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Donkey milk and fermented donkey milk: are there differences in the nutritional value and physicochemical characteristics?

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ABSTRACT

This study aimed to evaluate the nutritional value and physicochemical characteristics of fresh donkey milk and fermented donkey milk. Furthermore, the impact of the refrigerated storage (4 °C, 21 days) on the characteristics of the fermented milk was evaluated. The fermented milk had higher acidity (lower pH values and higher titratable acidity) and presented lower lactose, mineral (calcium, phosphorous, magnesium, sodium, and zinc), and amino acid (mainly essential amino acids) contents than milk. Otherwise, it had a better fatty acid profile (lower saturated fatty acid content, and higher monounsaturated and polyunsaturated fatty acid contents). The fermented milk storage resulted in decreases in the lactose content, improvements in the amino acid profile, and maintenance of the mineral content. However, negative impacts were observed on the fatty acid profile. It can be concluded that both donkey milk and fermented milk donkey present important nutritional value, and the fermented milk could be refrigerated stored up to 21 days.

1. Introduction

Donkey milk has a chemical composition similar to that of human milk and, for this reason, it is recommended as a substitute for infant nutrition in case of allergy to cow's milk protein (Aspri, Leni, Galaverna, & Papademas, 2018). It presents low protein content (1.5–2.2%), being particularly high in whey protein compared to casein. Furthermore, it presents high concentrations of lactose (7%), and the main oligoelements are calcium, potassium, phosphates, magnesium, and sodium (Altomonte, Salari, Licitra, & Martini, 2019; Massouras, Triantaphyllopoulos, & Theodossiou, 2017). The fat content is low (0.1–3.8%), and it has a specific fatty acid profile, with a higher concentration of C18:3n-3 and n-3 fatty acids, and a lower content of saturated fatty acids (SFA) than cow milk, as well as a lower n-6 to n-3 fatty acid ratio (Valle et al., 2018). The presence of substantial amounts of lysozyme, immunoglobulins, ω-3 fatty acids, lactoferrin, and bioactive peptides results in a milk with several functional properties, such as antimicrobial, antioxidant, antiviral, anti-inflammatory, anti-diabetic, and immunomodulatory activities (Aspri, Bozoudi, Tsaltas, Hill, & Papademas, 2017; Li, Kang, et al., 2020; Li, Liu, & Guo, 2018; Madhusudan et al., 2017; Yvon et al., 2018).

The chemical composition of donkey milk and its functional properties are dependent on a combination of different factors, including animal species, season, diet, physiological status, lactation period, among others (Li, Fan, et al., 2020). The functions and nutrition of donkey milk are still not well studied, and there is a need of collecting basic data; thus, the donkey milk industry could develop (Aspri, Economou, & Papademas, 2017; Li et al., 2018). Previous studies carried out the characterization of donkey milk from China (Guo et al., 2007; Li et al., 2018, 2020a), Greece (Massouras et al., 2017), Turkey (Ozturkoglu-Budak, 2018), India (Nayak et al., 2020) and Italy (Martini, Licitra, Altomonte, & Salari, 2020; Valle et al., 2018). However, as far as the authors know, there are no studies that characterized donkey milk produced in the Semiarid region of Brazil.

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Fermented milk is the most commercialized dairy product due to its suitable nutritional value; therefore, the dairy industry produces several types of fermented milk with different physicochemical characteristics (Miao et al., 2020). Donkey milk has a white color, low consistency, a slightly sweet and pleasant taste, and a milky aroma (Malissiova et al., 2016). The fermentative process of donkey milk can improve its sensory and functional properties; therefore, the production of fermented milk from donkey milk could be an interesting approach (Aspri et al., 2018; Chiavari, Coloretti, Nanni, Sorrentino, & Grazia, 2005). Studies involving the development and characterization of fermented milk from donkey milk are still scarce (Aspri et al., 2018; Miao et al., 2020; Tidona et al., 2015), and they did not perform a complete characterization of the nutritional value of the products (gross composition, sugars, minerals, amino acid profile, and fatty acid profile). Furthermore, the effect of fermented milk processing (heat treatment and fermentation) and storage on the characteristics of the products has not been reported.

The intake of donkey milk is still uncommon and restricted, despite having important nutritional characteristics and pleasant sensory aspects. The development of new technologies using donkey milk in the manufacture of dairy products represents an innovative alternative in the market, contributing to increasing its consumption. Thus, studies aiming at investigating the characteristics of milk and dairy products from donkey milk are of fundamental importance to disseminate the nutritional, technological, and socioeconomic advantages of this milk, thus valuing the dairy activity and the development of new attributions to donkeys. Therefore, the present study aimed to characterize fresh donkey milk, and evaluate the nutritional value and physicochemical and microbiological characteristics of donkey fermented milk during refrigerated storage.

2. Material and methods

2.1. Chemicals

DL-2-aminobutyric acid, acetonitrile, phenyl isothiocyanate (Merck, P-1034) 4-dimethylaminobenzaldehyde (DAB); Pronase E enzyme (Merck, ref.1074330001), sugar standards (galactose (G0750, >99%), lactose (L3625, >99%) and glucose (G47829, >99.5%)), HCl and phenol were supplied by Sigma-Aldrich® (St. Louis, MO). Thermophilic culture (YF-L903, Christian Hansen®, Valinhos, Minas Gerais, Brazil, *Streptococcus thermophillus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*), amino acids standard (Amino Acid Standard H, Thermo Scientific®, Rockford, IL, USA), commercial fatty acid methyl esters (FAME from Supelco® Inc., Bellefont, PA, USA, containing 37 fatty acid methyl esters from C4:0 to C22:6n-3), mineral standards (Specsol - Quimlab®, Jacareí, Brazil and Titrisol - Merck®, Darmstadt, Germany), de Man, Rogosa and Sharp (MRS) agar (Himedia®, Mumbai, India) and argon gas (99.996%, Air Liquide®, Brazil) were also used in the experiment. All the other reagents were of analytical grade.

2.2. Donkey milk

The milk samples were obtained from animals of the "Nordestina" donkey breed raised in the Northern Forest in the city of Carpina – Pernambuco, Brazil. The animals were aged between 3 and 10 years old, were healthy primiparous and multiparous, and were kept in a semi-confinement system, fed in collective feeders' *ad libidum*, and released daily to graze freely in native pasture. The animal's diet consisted of fresh forage composed by elephant grass, brachiaria, maniva (1:1:1), in addition to corn silage (300 g/head). All animals had access to fresh and clean water. Milk samples were collected by hand milking, packaged in polyethylene bottles, and transported under refrigerated temperature (4 ± 1 °C).

2.3. Fermented milk processing

The donkey milk was subjected to heat treatment (65 ± 2 °C/15 min), cooled to 45 ± 2 °C, added with the thermophilic culture (0.4%, *Streptococcus thermophillus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) and fermented at 28 ± 2 °C for 4 h in an incubator. Then, the fermented milk was refrigerated (4 ± 1 °C) for 24 h, and slowly homogenized by hand stirring with a glass stick. Finally, the fermented milk was packed in polyethylene bottles and stored (4 ± 1 °C) for 21 days, which is the shelf life of yogurts and fermented milks obtained from cow milk (Keshavarzi, Sharifan, & Yasini Ardakani, 2020; Mantovani et al., 2020).

2.4. Chemical composition and physicochemical characteristics

The chemical composition (moisture, protein, lipids, and ash) was determined according to the methodologies recommended by the Association of Official Analytical Chemist Methods (AOAC, 2016). The pH was measured by a digital potentiometer (model Q400AS®) and the titratable acidity (TA) was determined by titration and expressed in % lactic acid (AOAC, 2016).

2.5. Sugar profile

The sugar profile (lactose, glucose, and galactose) was determined according to the methodology described by Zeppa, Conterno, and Gerbi (2001). 2 g of lyophilized sample were weighed, followed by dilution in 10 mL of ultrapure water, centrifugation (6000×*g*, 4 °C) for 10 min, and filtration in a 0.45 μm cellulose filter (Whatman®, Chicago, USA). The sugars were quantified using a high-performance liquid chromatograph (HPLC, VARIAN, Waters 2690, California, USA) with a refractive index detector, coupled with a Hi-Plex Ca column (7.7 × 300 mm, 8 μm), at a temperature of 85 °C, using the ultra-pure water as mobile phase at a flow rate of 0.6 mL/min. The analysis lasted 30 min, and the quantification was performed by the injection of standards. The calibration curves showed correlation coefficient of 0.9991 for glucose, 0.9974 for galactose, and 0.9982 for lactose, and the detection limit was 0.1 g/100 mL. The detection limit was calculated using three points to the sugars (lactose, galactose, and glucose) with concentrations below the smallest point on the calibration curve, injected in triplicate at concentrations below the first concentration of the analytes on the analytical curve.

2.6. Free amino acid profile

The free amino acids of the evaluated samples were extracted by orbital shaking (100 rpm) for 30 min with methanol: 0.1 M chloric acid (60:30) and addition of internal standard followed by pre-column phenyl isothiocyanate (PITC) derivatization according to White, Hart, and Fry (1986) and Hagen, Frost, and Augustin (1989). The separation of phenylthiocarbamyl amino acid (PTC-aa) derivatives was performed in an HPLC system (Shimadzu Corporation, Tokyo, Japan) and Luna C18 reversed-phase column (250 mm 4.6 mm, 5 lm; Phenomenex Inc., Torrence, CA, USA). The mobile phases consisted of an acetate buffer at pH 6.4 and a 40% acetonitrile solution. The sample was injected automatically (50 μL), and detection was performed at 254 nm. The chromatographic separation was performed at a constant flow rate of 1 mL/min at a temperature of 50 °C. The chromatographic run time was 45 min and the results were expressed in mg of amino acid per 100 g sample. Amino acids were quantified by comparison with amino acids standard Thermo Fisher Scientific (code 20088), and the DL-2-aminobutyric acid was used as internal standard. A representative chromatogram (Fig. 1) and the data of the standard curve are provided in Supplementary material. A calibration curve was constructed with five points for the 18 individuals' amino acids of the standard in the range of 0.07–1.50 mg/mL (stock solution) and diluted to the concentration specified below for each point on the curve. The detection limit was 0.1–5.0 mg/100 mL depending on the amino acid.

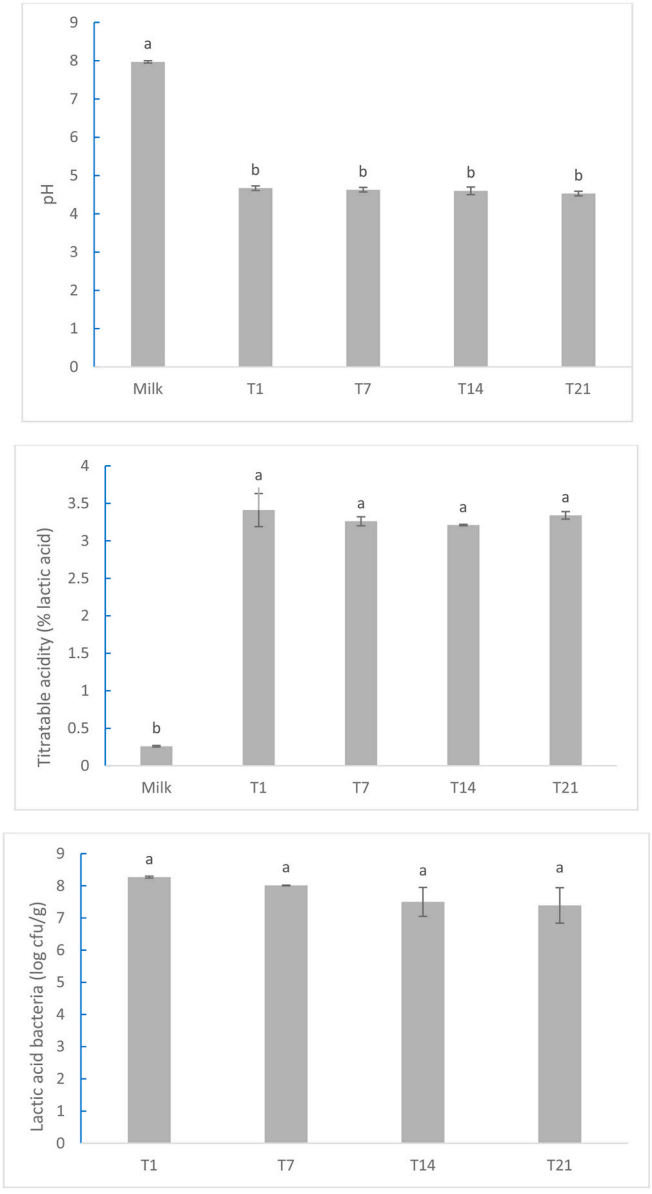

Fig. 1. Physicochemical and microbiological characteristics of fresh donkey milk and fermented milk during cold storage for 21 days. T1 = 1st, T7 = 7th, T14 = 14th, and T21 = 21st day of storage.

The calibration curve was constructed by plotting the peak areas obtained by injecting 50 μL of the amino acid solution prepared in a range of 0.41–10.2 mg/100 mL for aspartic acid; 0.45–11.3 mg/100 mL for glutamic acid; 0.32–8.1 mg/100 mL for serine; 0.2306–6.9 mg/mL for glycine; 0.48–11.90 mg/mL for histidine; 0.5348–13.36 mg/mL for arginine; 0.3657–9.1345 mg/mL for threonine; 0.2735–6.8336 mg/mL for alanine; 0.3534–8.6277 mg/mL for proline; 0.5631–13.8973 mg/mL for tyrosine; 0.3598–8.9887 mg/mL for valine; 0.4581–21.0094 mg/mL for methionine; 0.3687–9.558 mg/mL for cysteine; 0.4028–10.0625 mg/mL for isoleucine; 0.4028–10.0625 mg/mL for leucine; 0.5092–11.6704 mg/mL for phenylalanine; 0.6588–16.314 mg/mL for tryptophan, and 0.4489–11.2129 mg/mL for lysine. The LOD was obtained considering the instrument performance to the used method, where the signal/noise ratio (3:1) was established for the minimum concentration at which the specific amino acid can be reliably quantified (Nata, Technical Note 17 2013). A representative chromatogram is provided in Supplementary Fig. 1.

2.7. 7 Fatty acid profile

The fatty acid profile was obtained using the method described by Molkentin and Precht (2000), with modifications. Initially, the samples were directly transesterified to produce fatty acid in the form of methyl esters. The fatty acid methyl esters were analyzed by gas chromatography using a GC Shimadzu 2010-plus (Shimadzu Corp., Kyoto, Japan) equipped with a flame ionization detector (FID) and a SP-2560 capillary column (100 m × 0.25 mm i. d. and 0.20 μm film thickness, Supelco Inc., Bellefonte, PA, USA). Injection volume of 1 μL was used, and the chromatographic conditions were as follows: injector and detector temperatures were set at 220 °C and 250 °C, respectively; helium was used as the carrier gas at 1 mL/min constant flow; the initial oven temperature was of 50 °C, which was held for 1 min, increased at 50 °C/min to 150 °C and held for 20 min. Then, the temperature was increased at 1 °C/min to 190 °C, and then, increased at 2 °C/min to 220 °C and held for 30 min (total run time: 108 min). Identification of fatty acid methyl esters was achieved by comparison of their retention times with those of commercial standard mixtures. The methyl nonadecanoate was used an internal standard at 1 mg/mL. Fatty acid areas were corrected using theoretical relative FID correction factors and expressed as a percentage of the sum of the detected fatty acids (g/100 g of total fatty acids). A representative chromatogram is provided in Supplementary Fig. 1. The atherogenic index (AI), thrombogenic index (TI), desirable fatty acid (DFA) index, and hypercholesterolemic fatty acid (HSFA) index were calculated according to Sperry et al. (2018).

2.8. Mineral profile

For the analysis of minerals (calcium, phosphorus, magnesium, manganese, potassium, sodium, and zinc) a test method based on AOAC (2016) was used. The sample was carbonized and incinerated in a muffle (450 °C, 10 h), treated with hydrochloric acid, filtered, and analyzed by inductively coupled plasma optical emission spectrometry (ICP OES 5100 VDV, Agilent Technologies®, Tokyo, Japan) with liquid argon as gas. The analysis conditions were radio frequency power (1.2 kW); plasma flow (12 L/min); auxiliary flow rate (1.0 L/min); nebulization flow (0.7 L/min); plasma view (axial for Mn and Zn; radial for Ca, Mg, P, Na); and wavelengths: Ca (317,933 nm), Mg (279,553 nm), Mn (257, 610 nm), P (213,618 nm), Na (589,592 nm), K (766,491 nm), and Zn (206,200 nm). The analytical curves for minerals were prepared from dilutions of 10 mg/100 mL and 1000 mg/100 mL of the analytical standards in the ranges of 0.041–41.0 mg/100 mL for Ca and Na, 0.062–62.0 mg/100 mL for P, 0.015–14.5 mg/100 mL for Mg, 0.0003–0.100 mg/100 mL for Mn, 0.061–61.0 mg/100 mL for K, and 0.001–1.0 mg/100 mL for Zn, with a correlation coefficient (r) greater than 0.9999. The detection limits were: Ca e Na = 0.4 mg/100 mL; P e K = 0.6 mg/100 mL; Mg = 0.1 mg/100 mL; Mn = 0.003 mg/100 mL and Zn = 0.01 mg/100 mL. For this, the signal-to-noise ratio (3:1) was performed by comparing measured signals from samples with known low concentrations of analyte (Nata, Technical Note 2013).

2.9. Lactic acid bacteria viability

The total counts of lactic acid bacteria (LAB, *Streptococcus thermophillus* and *Lactobacillus delbrueckii* subsp. *bulgaricus,* log cfu/g) were performed on MRS agar and aerobic incubation for 48 h at 37 ± 2 °C (International Dairy Federation, 1988).

2.10. Statistical analysis

The donkey milk samples were evaluated on the first day of storage and the analyzes were performed in triplicates. The fermented milk was evaluated in three independent experiments and the analyses were carried out in triplicates. The fatty acid profile was evaluated on the first (1) and last day of storage (21), and the physicochemical analyzes and

viability of the LAB were performed on days 1, 7, 14 and 21 of storage. The results were analyzed using the Sigma Stat 3.5 Program (Systat Software Inc., Chicago, Illinois), being subjected to a one-way analysis of variance (ANOVA) and Tukey's test for comparison of means, considering $p < 0.05$.

3. Results and discussion

3.1. Chemical composition and physicochemical characteristics

Table 1 presents the chemical composition and physicochemical characteristics of the donkey milk and fermented donkey milk. The donkey milk presented chemical composition of 91.34 g/100 g moisture, 1.5 g/100 g protein, 0.43 g/100 g fat, 0.67 g/100 g ash, and 7.32 g/100 g lactose. Furthermore, it presented pH of 7.97 and TA of 0.26% lactic acid (Fig. 1). Therefore, based on the results, donkey milk was characterized by higher concentration of lactose compared to protein and fat. These results suggest that this type of milk present low allergenic properties and could contribute to the development of the intestinal microbiota, mainly in infants (Martini et al., 2020). The high lactose content may contribute to its good taste and palatability and can be an important energy source (Martini, Altomonte, Licitra, & Salari, 2018). The low acidity is associated with the low content of phosphate and casein (Altomonte et al., 2019) and results in a very perishable product, therefore, the pasteurization process should be carried out fast, and its transformation to derived products, as fermented milk, may be interesting.

The chemical composition and physicochemical characteristics of donkey milk of the present study are similar to those observed for donkey milks in previous studies in different countries (Guo et al., 2007; Li et al., 2018; Martini, Altomonte, Manica, & Salari, 2015; Massouras et al., 2017, 2020; Valle et al., 2018), as demonstrated in Supplementary Table 1. The differences may be associated to breed, feed, and other environmental factors, which may alter the chemical composition and physicochemical characteristics of the products. However, in an overall view, it seems that they fall in the same range. Furthermore, the donkey milk presented a chemical composition more similar to the human milk (87.8 g/100 g moisture, 1.2 g/100 g protein, 3.5–4.0 g/100 g fat, 0.25 g/100 g ash, and 6.3–7.0 g/100 g lactose) than the cow milk (87–88 g/100 g moisture, 3.0 g/100 g protein, 3.2 g/100 g fat, 0.7 g/100 g ash, and 4.6 g/100 g lactose) (Li et al., 2018; Martini et al., 2018). This is important from the nutritional point of view and opens opportunities for using this milk in the preparation of formulas for babies that cannot breastfeed.

The fermented milk presented chemical composition of 92.06 g/100 g moisture, 1.48 g/100 g protein, 0.43 g/100 g fat, 0.61 g/100 g ash, 6.41 g/100 g lactose, and 0.17 g/100 g galactose at day 1. Furthermore, it presented pH 4.67 and TA of 3.41% lactic acid (Fig. 1). Therefore, the fermented milk presented higher moisture and galactose contents and TA, and lower lactose content and pH values ($p < 0.05$) than the milk. During fermented milk processing, the LAB use lactose as substrate for their metabolism and produce lactic acid, increasing the acidity of the products (Tidona et al., 2015). At the same time, lactose is hydrolyzed in its moieties (galactose and glucose), and galactose is not completely metabolized by LAB (Ohlsson et al., 2017). This explains the higher concentration of galactose in the fermented milks if compared to milk. The higher moisture content in the fermented milk may be associated with the reduction in the sugar content during fermentation, which reduces the dry matter of the product (Santos et al., 2018).

During fermented milk storage, there was the maintenance of the chemical composition and physicochemical parameters (pH and TA) ($p > 0.05$), except for sugars. A decrease in the lactose content with a consequent increase in galactose and glucose contents ($p < 0.05$) was observed. The results show that the LAB hydrolyzed lactose during storage, but they had no substantial metabolic activity at refrigerated conditions, resulting in an increase in the concentration of glucose and galactose. This result is interesting from the industry point of view, as the products stored for 21 days would have similar characteristics to the new processed ones.

Table 1
Chemical composition of fresh donkey milk and fermented milk during cold storage for 21 days.

Variables	Milk	Fermented milk			
		T1	T7	T14	T21
Moisture	91.34 ± 0.0[b]	92.06 ± 0.02[a]	92.02 ± 0.01[a]	92.00 ± 0.01[a]	92.02 ± 0.02[a]
Protein	1.50 ± 0.07[a]	1.48 ± 0.01[a]	1.47 ± 0.01[a]	1.47 ± 0.01[a]	1.47 ± 0.01[a]
Fat	0.43 ± 0.01[a]	0.43 ± 0.06[a]	0.45 ± 0.01[a]	0.46 ± 0.06[a]	0.46 ± 0.06[a]
Ash	0.67 ± 0.01[a]	0.61 ± 0.01[a]	0.60 ± 0.01[a]	0.62 ± 0.03[a]	0.61 ± 0.01[a]
Lactose	7.32 ± 0.14[a]	6.41 ± 0.02[b]	NM	NM	5.70 ± 0.01[c]
Galactose	ND	0.17 ± 0.003[b]	NM	NM	0.52 ± 0.01[a]
Glucose	ND	ND	NM	NM	0.62 ± 0.02

[a-b]Mean ± standard deviation with different lowercase letters in the same line differed by Tukey's test ($p < 0.05$). Moisture, protein, fat, ash, lactose, galactose and glucose in g/100 g. Titratable acidity in % lactic acid. Lactic acid bacteria in log cfu/g. – NM= not measured. ND not detected (< 0.10 g/ 100 mL). T1 – day 1, T7– day7, T14– day14,T21 – day 21.

3.2. Fatty acid profile and health indices

Table 2 presents the fatty acid profile and health indices of the donkey milk and fermented donkey milk. The donkey milk presented 48.82 mg/100 g fatty acids (FA) of SFA, 37.67 mg/100 g FA of monounsaturated fatty acid (MUFA), and 14.87 mg/100 g FA of polyunsaturated fatty acid (PUFA). Furthermore, it presented significant concentrations of important fatty acids, such as C18:1c9 (29.20 mg/100 g FA), C18:2n-6 (7.80 mg/100 g FA), and C18:3n-3 (6.51 mg/100 g FA) and n6/n3 ratio of 1.19. MUFA consumption is associated with the reduction in cardiovascular diseases, mainly oleic acid (C18:1c9). This fatty acid increases the mobility of the fat globule and its metabolic activity. Furthermore, PUFA can reduce the risk of cardiovascular disease and enhance body metabolism. Linolenic acid (C18:3n-3) and linoleic acid (C18:2n-6) are fatty acids needed by the human body and they cannot be synthesized, therefore, they must be obtained by the diet (Li et al., 2018). In addition, donkey milk was characterized by low concentration of short-chain fatty acids (SCFA) and high concentration of long-chain fatty acids (LCFA), mainly with 16, 18, and 20 carbons. This behavior has already been reported for human milk (Altomonte et al., 2019). Finally, the n6/n3 ratio (1.19) was much lower than the maximum recommended value of 4, which considered the possible health effects of FA (Yoshimura et al., 2018). The results show that, although donkey milk presents a low concentration of fat (Table 1), this component was of good quality in the product.

The fermented donkey milk presented high concentrations of SFA (41.28 mg/100 g FA) and MUFA (41.02 mg/100 g FA), followed by PUFA (18.55 mg/100 g FA). Therefore, the fermented milk presented lower SFA and higher MUFA and PUFA levels ($p < 0.05$) than milk, mainly associated to the decrease in C8:0, C10:0, C12:0 and C14:0, and increase in C16:1c7, C17:1c9, C18:2n-6, C18:3n-3, C20:2n-6, among others ($p < 0.05$). Consequently, there was a decrease in AI and an increase in the DFA ($p < 0.05$). The consumption of products with lower AI and higher DFA is advisable, as it is associated with decreases in the total cholesterol and LDL cholesterol (Tidona et al., 2015). In this way, the fermented milk presented a better lipid profile and health indices than the milk. The changes in the fatty acid profile are mainly associated with the fermentation process, as the pasteurization temperatures (65 °C)

Table 2
Fatty acid profile (g/100 g of total fatty acids) and health indices of fresh donkey milk and fermented milk during refrigerated storage.

Fatty acid	Milk	Fermented milk	
		T1	T21
C4:0	0.162 ± 0.01[b]	0.526± 0.04[a]	0.500± 0.05[a]
C6:0	0.214± 0.02[c]	0.414± 0.04[b]	0.612± 0.05[a]
C8:0	4.713± 0.40[b]	3.088± 0.33[c]	6.998± 0.40[a]
C10:0	9.509± 0.95[b]	4.400± 0.42[c]	15.541± 0.99[a]
C10:1+[C11:0]	1.364± 0.10[b]	0.767± 0.08[c]	2.845± 0.30[a]
C12:0	6.574± 0.67[b]	3.011± 0.33[c]	15.996± 0.95[a]
C14:0	4.401± 0.42[b]	3.110± 0.29[c]	11.080± 1.08[a]
C14:1c9	0.233± 0.02[b]	0.193± 0.02[c]	0.594± 0.06[a]
C15:0	0.186± 0.02[c]	0.340± 0.03[a]	0.227± 0.04[b]
i-C16:0	0.072± 0.007[b]	0.151± 0.01[a]	0.089± 0.009[b]
C16:0	19.074± 1.65[b]	22.148± 2.24[a]	18.305± 2.28[b]
i-C17:0	0.623± 0.06[a]	0.711± 0.08[a]	0.176± 0.02[b]
C16:1c7	0.747± 0.08[c]	2.897± 0.30[b]	4.144± 0.42[a]
C16:1c9	4.030± 0.38[a]	4.997± 0.50[a]	2.250± 0.30[b]
a-C17:0	0.067± 0.002[b]	0.126± 0.002[a]	0.097± 0.009[ab]
C17:0	0.184± 0.01[b]	0.292± 0.02[a]	0.291± 0.03[a]
C17:1c9	0.325± 0.04[b]	0.416± 0.04[a]	0.365± 0.04[b]
C18:0	1.647± 0.20[b]	2.117± 0.23[a]	1.609± 0.16[b]
C18:1trans	0.176± 0.02[a]	0.099± 0.01[b]	0.000± 0.001[c]
C18:1c9	29.202± 2.23[a]	29.863± 2.99[a]	7.564± 1.05[b]
C18:1c11	1.594± 0.23[a]	1.790± 0.19[a]	0.382± 0.05[b]
C18:2n-6	7.798± 0.86[b]	10.571± 0.99[a]	5.715± 0.68[c]
C20:0	0.034± 0.004[b]	0.087± 0.009[a]	0.055± 0.005[ab]
C18:3n-6	0.061± 0.009[a]	0.070± 0.008[a]	0.000± 0.002[b]
C18:3n-3	6.507± 0.70[b]	7.280± 0.86[a]	4.279± 0.50[c]
C20:2n-6	0.138± 0.001[b]	0.190± 0.002[a]	0.054± 0.006[c]
C20:3n-6	0.046± 0.005[a]	0.015± 0.001[b]	0.055± 0.004[a]
C20:3n-3+[C22:1]	0.198± 0.06[a]	0.172± 0.04[a]	0.118± 0.06[b]
C20:4n-6	0.061± 0.007[b]	0.081± 0.009[a]	0.058± 0.004[b]
C20:5n-3	0.012± 0.001[b]	0.023± 0.002[a]	0.000± 0.001[c]
C22:5n-3	0.048± 0.005[a]	0.057± 0.008[a]	0.000± 0.001[b]
n-6:n-3	1.19 ± 0.10[b]	1.45 ± 0.10[a]	1.34 ± 0.10[a]
SFA	48.82 ± 5.02[b]	41.28± 5.03[c]	74.42± 6.63[a]
MUFA	37.67± 4.05[b]	41.02± 4.49[a]	18.14± 2.03[c]
PUFA	14.87± 1.55[b]	18.55± 2.03[a]	10.33± 1.09[c]
AI	0.85± 0.09[b]	0.64± 0.07[c]	3.07± 0.20[a]
TI	0.59± 0.06[b]	0.57± 0.06[b]	1.29± 0.02[a]
DFA	52.82± 5.33[b]	60.92± 5.99[a]	27.24± 3.05[c]
HSFA	30.05± 3.78[b]	28.27± 3.50[b]	45.38± 4.69[a]

[a-c]Mean ± standard deviation with different lowercase letters in the same line differed by Tukey's test ($p < 0.05$). T1 – day 1, T21 – day 21.

were extremely low if compared to those needed for non-oxidative decomposition of fatty acids (>200 °C) (Pestana, Gennari, Monteiro, Lehn, & De Souza, 2015). Stress factors, such as the fermentation process, can induce changes in the fatty acid profile, resulting in alterations in the fatty acid unsaturation, cyclization, and concentrations of LCFA (Vieira et al., 2015). The increase in the unsaturated fatty acids is related to the ability of LAB to increase the unsaturation degree of fatty acid, which is suggested as a universally conserved adaptation response (Do Espírito Santo et al., 2012). The fermented milk presented a slightly higher n6/n3 ratio compared to milk (1.45 *vs* 1.19) ($p < 0.05$), but the values were kept far from the maximum recommendation.

During fermented milk storage (from day 1–21), there was an increase in the SFA, and decreases in the MUFA and PUFA levels ($p < 0.05$). Consequently, the health indices were impacted, with increases in the AI, TI, and HSFA, and decreases in the DFA ($p < 0.05$). Therefore, the storage time impacted negatively on the lipid profile and health indices. The stress suffered by the LAB during storage (low pH values and presence of oxygen) can result in alterations in the fatty acid profile, with increases or decreases in the SFA, MUFA and PUFA. This is associated with the ability of LAB to reach suitable proportions of fatty acid unsaturation and fatty acid chain length in the membrane of the cell (Vieira et al., 2015). The LAB enzymes necessary for these changes are, mainly, intracellular enzymes (Ziarno, Bryś, Parzyszek, & Veber, 2020). Therefore, during storage, it can have occurred lysis of cells that allowed the lipolytic activity at a higher rate. No effect of refrigerated storage was observed on the n6/n3 ratio ($p > 0.05$).

The results of the fatty acid profile and health indices indicate that the processing of milk for the production of fermented donkey milk improves the fatty acid profile of the products, but it is advisable to consume it in the first days of storage for the supply of a better-quality lipid.

3.3. Mineral content

Table 3 presents the mineral content of the donkey milk and fermented donkey milk. Donkey milk was characterized by higher contents of the macro minerals sodium (902.90 mg/100 g) and calcium (846.56 mg/100 g) when compared to phosphorous (580.74 mg/100 g). Concerning the micro mineral, magnesium (86.72 mg/100 g), and zinc (3.11 mg/100 g) were detected in higher contents compared to manganese (0.01 mg/100 g). For children, it is recommended a product with calcium and phosphorous ratio of 1–2:1 and sodium and potassium ratio of up to approximately 1 (WHO, 2012; Altomonte et al., 2019). The donkey milk presented 1.46 for the former, and 1.02 for the latter, which confirms its importance for young nutrition. A low sodium and potassium ratio is recommended considering the role of sodium in the development and severity of hypertension (Altomonte et al., 2019).

The consumption of 100 g of donkey milk would provide 84.65% of the recommended daily intake of calcium, 82.96% of phosphorous, 60.19% of sodium, and 20–28% of magnesium, potassium, and zinc. Calcium consumption is related to improvements in the teeth and bone structures, and this mineral act in the development and contraction of the muscles, being also important for the regulation of heart beating and blood pressure (Matera et al., 2018). Magnesium has an important role in several enzymatic reactions, helps the nervous system, and also the muscles (Satir & Guzel-Seydim, 2016). Phosphorus is important in the processes of gluconeogenesis, skeletal mineralization, glycolysis, cellular signal transduction, and energy metabolism (Takeda, Yamamoto, Yamanaka-Okumura, & Taketani, 2012). Sodium is important in the regulation of extracellular fluid volume and molecules' active transport through the cell membranes. However, its high consumption may increase the prevalence of hypertension (Doyle & Glass, 2010). Therefore, donkey milk is a product with important mineral content.

The fermented donkey milk presented higher concentrations of calcium (666.07–669.57 mg/100 g), potassium (845–848 mg/100 g), sodium (845.77–848.66 mg/100 g), and phosphorous (528.30–537.73 mg/100 g) compared to magnesium (72.97–73.34 mg/100 g), zinc (2.51–2.70 mg/100 g), and manganese (0.07 mg/100 g). Furthermore, it presented 1.25 for the calcium and phosphorous ratio, and 0.64 for the sodium and potassium ratio, which confirms its importance for young nutrition. The fermented milk presented higher manganese content, and lower calcium, phosphorous, magnesium, sodium, and zinc contents ($p < 0.05$) than the milk. The lower concentrations of minerals in fermented milk may be associated with the utilization of minerals by LAB for their metabolic and physiological activities during fermentation (Afoakwa, Kongor, Takrama, & Budu, 2013). Furthermore, it can be associated with the reduction of the solubility of the minerals after heating treatment (De La Fuente, Olano, Casal, & Juárez, 1999). Even though the lower mineral content, the consumption of 100 g of fermented donkey milk would provide 66% of the recommended daily intake of calcium, 75–76% of phosphorous, 35–36% of sodium, and 17–24% of magnesium, potassium, and zinc. During fermented milk storage, there was maintenance of the mineral content of the products ($p > 0.05$).

The results of the mineral content suggest that the processing of milk for the production of fermented donkey milk decreases the mineral content of the products, and the storage time had no impact on it.

Table 3
Mineral content (mg/100 g) of fresh donkey milk and fermented milk during refrigerated storage.

Mineral (mg/100 g)	Mineral content			Recommended daily intake (%) (portion – 100 g)			Recommendation (mg)*
	Milk	Fermented milk		Milk	Fermented milk		
		T1	T21		T1	T21	
Calcium	846.56 ± 2.53[a]	669.57 ± 4.91[b]	666.07 ± 6.31[b]	84.65	66.96	66.61	1000[1]
Phosphorous	580.74 ± 3.81[a]	537.73 ± 3.60[b]	528.30 ± 5.85[b]	82.96	76.82	75.47	700[2]
Magnesium	86.72 ± 0.77[a]	73.34 ± 0.25[b]	72.97 ± 0.62[b]	20.65	17.46	17.37	420[2]
Manganese	0.01 ± 0.01[b]	0.07 ± 0.01[a]	0.07 ± 0.01[a]	0.43	3.04	3.04	2.3[1]
Potassium	880.75 ± 37.46[a]	845.77 ± 8.08[a]	848.66 ± 19.20[a]	25.90	24.88	24.96	3400[1]
Sodium	902.90 ± 20.81[a]	540.25 ± 9.17[b]	528.12 ± 8.38[b]	60.19	36.01	35.20	1500[1]
Zinc	3.11 ± 0.12[a]	2.70 ± 0.11[b]	2.51 ± 0.02[b]	28.27	24.54	22.82	11[2]

*Based on National Academies of Sciences, Engineering, and Medicine (2019, adult man aged 31-50 years)Guideline: Sodium intake for adults and children. Geneva, World Health Organization (WHO).
[a-b]Mean ± standard deviation with different lowercase letters in the same line differed by Tukey's test ($p < 0.05$). T1 – day 1, T21 – day 21.

3.4. Amino acid profile

Table 4 presents the amino acid profile of the donkey milk and fermented donkey milk. The donkey milk presented all of the essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, and tryptophan). It presented higher concentrations of glutamic acid (73.58 mg/100 g), histidine (36.85 mg/100 g), and serine (21.68 mg/100 g) compared to lysine (14.38 mg/100 g), and alanine (13.67 mg/100 g), and these were the main amino acids. The amino acid profile of donkey milk was substantially more similar to that reported for human milk (mg/100 g; 18.8; 27 histidine, 48 serine, 7.3 lysine, and 4.2 alanine) if compared to cow milk (Altomonte et al., 2019). The composition of infant feed is usually evaluated by an amino acid score, which is based on the human milk amino acid composition (Altomonte et al., 2019). Therefore, the similarity of donkey milk with human milk is important and suggests that donkey milk can be used in infant formulas.

The fermented donkey milk presented higher concentrations of glutamic acid (109.18–109.29 mg/100 g), histidine (48.28–50.07 mg/100 g), serine (26.58–26.87 mg/100 g), and alanine (20.75–24.39 mg/100 g) compared to lysine (9.17–9.58 mg/100 g), and these were the main amino acids. The fermented milk processing had an impact on the amino acid profile, promoting increases in aspartic acid, glutamic acid, serine, histidine, arginine, alanine, proline, tyrosine, and phenylalanine, and decreases in the other amino acids (glycine, threonine, valine, methionine, isoleucine, lysine) ($p < 0.05$). Therefore, fermented milk processing increased the concentration of a higher number of amino acids. However, it is important to mention that it decreased the content of 7 amino acids that are considered as essential (threonine, valine, methionine, isoleucine, leucine, lysine, and tryptophan). LAB have the capacity of degrading large proteins into peptides, and then, to amino acids (Miao et al., 2020). However, LAB need amino acids to ferment milk (Ma et al., 2016). Therefore, the differences in the amino acid profile between donkey milk and fermented donkey milk may be associated with proteolysis during fermentation and amino acid utilization by LAB. Furthermore, some amino acids may have been lost during heat treatment (Melini, Melini, Luziatelli, & Ruzzi, 2017).

During fermented milk storage, there was reduction in the proline and tyrosine contents, and increases in the other amino acids (arginine, threonine, alanine, valine, methionine, isoleucine, leucine, lysine, and tryptophan) ($p < 0.05$). Therefore, the storage time was important to improve the amino acid profile of the fermented milks. The results suggest that LAB continued the proteolysis process during refrigerated storage, increasing the concentration of some amino acids. Furthermore, storage can promote chemical modification of the amino acids, being the effect dependent on the conditions (temperature and time), and product characteristics (mainly water activity) (Lieshout, Van, Lambers, Bragt, & Hettinga, 2020).

The results of the amino acid profile suggest that the processing of donkey milk for production of fermented donkey milk decreased the amino acid content of the products, mainly of the essential ones. However, fermented milk still provides important amino acid contents, mainly at the end of the storage time.

Table 4
Free amino acid (mg/100 g) of fresh donkey milk and fermented milk during refrigerated storage.

Mineral (mg/100 g)	Milk	Fermented milk	
		T1	T21
Aspartic acid	4.67 ± 0.11[b]	8.03 ± 0.01[a]	7.72 ± 0.54[a]
Glutamic Acid	73.58 ± 0.62[b]	109.18 ± 0.30[a]	109.29 ± 1.68[a]
Serine	21.68 ± 0.10[b]	26.87 ± 0.08[a]	26.58 ± 0.09[a]
Glicine	13.49 ± 0.45[a]	4.22 ± 0.02[b]	5.04 ± 0.01[b]
Histidine	36.85 ± 0.69[b]	50.07± 0.05[a]	48.28 ± 0.43[a]
Arginine	9.85 ± 0.01[c]	15.10 ± 0.01[b]	16.05 ± 0.03[a]
Threonine	6.45 ± 0.01[a]	4.62 ± 0.01[c]	4.75 ± 0.01[b]
Alanine	13.67 ± 0.03[c]	20.75 ± 0.01[b]	24.39 ± 0.02[a]
Proline	3.36 ± 0.01[c]	11.94 ± 0.04[a]	10.74 ± 0.13[b]
Tyrosine	3.87 ± 0.01[c]	5.60 ± 0.01[a]	5.20 ± 0.11[b]
Valine	5.66 ± 0.04[a]	1.45 ± 0.01[c]	2.08 ± 0.01[b]
Methionine	2.27 ± 0.03[b]	1.84 ± 0.01[c]	2.62 ± 0.01[a]
Cistine	4.99 ± 0.01[a]	4.43 ± 0.01[a]	5.34 ± 0.50[a]
Isoleucine	5.33 ± 0.02[a]	1.28 ± 0.01[c]	2.23 ± 0.01[b]
Leucine	4.46 ± 0.01[b]	4.03 ± 0.01[c]	4.81 ± 0.04[a]
Phenylalanine	4.50 ± 0.04[b]	7.28 ± 0.04[a]	7.88 ± 0.30[a]
Tryptophan	5.04 ± 0.05[a]	3.74 ± 0.10[c]	4.50 ± 0.15[a]
Lysine	14.38 ± 0.06[a]	9.17 ± 0.12[c]	9.52 ± 0.10[b]

[a-c]Mean ± standard deviation with different lowercase letters in the same line differed by Tukey's test ($p < 0.05$). T1 – day 1, T21 – day 21.

3.5. LAB viability

Fig. 1 presents the LAB viability in the fermented donkey milk. The products presented 7.39–8.27 log cfu/g of LAB, and no effect of storage time was observed ($p > 0.05$). Brazilian law establishes some minimum requirements for counts of specific microorganisms in fermented milks, which must be met throughout the shelf life of the products. Therefore, the products must have at least 6 log cfu/g of LAB (Brasil, 2007). The products of the present study complied with the legislation. This maintenance of the LAB viability corroborates the other results of the present study, as maintenance of the physicochemical characteristics, chemical composition (except sugars), and mineral profile was observed.

4. Conclusion

This is the first study to determine the complete nutritional value of donkey milk and fermented donkey milk, and it provides important results to the dairy industry. The fermented milk presented different physicochemical characteristics (moisture and acidity) and lower nutritional value (lower lactose, and mineral and amino acid contents)

than milk. However, it had a better fatty acid profile. The fermented milks could be refrigerated stored for 21 days without negative impacts on the nutritional value of the products, except for the fatty acid profile. It can be concluded that both donkey milk and fermented donkey milk present important nutritional value, and the fermented milk could be refrigerated stored for 21 days. Donkey milk is still underutilized, and the results of the present study can be used to encourage its consumption or processing into fermented milks. This study presented important information about the nutritional value of donkey milk produced in the Semiarid region of Brazil and the fermented milk produced with it, addressing the modifications after processing and storage. Furthermore, it contributes to disseminating the nutritional and technological advantages of this milk, valuing the dairy activity, as well as its utilization in the development of dairy products.

CRediT authorship contribution statement

Natália Sufiatti Holanda Cavalcanti: Conceptualization, performed the experiments, Formal analysis, Writing – original draft. **Tatiana Colombo Pimentel:** Formal analysis, Writing – original draft. **Marciane Magnani:** Conceptualization, Formal analysis, Writing – original draft. **Maria Teresa Bertoldo Pacheco:** performed the experiments. **Susana Paula Alves:** performed the experiments, Formal analysis. **Rui José Branquinho Bessa:** Formal analysis. **Amanda Marília da Silva Sant'ana:** performed the experiments, Formal analysis, Resources, Supervision. **Rita de Cássia Ramos do Egypto Queiroga:** Writing – original draft, Project administration, Resources, Supervision, Formal analysis.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.111239.

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