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Fatty acids, essential amino acids, minerals and proteins profile in whey from goat cheese: Impacts of raising system

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ABSTRACT

This study aimed to evaluate the nutritional quality of goat whey considering the impacts of raising system. Goat whey from *Coalho* cheese processing was assessed for the minerals, organic acid and fatty acid profiles, amino acid composition, and protein pattern. The pasture production system resulted in products with a better mineral composition (higher concentration of calcium, iron, and potassium and lower sodium concentration) and improved fatty acid profile (lower proportions of medium-chain fatty acids, higher concentration of poly-unsaturated fatty acids, lower n6-n3 ratio, and better health indices). Furthermore, a greater intensity of protein bands and proteins of higher molecular weight was observed. On the other hand, the confinement production system resulted in higher concentrations of essential amino acids, glutamic and aspartic acids, alanine, and proline. The results demonstrated that goat whey has a high nutritional value and potential as an ingredient to the food industry, regardless of the raising system.

1. Introduction

Goat milk and its derivatives have gained importance in the human diet due to their composition and associated health effects (Chávez-Servín et al., 2018). As a result, the goat milk market has been valued at US$ 8.5 billion, and it is expected to achieve US$ 11.4 billion by 2026, with an annual growth rate of 3.8 % in the years 2019–2026 (Bezerril et al., 2021).

Goat whey is the aqueous portion obtained after coagulation and curd removal in cheesemaking (Galdino et al., 2020). Historically, milk whey is processed into dairy products very appreciated for their taste and opportunity of exploitation of by-products by the dairy sector (farmhouse or industry). In some countries such as France, Greece, Portugal, Spain, Italy, Norway, Sweden, Turkey and Lebanon whey 'is employed in the production of whey cheeses. Normally, these products are typically obtained from ovine, caprine, bovine or buffalo cheese whey. In Italy, Ricotta cheese, particularly the ovine type, is an Italian typical dairy product obtained by heat-coagulation of whey proteins (Pirisi et al., 2011). In Lebanon, there is a very old production linked to the Baladi breed, of a cheese produced with goat whey, called Archichi cheese (Hayaloglu and Karagul-Yuceer, 2011). In rural properties in Sweden, "red cheese" is produced with goat whey, through boiling whey for a full day, which caramelizes the lactose and the whey turns into a red, sweet thick mass (Eriksson and Bull, 2017). It comes in several varieties differing in the degree of browning. In Norway, it is usually called brown whey cheese (Brunost) and in Sweden it is called "Mesost"

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(Skeie and Abrahamsen, 2017).

The whey has been increasingly applied in food products because of its nutritional properties (Gojkovic et al., 2019). For example, it may contain functional compounds, such as sialic acid, oligosaccharides, proteins, and peptides (Sousa et al., 2019). In addition, its consumption has been associated with several health effects, such as anti-inflammatory effects (Araújo et al., 2017), antioxidant activity (Gojkovic et al., 2019), antitumor properties (Medeiros et al., 2018), and modulation of the intestinal microbiota (Paulino et al., 2019).

Goat whey composition depends mainly on the processing techniques used in cheese production and on the original composition of the milk used. Thus, animal breed, lactation stage, geographical location, the season of the year, and the production system can influence the composition of whey (Chávez-Servín et al., 2018). The Brazilian semiarid region is mainly covered with the Caatinga Biome and is one of the less developed regions of the country. Therefore, promoting local agro-industrial activities, including goat milk production, has a significant role in developing the local economy and sustainability of these rural areas. The most common production systems used are the free-range grazing (pasture extensive production system) and the permanent confinement (confinement intensive production system) (Chávez-Servín et al., 2018). In the free-range grazing, the goats graze and browse the plants in the Caatinga, while in the permanent confinement, the goats are confined and fed typical diets (Santos et al., 2021).

A significant effect of feeding system on the fatty acid, volatile and sensory profile of goat milk and cheese has previously been shown (Sant'Ana et al., 2019), and a previous study observed that goat whey from free range grazing milk presented a higher concentration of phenolic compounds and antioxidant activity compared to permanent confinement (Chávez-Servín et al., 2018). However, as far as the authors know, no previous study evaluated the impact of raising system on the chemical composition and nutritional value of goat whey obtained by milk from goats of semiarid region of Brazil. Thus, the present study aimed to assess the impact of raising system on the chemical composition, fatty acids, amino acids, protein profile and minerals of goat whey. obtained after Coalho cheesemaking process.

2. Material and methods

2.1. Animals and production systems

Twelve crossbreds Sannen×American-Alpine goats, averaging 51.4 ± 3.75 kg of live weight. The goats producing on average 1.3L of milk per day and with approximately and were maintained at the Experimental Station of the Federal University of Paraíba in the municipality of São João do Cariri in the Paraíba State. The Experimentation Station was located in the Northeastern Region of Brazil at latitude 07° 23 '27 "S, longitude 36° 31' 58" W, 458 m of altitude, and a mean temperature of 24 °C. According to Koppen's climatic classification, the region's climate is hot semiarid, with an average annual rainfall of less than 600 mm (Alves et al., 2014).

The production systems and the diets fed to the animals corresponded to the usual practice in the region. Two types of a production systems were studied: PAS (pasture production system) and CON (confinement production system). In PAS, the goats had daily access to Caatinga natural pasture, which consists of species belonging to the families *Asteraceae (11 %)*, *Fabaceae (11 %), Poaceae* (11 %), *Malvaceae* (7 %), *Euphorbiaceae* (6 %), *Cactaceae* (4 %), and *Apocynaceae* (2 %) (Sant'Ana et al., 2019).

The twelve animals, six CON and six PAS, were submitted to the feed adaptation system for 40 days, so that the first sample of milk from each treatment was performed after 70 days of lactation. The next four days were used to collect milk samples. The animals were released after the first milking in the morning (7 am) and collected at the end of the afternoon for the second milking (4 pm). They were then taken to individual stalls where they received a concentrate supplement in the amount of 1.5 % of live weight. In CON, the goats were managed in individual stalls of a 3.75 m^2 area, providing feeders and drinkers. The diet for this system was composed of 51 % of elephant grass (*Pennisetum purpureum*) hay and 49 % of concentrate supplementation at 1 kg per animal per day. The diet composition of both production systems is shown in Table 1. Caprine milk was collected during three consecutive days and twice a day, kept under refrigeration (4 °C ± 1 °C), homogenized, and bottled.

2.2. Sampling goat whey

The *Coalho* cheese production was performed according to the procedure described by Sant'Ana et al. (2019). First, 30 L of goat milk for each experimental group was pasteurized (65 °C/ 30 min), cooled down (37 °C), and added with calcium chloride (0.5 mL/L) and a commercial coagulating agent (0.9 mL/L, Ha-la, Christian Hansen®, Valinhos, Brazil, Force: 1:3000/75 IMCU). The mixture was incubated at 36 °C until the formation of the curd (approximately 40 min). Then, the gel was cut into cubes (1.5 cm) and allowed to drain, placed in rectangular moulds (approximately 250 g capacity, 8.5 × 9.0 × 15.5 cm - h × w × l), salted (1 g/100 g), and maintained at 36 °C under pressure for 4 h. Six cheeses from each experimental group (PAS and CON) were produced. All goat whey obtained during desorption was bottled, frozen at − 80 °C, and lyophilized for the chemical composition and sugar and fatty acid profiles analyses (dry basis). For protein profile, the lipid phase was removed by centrifugation (2900×*g* for 20 min at 4 °C) and dialyzed against distilled water for 24 h using dialysis membranes (14 kDa cut-off) before lyophilization. For the physicochemical analysis (pH and titratable acidity), liquid goat whey was used (wet basis).

2.3. Chemical composition and physicochemical analysis

The chemical composition (moisture, fat, protein, and ash) was determined using the methodologies described by the Association of Official Analytical Chemistry (AOAC, 2016). The pH was measured using a potentiometer (Q400As - Quimis®). The titratable acidity was performed following AOAC (2016).

Sugar and organic acid profile were determined using a soluble extract, obtained using the method described by according to the methodology described by Zeppa et al. (2001). The samples (2 g) were diluted in 10 mL of ultrapure water, mixed, centrifuged for 10 min, and

Table 1
Composition (%) of dietary components for the two production systems and the chemical composition of the individual ingredients of the experimental diets (based on dry matter; g kg^{-1}).

Dietary components	System	
	PAS[a]	CON[b]
Native "Caatinga" vegetation	ad libitum	–
Elephant grass (*Pennisetum purpureum*) hay	–	51 %
Soybean meal	30 %	12.5 %
Corn meal	69 %	36 %
Mineral supplement	1 %	0.5 %

Chemical composition[c] (%)	DM	CP	EE	FDN	TC	NFC	Ash
Elephant grass (*Pennisetum purpureum*)	87.25	7.30	1.15	70.49	83.45	12.95	8.10
Soybean meal	88.88	51.73	1.33	11.47	40.88	29.42	6.05
Corn meal	88.63	9.41	4.53	18.40	84.79	66.39	1.27

[a] Percentage of the concentrate supplementation based on animals' weight (1.5 %).
[b] Percentage of the concentrate supplementation (based on 1 kg per animal per day).
[c] DM: Dry matter; CP: Crude protein; EE: Ethereal extract; FDN: Fiber in neutral detergent; TC: Total carbohydrates; NFC: Non-Fibrous Carbohydrates.

filtered on a 0.45 μm cellulose filter. Sugars were quantified using high-performance liquid chromatography (HPLC, Varian, Waters 2690, California, USA) with a refractive index detector coupled with a Hi-Plex Ca column at a temperature of 85 °C and using ultrapure water as the mobile phase at a flow rate of 0.6 mL/min. The same equipment was used for the organic acids with a UV detector coupled with a Hi-Plex H column, set at 220–275 nm wavelength, temperature of 25 °C, and using sulfuric acid at 0.009 mol/L as the mobile phase at a flow rate of 0.7 mL/min. The running time for both analyses was 30 min. The data processed in Galaxie Chromatography Data System software and the quantification of the organic acids profile (oxalic, citric, acetic, lactic, succinic, pyruvic and formic acids) was obtained by injection of standard curve acids and expressed as g/100 g of whey.

2.4. Mineral profile

The AOAC method n. 999.10 was used for the mineral analysis (Horwitz and Latimer, 2012). The samples were prepared using acid-assisted microwave digestion by weighing 0.5 g of the sample in Teflon tubes and adding 7 mL of nitric acid purified by sub-boiling distillation (Distillacid, Berghof, Eningen, Germany) and 1 mL of 30 g/100 mL hydrogen peroxide (Merck, Darmstadt, Germany). The digestion program used a maximum temperature of 170 °C for 37 min, and the tube contents were transferred with purified water by reverse osmosis (Gehaka, São Paulo, Brazil) into a 25 mL flask. The analytical blanks were prepared according to the same procedure without the sample. Mineral determination was performed in an atomic emission spectrometer with inductive coupling plasma (ICP OES 5100 VDV, Agilent Technologies, Tokyo, Japan).

2.5. Fatty acid profile

The sample preparation for fatty acid profile analysis was performed following Sant'Ana et al. (2019). Gas chromatograph (GC) fatty acid methyl esters were analyzed with flame ionization detection (GC-FID) using a Shimadzu QP2010-plus (Shimadzu, Kyoto, Japan) equipped with a fused silica capillary column (SP-2560, 100 m × 0.25 mm × 0.20 μm, Supelco, Bellefonte, PA, USA). The injector and detector were kept at 250 and 280 °C, respectively. Helium was used as the carrier gas at 1 mL/min constant flow rate, and 1 μL of the sample was injected. The temperature program was as follows: 50 °C for 1 min; ramped to 150 °C at 50 °C/min and held for 20 min; then ramped to 190 °C at 1 °C/min, held for 1 min; and ramped to 220 °C at 2 °C/min, held for 30 min. For additional identification of fatty acid methyl esters, including the branched-chain fatty acids (BCFA), was used gas chromatography-mass spectrometry (GC-MS) using a GC-MS QP2010-plus chromatograph (Shimadzu, Kyoto, Japan), equipped with a SP-2560 column. The GC conditions were similar to the GC-FID analysis; the MS conditions were as follows: 200 °C ion source temperature; 240 °C interface temperature; 70 eV emission voltage. Identification of fatty acid (FA) methyl esters was achieved by GC-MS analysis, comparing the retention times with those of authentic standards (FAME mix 37 components from Supelco Inc., Bellefont, PA, USA) and by comparison with published chromatograms (Alves and Bessa, 2014).

The atherogenic index (AI), thrombogenic index (TI), desired fatty acid (DFA), and hypercholesterolemic saturated fatty acid (HSFA) were calculated according to Ulbricht and Southgate (1991).

2.6. Protein profile

The lyophilized goat whey (1 mg) was re-suspended in 1 mL of water and soluble proteins were determined using the Bradford (1976) method. Then, the obtained data were compared with a standard curve constructed with bovine serum albumin (BSA).

The protein profile was obtained by SDS-PAGE electrophoresis and 2D-PAGE coupling isoelectric focusing (IEF) and SDS-PAGE. SDS-PAGE was performed according to the technique described by Laemmli (1970), with the use of 15 g/100 g polyacrylamide separation gel in 3 mol/L Tris-HCl buffer, pH 8.8, and 1 g/100 g SDS, while the concentration gel was prepared with 3.5 g/100 g polyacrylamide in 0.5 mol/L Tris-HCl buffer, pH 6.8 and 1 g/100 g SDS. Samples (1 mg/mL) were dissolved in 0.625 mol/L Tris-HCl buffer, pH 6.7, in the presence of 2 g/100 g SDS, 10 g/100 mL glycerol, 5 g/100 g β-mercaptoethanol, and 0.02 g/100 g bromophenol blue. Then, they were heated to 100 °C for 10 min, and 10 μL and 20 μL volumes for PAS and CON whey were consecutively applied to the gels. A 12–225 kDa protein marker was used as the molecular weight marker (Amersham™ ECL™ Rainbow™ Marker - Full Range, GE Healthcare, RPN800E). The electrophoresis run was performed under the conditions of 200 V, 25 mA, and 15 W. After completing the run, the gels were then incubated in methanol, acetic acid, and water (40:10:50) solution for 45 min, then in 10 g/100 mL TCA and stained overnight with Coomassie Brilliant Blue R-250 at 0.005 g/100 g. Next, the gel was rinsed with methanol, acetic acid, and distilled water solution (20:20:70) and scanned (Image Scanner III da GE HealthcareLife Science) using Labscan 6.0 software.

The 2D-PAGE was adapted from the O'Farrel and Klose (1975) method. Sample preparation consisted of weighing 1 mg of lyophilized whey added with 2.5 mg of DTT (dithiothreitol), 250 μL of Destrick solution, and 2.5 μL of IPG (Immobilized pH gel) buffer (pH 3–10). The sample was then applied to a linear strip of 13 cm, pH 3–11 (GE Healthcare Life Science) in an IPG Box with mineral oil on the surface and remained at 25 °C for 18 h. Then the strip was removed and taken to the isoelectric focusing system (IPGPhor III - GE Healthcare®), configuring the first dimension of the electrophoresis. Next, the strip was equilibrated with DTT and iodoacetamide and then placed over the gel made with 15 g/100 g polyacrylamide in the presence of SDS for executing the second dimension (SDS-PAGE). The protein marker used as the standard was the same one used for the one-dimensional SDS-PAGE electrophoresis. After applying the current (100 V, 30 mA, and 50 W), the gel was fixed and rinsed under the same conditions as SDS-PAGE and then scanned (GE Healthcare Life Science Image Scanner III) using Labscan 6.0 software. ImageMaster™ 2D Platinum 7.0 software and the *TagIdent* tool of the ExPASy (Expert Protein Analysis System) with the Swiss-Prot database (Bairoch and Apweiler, 2000) were used to identify the isoelectric points and the molecular weights of proteins from the spots.

2.7. Total amino acid profile

The total amino acid composition was determined by pre-column derivatization of the amino acids released after acid hydrolysis of the proteins, which occurred by diluting 0.15 g of the sample in 9 mL of HCl at 6 mol/L under heating (110 °C for 22 h). Amino acid analysis was performed on HPLC (Shimadzu Corporation, Kyoto, Japan) using a C18 LUNA 100 Å reversed-phase column (4.6 mm × 250 mm; 5 μm particle) (Phenomenex, Torrance, CA, USA), according to the method described by White et al. (1986). The amino acids were quantified by comparison with standard Thermo Scientific amino acids (Rockford, USA). DL-2-aminobutyric acid (Sigma-Aldrich®, St. Louis, MO, USA) was used as an internal standard.

2.8. Statistical analysis

All analyses were performed in triplicates. The data were submitted to analysis of variance (ANOVA) with the two production systems as a variation source, as well as the F test performed at a 5 % significance level ($P < 0.05$) using the Statistical Analysis System (SAS) version 9.0 software (SAS Institute Inc, Cary, NC, USA).

3. Results and discussion

3.1. Gross composition and physicochemical parameters

The goat whey showed chemical composition for PAS and CON, (g/100 g, dry basis), respectively: moisture (13.12 and 13.42), total dry matter (86.88 and 86.58), fat (7.87 and 6.98), protein (14.43–16.64), lactose (56.45 and 54.25), and ash (7.94 and 7.35). Furthermore, it showed a pH of 7.19 for PAS and 6.88 for CON. For titratable acidity, the results are 0.11 % and 0.10 % lactic acid for PAS and CON, respectively (Table 2). Similar chemical composition (on a dry basis) and pH values were observed in previous studies with goat whey from rennet-type cheese (Silveira et al., 2015; Galdino et al., 2020).

The production system type did not affect the gross chemical composition (moisture, total dry matter, fat, protein, lactose, and ash) and physicochemical parameters (pH and titratable acidity) of the goat whey ($P > 0.05$, Table 2).

The whey composition may vary depending on the cheese processing, cheese type, original milk composition, that can be affected by other factors such as, lactation phase, breed, seasonality and feed (Pires et al., 2021). The Lactose consisted of a high proportion (62–65 %) of the total whey solids in this study and was the only sugar identified in the goat whey. This result may be due to cheese technology because we used goat whey from Coalho cheese, obtained after the coagulation of casein using enzymes, processed without using starter cultures.

A previous study reported the presence of lactose, glucose, and galactose in goat whey (Thum et al., 2015). However, they used goat whey from Camembert-type cheese, which was processed using starter culture (*L. lactis* subsp *lactis*, *L. lactis* subsp *cremosis*, and *Streptococcus thermophilus*) and fungus (*Geotrichum candidum*). The starter culture may have partially metabolized the lactose present in the medium, increasing the concentration of glucose and galactose in the whey (Sousa et al., 2019). Therefore, the differences in the sugar profile may be associated to the coagulation process (rennet or acid coagulation).

The highest pH value observed in the present study (6.88 for PAS and 7.19 for CON) also is an important indicator of whey quality. Whey obtained after the coagulation of milk using enzymes is called sweet. The highest concentrations of lactose and pH values in the sweet whey may be interesting from the industry point of view, because this ingredient would have a higher lactose yield and applications in food products as the production of lactose concentrates with several potential industrial applications. The content Lactose in whey can be recovered from selective membrane separation technologies such as ultrafiltration and nanofiltration, and be used, for example, an important ingredient for infant formula, as sweetener in food products such as ice-creams and baby food (Pires et al., 2021; Pisponen et al., 2013).

Table 2
Chemical composition (% DM) and physicochemical characteristics of the goat whey from pasture production system (PAS) and confined production system (CON) (mean value ± S.d.).

Variables	Goat whey		
	PAS	CON	*P-value
Moisture (g/100 g)	13.12 ± 1.44	13.42 ± 1.21	0.229
Total dry matter (g/100 g)	86.88 ± 1.44	86.58 ± 1.21	0.229
Fat (g/100 g)	7.87 ± 1.29	6.98 ± 0.96	0.382
Protein (g/100 g)	14.43 ± 0.62	16.64 ± 0.92	0.103
Lactose (g/100 g)	56.45 ± 0.40	54.25 ± 0.40	0.589
Ash (g/100 g)	7.94 ± 0.32	7.35 ± 0.41	0.284
Acidity (% lactic acid)	0.11 ± 0.03	0.10 ± 0.02	0.735
pH	7.19 ± 0.12	6.88 ± 0.29	0.133

Sd = Standard deviation.
* Considering significance at $P < 0.05$.

3.2. Organic acid profile

The goat whey showed succinic acid (0.65–0.66 g/100 g), pyruvic acid (0.27–0.28 g/100 g), lactic acid (0.07–0.08 g/100 g), citric acid (0.10–0.11 g/100 g), acetic acid (0.03–0.05 g/100 g), oxalic acid (0.02 g/100 g), and formic acid (0.07 g/100 g) as organic acids (Table 3). Organic acids are commonly found in milk and derivatives, such as citric, pyruvic, lactic, acetic, propionic, and hippuric acids (Rocha-Mendoza et al., 2021). Citric acid is commonly retained in whey during cheese processing, mainly because of its high solubility (Yadav et al., 2015), while succinic acid can be produced as an intermediate product of the citric acid cycle (Wan et al., 2008). The low concentrations of lactic acid can be explained by type of the manufacture of cheese, with milk pasteurization and addition of rennet to milk, that works by curdling the casein present in the milk leading to the formation of curd (Pires et al., 2021), without the presence of the activity of lactobacilli that convert lactose to lactic acid.

The production system type did not affect the organic acid profile of the goat whey ($P > 0.05$, Table 3). Organic acids have received significant interest by the food industry due to their role as antimicrobial agents in food packaging, shelf-life extension, and food safety (Deng et al., 2016; Adeleke et al., 2017). Thus, strategies for recovering organic acids from different lower-cost and high-volume biological sources have been researched (Saxena et al., 2017). Our results demonstrate that goat whey could be a source of organic acids for food applications, regardless the production system used.

3.3. Mineral profile

The goat whey showed potassium (2967.20–3079.40 mg/100 g), sodium (676.90–722.75 mg/100 g), phosphorous (484.24–489.60 mg/100 g), calcium (460.83–484.24 mg/100 g), magnesium (115.53–117.29 mg/100 g), zinc (0.12–0.13 mg/100 g), iron (0.06–0.09 mg/100 g), and copper (0.06 mg/100 g) as minerals (Table 4). Like other milk components, the concentrations of minerals are influenced by several factors including by the mineral composition of feed. In milk, the mineral composition is affected was found not only between the different feeding systems but also within different pasture-based systems (Gulati et al., 2018). At the same time, the mineral content of milk is highly affected by the developmental stage of the plants consumed by the animals (Gabryszuk et al., 2010). However, during cheese manufacture, minerals are lost to the whey rapidly as whey pH decreases (Poulsen et al., 2015). Thus, the minerals in goat milk are dispersed in the whey or associated with proteins, and the total content is influenced by the mineral composition of the original milk (Moreno-Montoro et al., 2015).

The production system type had a significant effect on the goat whey mineral content ($P > 0.05$, Table 4). The PAS whey presented higher ($P < 0.05$) concentrations of potassium, calcium, and iron and lower concentrations of sodium and magnesium than CON whey ($P < 0.05$; Table 4).

Table 3
Organic acid composition of the goat whey from pasture production system (PAS) and confined production system (CON) (mean value ± S.d.).

Organic acids (g/100 g)	Goat whey		
	PAS	CON	*P-value
Succinic	0.65 ± 0.30	0.66 ± 0.31	0.317
Pyruvic	0.28 ± 0.01	0.27 ± 0.10	0.282
Lactic	0.10 ± 0.01	0.08 ± 0.01	0.114
Citric	0.10 ± 0.03	0.11 ± 0.01	0.246
Acetic	0.03 ± 0.02	0.05 ± 0.04	0.500
Oxalic	0.02 ± 0.00	0.02 ± 0.00	0.524
Formic	0.07 ± 0.01	0.07 ± 0.01	0.352

Sd = Standard deviation.
* Considering significance at $P < 0.05$.

Table 4
Mineral profile of the goat whey from pasture production system (PAS) and confined production system (CON) (mean value ± S.d.).

Minerals (mg/100 g)	Goat whey		
	PAS	CON	*P*-value
Potassium	3079.40[a] ± 1.64	2967.20[b] ± 5.44	< 0.001
Sodium	676.90[b] ± 3.81	722.75[a] ± 3.90	< 0.001
Phosphorus	484.24 ± 3.15	489.60 ± 2.32	0.075
Calcium	474.14[a] ± 3.30	460.83[b] ± 1.40	0.001
Magnesium	115.53[b] ± 0.90	117.29[a] ± 0.43	0.030
Zinc	0.12 ± 0.03	0.13 ± 0.02	0.617
Iron	0.09[a] ± 0.01	0.06[b] ± 0.00	0.019
Copper	0.06 ± 0.02	0.06 ± 0.00	0.446
Manganese	0.007 ± 0.00	0.007 ± 0.00	0.426

[a, b]Different letters on the same line indicate a significant difference (F test, $P < 0.05$)
Sd = Standard deviation.

Potassium is a mineral found in higher amounts in goat milk than human, bovine, or ovine milk, resulting in high concentrations in goat whey (Raynal-Ljutovac et al., 2008). Potassium, sodium, and magnesium are in the aqueous milk phase, and, consequently, they can be observed in higher concentrations in whey (Sousa et al., 2019). Sodium presence in whey is also associated with salt added in cheese processing. On the other hand, copper, iron, and zinc are more closely linked to caseins in ruminant milk, and therefore present in lower amounts in the whey (Sousa et al., 2019). Therefore, pasture consumption promoted a higher concentration of important minerals (potassium, calcium, and iron) in whey and decreased sodium, which is an important results as reduced sodium consumption has been commonly recommended.

3.4. Fatty acid profile

The fatty acid profile of goat whey is presented in Table 5. The most abundant fatty acids present in whey were in descending order, the palmitic (16:0), oleic (18:1c9), stearic (18:0), myristic (14:0), capric (10:0), lauric (12:0), caprylic (8:0), caproic (6:0), butyric (4:0) and linoleic (18:2n-6). This fatty acid profile is similar to that reported for goat milk (Sant'Ana et al., 2019), that originated the whey of this study.

The production system type had a significant effect on the fatty acid profile of the goat whey ($P < 0.05$, Table 5). The PAS whey presented higher ($P < 0.05$) proportions of UFA and *trans* fatty acids than CON, represented by the higher concentration of 18:1c9, and most *trans*-18:1 isomers, 18:2n-6, and 18:3n-3. Furthermore, it presented lower concentrations of medium-chain fatty acids (C12:0 and C14:0), caprylic (8:0), caproic (6:0), and 18:2c9,t11 fatty acids ($P < 0.05$. The lower metabolized energy availability in the pasture diet may have decreased their concentration of medium-chain fatty acids in PAS (Sant'Ana et al., 2019), and may have mobilized more 18:0 and 18:1c9 from adipose tissue reserves to the milk, resulting in higher concentrations of these fatty acids in PAS (Sant'Ana et al., 2019; Palma et al., 2017). The concentration of rumenic acid (18:2c9t11), the main CLA isomer, was higher in CS group compared with the PS group is which surprising, as grazing ruminants usually produce milk enriched with 18:1t11 (Claps et al., 2018). Pasture has a higher concentration of long-chain fatty acids, which enter the digestive tract of the goats, pass into the bloodstream, and are incorporated into the milk fat (Timlin et al., 2021). In this way, pasture may contribute with increases in 18:2n-6 and 18:3n-3 fatty acids (Sant'Ana et al., 2019), which may form trans-18:1 by ruminal biohydrogenation (Kilcawley et al., 2018). Finally, higher concentration of tannins in PAS may induce incomplete biohydrogenation in the rumen, resulting in the accumulation of trans-18:1 intermediate (Vasta and Bessa, 2012).

The changes in the fatty acid profile promoted by PAS may be significant from the health and sensory point of view. PUFA consumption has been associated with a reduction in total and low-density lipoprotein cholesterols, increase in high-density lipoprotein cholesterol, antihypertensive properties, regulation of hormonal secretion, among others (Claps et al., 2018). At the same time, lower consumption of MCFA fatty acids is recommended due to their hypercholesterolemic

Table 5
Fatty acid profile and health indices of the goat whey from pasture production system (PAS) and confined production system (CON) (mean value ± S.d.).

Fatty acids (g/100 g fat)	Goat whey		*P*-value
	PAS	CON	
C4:0	1.60 ± 0.26	1.88 ± 0.30	0.116
C6:0	1.95[b] ± 0.29	2.28[a] ± 0.10	0.022
C8:0	2.13[b] ± 0.33	2.67[a] ± 3.91	0.005
C10:0	7.76[b] ± 1.26	9.95[a] ± 0.67	0.004
C12:0	2.60[b] ± 0.41	3.91[a] ± 0.29	< 0.001
*iso*C14:0	0.12 ± 0.03	0.14 ± 0.02	0.216
C14:0	9.19[b] ± 1.31	11.78[a] ± 0.59	0.001
*iso*C15:0	0.24 ± 0.31	0.27 ± 0.06	0.119
*anteiso*C15:0	0.37 ± 0.04	0.42 ± 0.05	0.294
C14:1c9	0.10 ± 0.08	0.15 ± 0.06	0.283
C15:0	0.82 ± 0.11	0.91 ± 0.12	0.231
*iso*C16:0	0.37 ± 0.10	0.33 ± 0.09	0.146
C16:0	34.00 ± 2.53	32.33 ± 2.64	0.238
C16:1c7	0.39[a] ± 0.10	0.21[b] ± 0.04	0.002
C16:1c9	0.61 ± 0.29	0.60 ± 0.10	0.693
C17:0	0.66[a] ± 0.07	0.48[b] ± 0.04	0.003
*iso*C17:0	0.31 ± 0.03	0.35 ± 0.09	0.572
*anteiso*C17:0	0.42 ± (0.04	0.41 ± 0.04	0.080
Phytanic	0.31[b] ± 0.05	0.22[a] ± 0.06	0.017
C17:1c9	0.28 ± 0.08	0.20 ± 0.03	0.078
C18:0	10.10[a] ± 1.60	7.50[b] ± 1.03	0.001
C18:1t16 +c14	0.07[b] ± 0.01	0.09[a] ± 0.01	0.012
C18:1t6 +t8	0.14[a] ± 0.01	0.11[b] ± 0.01	0.026
C18:1t9	0.15 ± 0.03	0.13 ± 0.01	0.085
C18:1t10	0.16[a] ± 0.03	0.10[b] ± 0.44	0.001
C18:1t11	0.53 ± 0.09	0.44 ± 0.01	0.054
C18:1t12	0.18[a] ± 0.02	0.13[b] ± 0.02	0.002
C18:1c9	19.94 ± 1.80	19.31 ± 1.90	0.783
C18:1c11	0.40[a] ± 0.09	0.29[b] ± 0.04	0.026
C18:1c12	0.12[a] ± 0.02	0.07[b] ± 0.00	0.024
C18:1t16 +c14	0.14[a] ± 0.01	0.09[b] ± 0.02	0.012
C18:2n6	1.80[a] ± 0.50	1.21[b] ± 0.08	< 0.001
C18:3n3	0.26[a] ± 0.07	0.08[b] ± 0.01	<0.001
C20:0	0.28[a] ± 0.02	0.24[b] ± 0.04	0.001
C20:1	0.04 ± 0.00	0.03 ± 0.00	0.651
C20:4n6	0.18[a] ± 0.04	0.13[b] ± 0.01	0.028
C21:0	0.07 ± 0.00	0.07 ± 0.01	0.447
C22:0	0.11[a] ± 0.02	0.07[b] ± 0.00	0.005
C22:5n3	0.06[a] ± 0.01	0.04[b] ± 0.00	0.004
C23:0	0.03 ± 0.00	0.02 ± 0.00	0.432
C24:0	0.04[a] ± 0.01	0.02[b] ± 0.00	0.002
CLAc9t11	0.27[b] ± 0.08	0.35[a] ± 0.03	0.036
SCFA	5.68 ± 0.07	6.82 ± 0.06	0.101
MCFA	21.20[b] ± 0.04	27.54[a] ± 0.04	< 0.001
LCFA	62.91 ± 0.02	65.59 ± 0.01	0.79
SFA	74.28 ± 0.03	76.35 ± 0.03	0.122
MUFA	23.14 ± 0.02	21.84 ± 0.03	0.302
PUFA	2.51[a] ± 0.02	1.81[b] ± 0.02	< 0.001
Trans	1.65[a] ± 0.02	1.30[b] ± 0.01	0.002
MUFAs/SFAs	0.31 ± 0.03	0.29 ± 0.02	0.248
PUFAs/SFAs	0.003[a] ± 0.02	0.002[b] ± 0.02	< 0.001
UFA/SFA	0.35 ± 0.04	0.31 ± 0.04	0.128
n6-n3	6.92[b] ± 0.04	15.12[a] ± 0.04	0.001
DFA	35.93[a] ± 0.01	31.14[b] ± 0.01	0.005
AI	2.96[b] ± 0.03	3.52[a] ± 0.03	0.005
TI	3.98[b] ± 0.04	4.36[a] ± 0.03	0.005
HSFA	45.79[b] ± 0.05	48.02[a] ± 0.04	0.006

SCFA - Short chain fatty acids (C4:0-C9:0); MCFA - Medium chain fatty acids (C10:0-C15:1); LCFA - Long chain fatty acids (C16:0-C24:0); SFA -Saturated fatty acids; MUFA - Monounsaturated fatty acids; PUFA - Polyunsaturated fatty acids; Trans; MUFAs/SFAs; PUFAs/SFAs; DFA - Desirable fatty acids (MUFA+PUFA+C18:0), AI- Atherogenic index (C12:0 + 4 *C14:0 + C16:0)/(C18:2n6 + C18:3n3), TI- Thrombogenic index (C18:0 + C16:0 + C14:0)/(0.5 *MUFA) + (3 * C18:3n3) + (C18:3n3/ C18:2n6), HSFA- Hypercholesterolemic saturated fatty acid (C12:0 + C14:0 + C16:0). Sd = Standard deviation.

effect (Sepe and Argüello, 2019). The beneficial effect of PAS whey may also be observed by linoleic acid (n-6) and α-linolenic acid (n-3) ratio and health indices. In our study, the PAS whey showed a lower n6-n3 ratio (6.92) compared to CON (15.12) ($P < 0.05$). Furthermore, PAS whey showed better health indices than CON, represented by higher levels of DFA and lower levels of AI, TI, and HSFA ($P < 0.05$). Low AI, HSFA and TI levels may inhibit the platelets aggregation, and prevent the appearance of coronary diseases (Costa et al., 2019).

The presence of lower concentrations of caprylic (8:0) and caproic (6:0) fatty acids in PAS could be attractive from the sensory point of view. These fatty acids are compounds associated with the characteristic aroma and flavor of goat dairy products, which may be unpleasant for some consumers (Kilcawley et al., 2018). In addition, lower amounts of this group of acids may lead to a milder goat flavor and residual flavor in food products, resulting in greater acceptability (Borba et al., 2014). In this sense, it may be advantageous to use whey from grazing goats for products with a softer flavor and aroma.

The results suggest that pasture feeding optimizes the balance between valuable and detrimental fatty acids in goat milk, resulting in goat whey with higher possible beneficial effects to consumers. Furthermore, the verification that goat whey fatty acid profile is closely linked to the goat diet indicates that it is possible to perform animal dietary interventions to improve the nutritional value of goat milk. In this way, pasture may be a low-cost and natural way to modulate the fatty acid profile of goat milk sharply and rapidly and, consequently, goat whey (Claps et al., 2018).

3.5. Protein

The protein profile by SDS-PAGE of whey samples (Fig. 1) includes the main proteins found in milk whey, with a predominance of the low molecular weight proteins β-lactoglobulin (17 kDa) and α-lactalbumin (11 kDa), and lower presence of high molecular weight proteins (> 52 kDa), as already described for goat whey (Ahmed et al., 2015). However, the quantity and proportion of goat whey proteins may be affected by different factors, especially by the genotype of animals and the diet (Madureira et al., 2013).

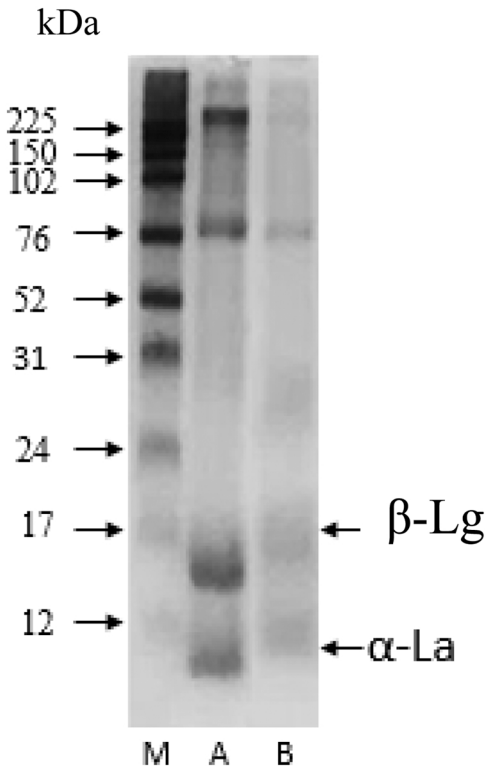

Fig. 1. SDS-PAGE of goat whey proteins originating from grazing and confinement systems. (A) Whey from pasture production system (PAS); (B) Whey from confined production system (CON). (M): Molecular weight standards; β-Lg: β-Lactoglobulin; α-La: α-Lactalbumin.

Soluble protein content analyzed by the Bradford method showed mean values of 0.85 mg/mL and 0.40 mg/mL for PAS and CON whey, respectively. These results follow the SDS-PAGE, which presented more intense protein bands for PAS whey (Fig. 1). A higher milk concentration of α-lactalbumin, lactoferrin and lysozyme in goats' milk in the spring-summer season (goats grazed on pastures) relative to autumn-winter season (goats were fed mainly with haylage) was demonstrated (Brodziak et al., 2014). The authors point out that restricting pasture in the diet led to a decrease of β-lactoglobulin and α-lactalbumin. The reason for such differences is not apparent, but at least for lactoferrin and lysozyme might be associated with higher immunity challenges in grazing animals than in confined animals. The higher concentration of proteins in PAS may be interesting as they may show health effects. Lactoferrin, for example, may improve iron bioavailability, and present antioxidant, anti-inflammatory, and anticarcinogenic activities (Tsermoula et al., 2021).

Further separation of whey proteins by 2D SDS-PAGE revealed a more number of spots (proteins and their isoforms) for the PAS whey than for CON whey (Fig. 2A). In addition, the protein profile of PAS whey presented spots with higher molecular weights equal to or greater than 225 kDa in the Ip (Isoelectric point) range of 6.6–7.4, respectively (spots 1–5), and spots with 71–72 kDa in Ip ranging from 6.5 to 7.9 (spots 6–14), which were not identified in the CON whey (Fig. 2A). Conversely, the most proteins found in CON whey range from 51 to 92 kDa, with the highest spot intensity found for proteins ranging from 63 to 65 kDa (spots 1 and 2) (Fig. 2B). Whey samples derived from the milk of grazing and confined goats also differ in the abundance of proteins with molecular weight ranging from 50 to 52 kDa (spots 15–21) in the neutral region of the Ip range, with higher molecular weight spots for PAS. In contrast, Ip was predominantly in the field of 8.3 for proteins and isoform spots of 51 kDa molecular weight in the CON whey but also varied to 3.3 (spots 3–11). In addition, proteins with 36–37 kDa were identified in PAS whey (spots 22–26) but not observed in CON whey.

Spots of proteins with a molecular weight of 23 and 24 kDa were identified in both types of whey (numbered from 27 to 29 in grazing and 12–14 in CON whey samples). The spots are within the low molecular weight proteins, β-lactoglobulin (17 kDa and 5.0–5.9 Ip) and α-lactalbumin (11 kDa and 4.7–5,0 Ip). Two isoforms for β-lactoglobulin (spots 30 and 31) were present in PAS whey. In comparison, three isoforms (spots 15, 16, and 17) were observed in CON whey, reflecting differences in the genotype of animals randomly allocated in each experimental group (Rahmatalla et al., 2020; Ezewud et al., 2019). Peptides with 9 kDa molecular weight and Ip of 3.4 (spot 19) and 8 kDa and Ip of 4.3 and 5.0 (spots 20 and 21) were only observed in CON whey. The intensity values are provided in supplementary material.

Anagnostopoulos et al. (2016) wrote the protein profile of goat whey characterized by an abundance of lower molecular weights protein and peptides, and somewhat similar to our samples from the confinement group and argue that these lower molecular weights proteins are susceptible to variations according to the breed, geographical location, animal diet, and genetic factors (Yang et al., 2013).

3.6. Total amino acid profile

Whey proteins are rapidly metabolized and absorbed. They are considered superior to eggs, red meat, soy, caseins, and fish, concerning the content of essential amino acids and bioactive peptides capable of bringing benefits to human health (Smithers, 2008). In our study, the essential amino acids of goat whey comprised about 44 % of the total amino acid content (Table 6). A high content of branched-chain amino acids (leucine, isoleucine, and valine) was observed, representing about

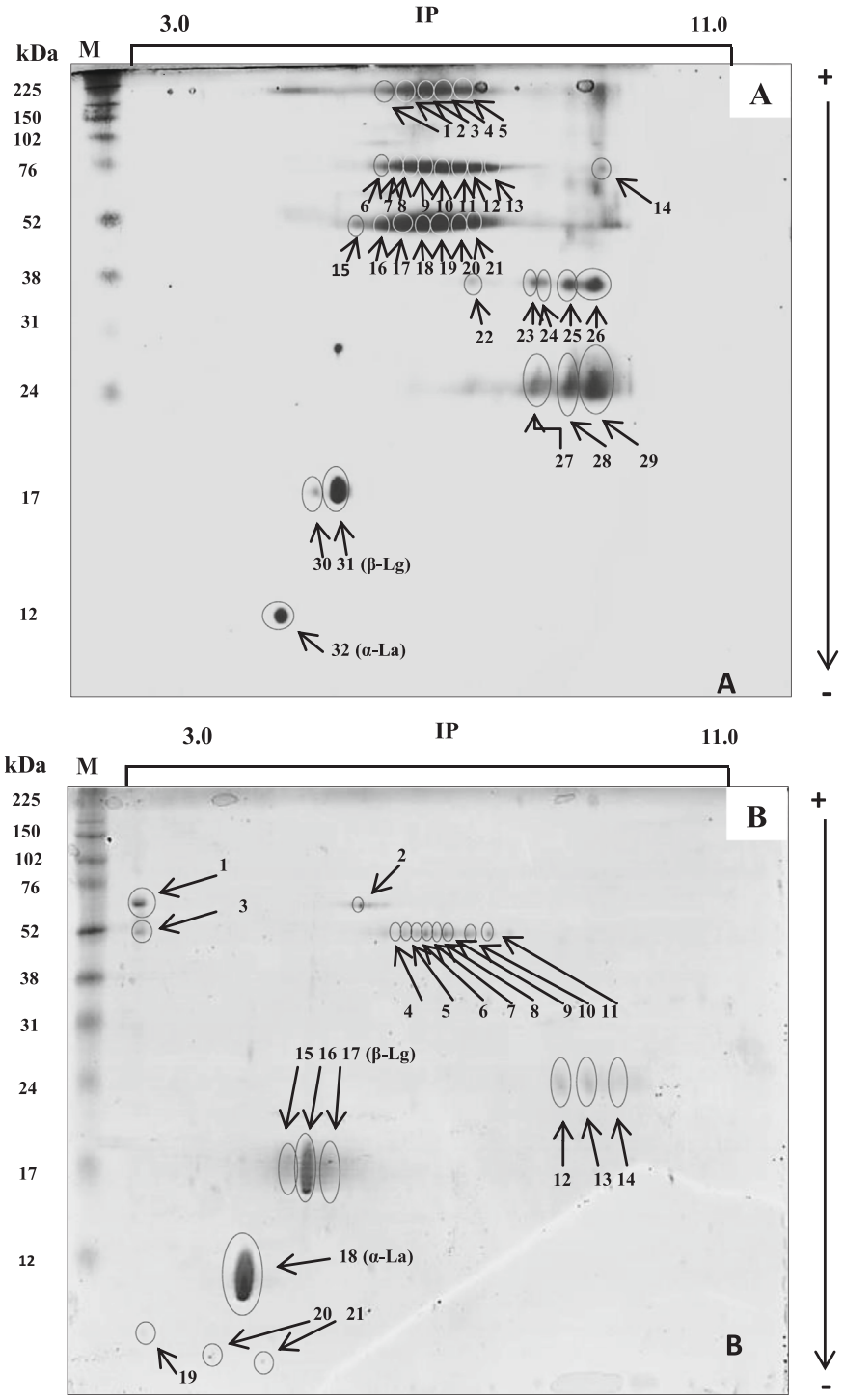

Fig. 2. SDS-PAGE of goat whey proteins originating from grazing and confinement systems. (A) Whey from pasture production system (PAS); (B) Whey from confined production system (CON). M: Molecular weight standards; IP: Isoelectric Point range; β-Lg: β-Lactoglobulin; α-La: α-Lactalbumin.

43 % of the total essential amino acids. All essential amino acids, except the sulfur-containing amino acids (methionine + cysteine), reach or exceed the reference chemical score value of 1, recommended by FAO/WHO/UNU (2007) (Table 5). In addition, the goat whey showed an important concentration of lysine (53.1–65.6 mg/g). This essential amino acid is often the limiting amino acid of widely consumed cereals such as rice and wheat (Teba et al., 2017). Among non-essential amino acids, aspartic and glutamic acids were present in large amounts in both types of goat whey (Table 6), consistent with the literature (Kareb et al., 2017).

The production system type significantly affected the total amino acid composition of the goat whey ($P < 0.05$, Table 6). The protein from CON whey presented a higher concentration of all essential amino acids, aspartic and glutamic acids, alanine, and proline than protein from PAS whey ($P < 0.05$). The highest concentration of protein in the CON diet can cause an increase in propionic acid in the rumen, increasing the amino acid availability to the mammary gland (Lima et al., 2021). Practical recovering amino acids from whey proteins can be accomplished by enzymatic, raising prospects of industrial applications for the whey amino acids and peptides. Furthermore, whey protein

Table 6
Amino acid profile (mg/g of protein) of the goat whey from pasture production system (PAS) and confined production system (CON) (mean value ± S.d.).

Amino acids (mg/g)	Goat whey		*P*-value*	Overall mean (mg/g of protein)		FAO[1] Standard (mg/g of protein)	AA Score[2]
	PAS	CON					
Essential Amino Acids							
Threonine	46.0^{b} ± 0.00	51.1^{a} ± 0.02	0.039	48.5	23	2.00–2.22	
Valine	37.2^{b} ± 0.00	41.3^{a} ± 0.02	0.014	39.2	39	0.95–1.05	
Methionine + cysteine	13.28^{b} ± 0.00	15.8^{a} ± 0.00	0.028	14.5	22	0.60–0.71	
Isoleucine	34.6^{b} ± 0.00	38.1^{a} ± 0.00	0.017	36.3	30	1.15–1.27	
Leucine	51.4^{b} ± 0.00	60.8^{a} ± 0.01	0.017	56.1	59	0.87–1.03	
Phenylalanine + tyrosine	39.0^{b} ± 0.00	43.0^{a} ± 0.00	0.024	41.0	38	1.02–1.13	
Lysine	53.1^{b} ± 0.00	65.6^{a} ± 0.02	0.028	59.3	45	1.18–1.46	
Histidine	14.2 ± 0.00	15.4 ± 0.01	0.095	14.8	15	0.95–1.03	
Non-essential amino acids							
Aspartic acid	77.1^{b} ± 0.00	81.1^{a} ± 0.01	0.005	79.1	NA[3]	NA	
Glutamic acid	116^{b} ± 0.03	125.7^{a} ± 0.01	0.019	120.8	NA	NA	
Serine	38.1 ± 0.02	40.0 ± 0.01	0.051	39.0	NA	NA	
Glycine	23.9 ± 0.03	21.1 ± 0.00	0.311	22.5	NA	NA	
Arginine	23.0 ± 0.01	23.5 ± 0.00	0.154	23.2	NA	NA	
Alanine	38.1^{b} ± 0.01	43.0^{a} ± 0.00	0.011	40.5	NA	NA	
Proline	34.6^{b} ± 0.01	39.0^{a} ± 0.00	0.016	36.8	NA	NA	

[1] Estimated amino acid requirement (Adults)/Standard reference for proteins FAO/WHO/UNU (2007).
[2] Amino acid score (mg/g of protein from the sample)/(mg/g of standard proteins FAO/WHO).
[3] Not applicable.
* Considered significant at $p < 0.05$. Sd = Standard deviation.

allergenicity may be reduced through partial protein hydrolysis, allowing its use as an ingredient in special-purpose foods.

4. Conclusion

This study was the first to perform a complete evaluation of the nutritional quality of goat whey considering the impacts of raising system. The results demonstrated that goat whey has a high nutritional value and potential as an ingredient to the food industry, regardless of the production system. However, detailed profiles of the minerals, fatty acids, and proteins revealed that the pasture system may result in goat whey with a richer fatty acid profile (higher proportions of PUFA and lower concentration of short and medium-chain fatty acids), and better health indices and n6-n3 ratio. Also, the pasture production system improved the mineral concentration (potassium, calcium, and iron) and total protein concentrations, mainly higher molecular weight proteins. Conversely, the confined system improved the concentration of essential amino acids. The present study results are significant as the identification of compositional differences may contribute to market differentiation strategies for applying goat whey in food products or as a source of functional compounds.

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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.smallrumres.2022.106842.

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