQ-0.030	<LOQ-0.018	<LOQ-0.010	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	

* According to previous research by the same authors (Do an *et al*., 2024). ** This is the mean value of positive samples, which was 0 for Sn, Co, Be, L, Sb, Ti and Hg; 8, 12, 13, and 17 for Cd, Pb, Mo and As; 25 for all other elements.

Fatty acids analysis by GC-FID

Sample preparation

Total crude lipids were extracted from approximately 4 g of milk using a modified Folch method. This involved homogenization with 40 mL of chloroform/methanol (2:1 v/v) solution, ultrasonication (LBS1, Falc Instruments, Bergamo, Italy) for 30 min, pH adjustment at 1.00 with 37% HCl, addition of 5 mL of 0.73% NaCl, vertexing for 2 minutes, and centrifugation at 3500 rpm for 15 minutes at 4°C (MF 20-R, Awel Industries, Blain, France). The lipid phase was filtered, and the solvent was evaporated (Rotavapor R-210, Büchi, Flawil, Switzerland). The lipids were determined gravimetrically. Fatty acid methyl esters (FAME) were then prepared by trans-methylation of 25 mg of the extracted lipids using 0.1 mL of methanolic KOH (2 N), vortexed for 2 minutes, and centrifuged for 1 minute at 3500 rpm. The FAME organic layer was transferred to a new vial for GC analysis.

Instrumental analysis

The fatty acid methyl esters were analyzed using a gas chromatograph (GC) equipped with a split/splitless injector and flame ionization detector (FID) (Dani Master GC1000, Dani Instrument, Milan, Italy). A SLB-IL100 capillary GC column (Supelco, Sigma Aldrich, USA) was used. The analysis involved a specific temperature program for the column (from 180 °C to 270 °C, hold time 2 min, at 3 °C/min) and used helium as carried gas. Injector and detector temperatures were set at 220 °C and 240 C, respectively. FAMEs were identified by comparing their retention to standards, and the percentage of each FAME was calculated based on the total area of the chromatogram.

Evaluation of the Health Benefits and Risks of milk from Barbaresca sheep

Mineral elements intake

The contributions of elements derived from consuming Barbaresca sheep milk were estimated by calculating the daily exposures. These were calculated by multiplying the amount of milk consumed (taken as 200 mL) by the mean concentrations of the elements in the samples. The resulting values were compared with the Nutrient Reference Value (NRV) for the essential elements established by European Food Safety Authority and by European Regulation. For toxic and potentially toxic elements, the estimate exposures (calculated for a 60 kg adult) were compared with toxicological reference values (TRVs) from the European Food Safety Authority and World Health Organization.

Lipid quality

Dietary reference values (DRVs) for polyunsaturated fatty acids (PUFAs) were established in 2009 by the European Food Safety Authority. These were set at 10 g/day for linoleic acid (C18:2 n-6) and 2 g/day for alpha-linolenic acid (C18:3 n-3). The contributions to these DRVs were calculated by considering the quantity of these acids derived from the consumption of 200 mL of milk from the Barbaresca sheep under analysis.

Another key indicator used was the ratio of PUFA n-6 to PUFA n-3. A balanced ratio in the diet is important for health, as an imbalance can lead to chronic diseases. Some studies suggest a ratio of 4:1 for the prevention of coronary heart disease. Consuming foods that are naturally lower in this ratio, such as those rich in PUFA n-3 and with a balanced PUFA n-6, is a good way to improve the dietary ratio.

Additional indices were used to assess lipid quality: the Atherogenicity Index (AI), which estimates the potential of a fat to contribute to the formation of atherosclerotic plaques, and the Thrombogenicity Index (TI), which assesses the tendency of a fat to form clots in blood vessels. The AI and TI indices are calculated using equations that consider the composition of saturated and unsaturated fatty acids. Specifically, the formulas for calculating these indices were derived from Ulbricht & Southgate [16], except for the substitution of C18:0 for C12:0, as suggested by Nudda et al. [17], and are as follows:

$$AI = \frac{\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}}{\text{PUFA} + \text{MUFA}}$$

$$TI = \frac{\text{C14:0} + \text{C16:0}}{[(0.5 \times \text{MUFA}) + (0.5 \times \text{PUFA n} - 6) + (3 \times \text{PUFA n} - 3) + (\text{PUFA n} - 3/\text{PUFA n} - 6)}$$

Another index used to assess the nutritional properties of fatty acids is the hypocholesterolaemic/hypercholesterolaemic ratio (h:H). It is calculated according to Fernández et al. [18] using the following equation:

$$h:H = \frac{\text{C18:1c9} + \text{C18:1c11} + \text{C18:2 n6} + \text{C18:3n6} + \text{C18:3n3} + \text{C20:3n6} + \text{C20:4n6} + \text{C20:5n3} + \text{C22:4n6} + \text{2C2:5n3} + \text{C22:6n3}}{(\text{C14:0} + \text{C16:0})}$$

RESULTS AND DISCUSSION

Mineral composition

Table 2 show the elements concentration in Barbaresca sheep milk.

Calcium and K were the most concentrated macroelements, with values ranging from 2345.7 to 3103.3 mg/L and 1125.6 to 1652.3 mg/L, respectively. Na and Mg were the other two elements found in high concentrations. The Na concentration ranging from 329.57 to 408.11 mg/L, while the Mg concentration ranging from 184.56 to 221.17 mg/L.

Essential trace elements such as Zn, Fe, Mn and Cu were around 2.07, 6.01, 0.197 and 0.159 mg/L, respectively; other essential elements like Cr, Ni and Mo were found at lower concentrations. As, Pb and Cd, according to previous research by the same authors, were around 0.017, 0.010 and 0.005 mg/L, respectively [14]. Cd, Pb, Mo and As were detected above the limit of quantification in only a percentage of samples (32-68%), while Sn, Co, Be, Li, Sb, Ti, and Hg were below the limit of quantification in all samples.

Importantly, the measured Pb and Cd concentrations were always below the legal limits set by Codex Alimentarius Commission and Commission Regulation (EU) 2023/915 for milk consumption (0.020 and 0.050 mg/L, respectively).

Comparing these findings to literature, calcium and potassium levels were found to be consistent with other studies [7-9,19,20]. Sodium and magnesium content also generally agreed with published data [7-9,20]. Zinc and iron levels in the examined samples were higher than those reported in several other studies, while copper and manganese levels were similar [8,9,19-23]. Chromium and nickel levels were lower than some findings [22,23].

The levels of As, Pb, Cd, Hg, and other potentially toxic elements found in this study agree with literature data for sheep milk from regions with low anthropogenic impact [9,21-23].

Fatty acids profile

The fatty acid composition (g/100g FAME) of the milk from Barbaresca sheep is presented in Table 3.

The average amount of total lipids in the examinate sheep milk samples was 5.95%. With an average of 61.51%, saturated fatty acids (SFA) were the most abundant fraction. Within this category, palmitic acid (C16:0) was the most represented (22.92%) followed stearic acid (C18:0) at 7.73% and myristic acid (C14:0) at 7.32%. Monounsaturated fatty acids (MUFA) were the second largest fraction with an average of 30.24 %. Oleic acid (C18:1 n-9) at 23.96 %. was the predominant monounsaturated fatty acid. Polyunsaturated fatty acids (PUFA) account for an average of 8.26%. These included omega-6 fatty acids (PUFA n-6) at 4.36% and omega-3 fatty acids (PUFA n-3) at 2.42%. The main omega-6 was linoleic acid (C18:2 n-6) while alpha-linolenic acid (C18:3 n-3) and eicosapentaenoic acid (C20:5 n-3) were the main omega-3. The isomer CLA cis-9, trans-11 was present at an average of 1.47%. Short chain fat-

Table 3 - Fatty acids composition (g/100g of FAME) and health-related indices of Barbaresca sheep milk.

Fatty acids	Mean ± S.D.	Fatty acids	Mean ± S.D.	Fatty acid grouping and health-related indices	Mean ± S.D.
C4:0	2.78 ± 0.30	C14:1	0.26 ± 0.02	SFA	61.51 ± 0.84
C6:0	2.03 ± 0.14	C15:1	0.02 ± 0.01	MUFA	30.24 ± 0.76
C8:0	2.71 ± 0.41	C16:1 n-9	0.23 ± 0.02	PUFA	8.26 ± 0.58
C10:0	6.45 ± 0.15	C16:1 n-7	1.14 ± 0.13	SCFA	13.98 ± 0.67
C11:0	0.07 ± 0.01	C17:1	0.35 ± 0.01	MCFA	40.70 ± 0.65
C12:0	3.47 ± 0.18	C18:1 n-9	23.96 ± 0.74	LCFA	45.33 ± 0.94
C13:0	0.08 ± 0.01	C18:1 trans-11	4.18 ± 0.03	OCFA	5.36 ± 0.22
C14:0	7.32 ± 0.96	C20:1 n-9	0.05 ± 0.01	BCFA	2.57 ± 0.05
iso C15:0	0.30 ± 0.02	C22:1 n-9	0.01 ± 0.01	OBCFA	1.52 ± 0.07
anteiso C15:0	0.72 ± 0.09	C24:1 n-9	0.04 ± 0.02	PUFA n-6	4.36 ± 0.19
C15:0	1.19 ± 0.08	C18:2 n-6	3.93 ± 0.12	PUFA n-3	2.42 ± 0.43
iso C16:0	0.33 ± 0.03	CLA cis-9, trans-11	1.47 ± 0.17		
C16:0	22.92 ± 0.52	C18:3 n-6	0.23 ± 0.01	C18:2 n-6 (% of DRF)	4.68 ± 0.34
iso C17:0	0.57 ± 0.02	C18:3 n-3	1.98 ± 0.39	C18:3 n-3 (% of DRF)	11.79 ± 2.10
anteiso C17:0	0.64 ± 0.04	C20:2 n-6	0.06 ± 0.02	PUFA n-6/PUFA n-3	1.84 ± 0.28
C17:0	1.08 ± 0.26	C20:3 n-6	0.02 ± 0.02		
C18:0	7.73 ± 0.44	C20:3 n-3	0.03 ± 0.02	AI	1.45 ± 0.09
C20:0	0.40 ± 0.02	C20:4 n-6	0.09 ± 0.05	TI	1.21 ± 0.09
C21:0	0.18 ± 0.01	C20:5 n-3	0.12 ± 0.04	h/H	1.45 ± 0.09
C22:0	0.25 ± 0.02	C22:2	0.03 ± 0.01		
C23:0	0.15 ± 0.01	C22:5 n-3	0.22 ± 0.05	Total lipids (g/100g)	5.95 ± 0.66
C24:0	0.12 ± 0.02	C22:6 n-3	0.07 ± 0.03		

SFA, Saturated Fatty Acids; MUFA, Monounsaturated Fatty Acids; PUFA, Polyunsaturated Fatty Acids; SCFA, Short-Chain Fatty Acids (from C4:0 to C10:0); MCFA, Medium-Chain Fatty Acids (from C11:0 to C17:0); LCFA, Long-Chain Fatty Acids From (from C18:0 to C22:6); OCFA, Odd-Chain Fatty Acids; BCFA, Branched-Chain Fatty Acids; OBCFA, Odd- And Branched-Chain Fatty Acids; PUFA n-6, n-6 Fatty Acids; PUFA n-3, n-3 Fatty Acids; DRF, Dietary Reference Values; AI, Atherogenic Index and TI, Trombogenic Index; h:H, hypocholesterolemic to hypercholesterolemic ratio.

ty acids (SCFA) had an average of 13.98%. Medium chain fatty acids (MCFA had an average of 40.70%. The average percentage of long-chain fatty acids (LCFA) was 45.33%.
The average percentages of odd-chain fatty acids (OCFA) and branched-chain fatty acids (BCFA) were 5.36% and 2.57% respectively. C11:0, C13:0, C15:0, C17:0, C21:0 and C23:0 acids were included in OCFA, whereas iso C15:0, anteiso C15:0, iso C16:0, iso C17:0 and anteiso C17:0 acids were included in BCFA. According to result findings, that Barbaresca sheep milk have a complex and rich lipid composition, with a good presence of beneficial fatty acids such as CLA and omega-3, as well as a good proportion of short-chain fatty acids, which contribute to the characteristic flavor of the milk and cheese products. The predominance of saturated fatty acids is typical of ruminant milk, but the balance of monounsaturated and polyunsaturated fatty acids makes this milk a food with a high nutritional value.
The average percentage of SFA found in this research (over 60%) was comparable with the most bibliographic studies [5,24-28], with some exception [29]. The percentages of C16:0 proved to be comparable to that obtained in milk from two indigenous Greek sheep breeds [26] and in milk from sheep from Tuscany and Sardinia [29]. However, there are some studies in the literature reporting higher levels of this fatty acid in sheep milk [30]. These differences may be related to the different diets that sheep can be exposed to. For example, increasing the amount of hay, fresh grass and maize silage in the diet may lead to a variation in C16:0 content [30].
Stearic acid (C18:0) showed and average content comparable to the literature [25,29], whereas the value of myristic acid (C14:0) seems to be slightly lower than reported in the literature [5,25,26,31,32].
The average MUFA content was also comparable to some data reported in literature [5, 24-25]. However, the values determined in this research were higher than those found by Sinanoglou et al., where the MUFA content varied according to the lactation stage of the sheep [26]. This trend was observed especially for oleic acid (C18:1 n-9), the most abundant of the MUFAs in the present study. PUFA average percentage proved to be twice as high as those reported in the literature [5,24]. Regarding the CLA isomer cis-9, trans-11, its value was also found to be in some cases comparable to literature data [25,29], but in other cases higher [5]. This difference in PUFA and CLA content is related to the type of diet fed to the sheep. In fact, a diet containing linseed oil can increase PUFA in milk, especially C18:3 n-3 and cis-9, trans-11 CLA. In addition, consumption of fresh grass also increases the concentration of cis-9, trans-11 CLA [25,32].
Regarding SCFA, MCFA and LCFA, comparable average percentages were observed with the published data [25,32].

Quality and safety characteristics of milk from Barbaresca sheep

Assessment of the health impact of the mineral elements

The mineral elements in Barbaresca sheep milk were assessed for their health impact, considering both essential elements and potentially toxic ones. Consumption of this milk contributes to the daily intake of minerals crucial for human physiological and metabolic functions.
The contribution of 200 mL of Barbaresca sheep milk to the recommended daily intake of essential elements and the exposure to potentially toxic and toxic elements is summarized in Table 4.
Barbaresca sheep milk provides a significant contribution to the recommended daily of several essential elements. It contributes at 67 % of the daily required intake of calcium and 44 % for zinc.

Table 4 - Essential, potentially toxic and toxic mineral elements with nutrient reference values (NRVs), toxicological reference values (TRVs) and percentage contributions from the samples of Barbaresca sheep milk (200 mL).

Element	Essential/Toxic	NRF	Contribution to NRF	TRV	Contribution to TRV
Ca	Essential	800 mg/day	67 %		
K	Essential	2000 mg/day	14 %		
Na	Essential	2000 mg/day	4 %		
Mg	Essential	375 mg/day	11 %		
Zn	Essential	10 mg/day	44 %		
Fe	Essential	14 mg/day	9 %		
Mn	Essential	2 mg/day	2 %		
Cu	Essential	1 mg/day	3 %		
Cr	Essential	40 µg/day	22 %		
Mo	Essential	50 µg/day	10 %		
Ni	Potentially Toxic			13 $\mu g/kg_{bw}/day$	0.7 %
Li	Potentially Toxic			2 $\mu g/kg_{bw}/day$	-
Sb	Potentially Toxic			6 $\mu g/kg_{bw}/day$	-
Sn	Potentially Toxic			14 $mg/kg_{bw}/day$	-
As	Toxic			0.3 $\mu g/kg_{bw}/day$	23.5 %
Pb	Toxic			10 $\mu g/kg_{bw}/day$	0.7 %
Cd	Toxic			2.5 $\mu g/kg_{bw}/week$	12.8 %
Hg	Toxic			4 $\mu g/kg_{bw}/week$	-

Regarding toxic elements, the levels of arsenic and cadmium measured contribute 23.5% and 12.8% respectively to the respective toxic reference values, suggesting a limited risk to human health, especially considering that the toxicological reference values are precautionary values. The levels of nickel and lead are negligible, contributing only 0.7%. The levels of lithium, antimony, tin and mercury are below the limit of quantification, indicating that there is no risk associated with the consumption of this milk regarding these elements.
In conclusion, Barbaresca sheep milk can be considered safe and healthy in terms of its mineral content. This is attributed to the sheep grazing in the Nebrodi area, an environment with rich endemic vegetation and far from pollution sources, which helps to limit the presence of toxic elements in the milk, while the variety of flora has a positive effect on the mineral composition, enriching it with valuable nutritional elements.

Nutritional evaluation based on fatty acid composition

The results, presented in Table 3, show that Barbaresca milk has important nutritional characteristics. Since 200 mL of milk from Barbaresca sheep contains 0.468 g of linoleic acid, it provides around 4.68% of the daily required consumption of linoleic acid (10 g/day). 200 mL of Barbaresca sheep milk provides 0.236 g of alpha-linolenic acid, that is approximately 11.8% of the daily recommended intake of 2 g. These results imply that milk from Barbaresca sheep may be useful in fulfilling daily PUFA requirements, contributing significantly to consumption of both linoleic and alpha-linolenic acids. The PUFA n-6/PUFA n-3 ratio was 1.84, indicating also a good balance between omega-6 and omega-3 fatty acids. The AI was 1.45 and the TI was 1.21; these were lower than those reported by Kasapidou et al., where the AI was 2.59 and a TI of 2.71 [5], as well as those reported by Paszczyk & Łuczy ska, where the AI was 1.63 ± 0.06 and the TI was 3.13 ± 0.13 [30]. Despite being higher than 1, these values are very good for a product of animal origin. Furthermore, a good balance between lipids that are favorable (hypo) and unfavorable (hyper) for heart health is indicated by the hypocholesterolaemic/hypercholesterolaemic (h/H) ratio of 1.45. The lipid profile of Barbaresca sheep milk, with its potential positive effects on cardiovascular health and balanced omega-6 to omega-3 PUFA ratio, could be a result of the sheep grazing on the diverse endemic vegetation of the Nebrodi area. This rich flora, including species like Graminaceae and Asteraceae, may contribute to the milk wealth of beneficial fatty acids.

CONCLUSIONS

According to the obtained results, Barbaresca sheep milk is characterised by a high content of essential minerals and a rich and complex lipid profile, qualities likely influenced by the sheep diet in the Nebrodi region. This area, known for its diverse endemic vegetation and relative lack of pollution, allows the sheep to produce milk that contributes significantly to the recommended daily intake of essential minerals such as calcium and zinc and can be considered safe in terms of the presence of toxic or potentially toxic elements, with negligible levels of some. With a good intake of linoleic and linolenic acids, a good balance of omega-6 and omega-3 fatty acids, and very low values for atherogenicity and thrombogenicity indices as well as the hypocholesterolaemic/hypercholesterolaemic ratio, Barbaresca sheep milk has a lipid profile that suggests benefits for cardiovascular health. This beneficial composition is potentially a direct result of the sheep grazing on the unique flora of the Nebrodi mountains.

Credit authorship contribution statement

Francesca Aragona: Formal Analysis, Investigation, Data Curation. **Vincenzo Nava**: Methodology, Validation, Formal Analysis, Investigation. **Angela Giorgia Potortì**: Data Curation, Writing-Original Draft Preparation, Writing-Review and Editing, Supervision. **Federica Litrenta**: Methodology, Validation, Investigation. **Antonino Costa**: Formal Analysis. **Francesco Fazio**: Conceptualization, Supervision. **Vincenzo Lo Turco**: Conceptualization, Writing-Review and Editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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