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Comparison of Nutritional Characteristics of Fresh and Freeze-Dried Donkey Milk

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Research Article

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Abstract

This study was performed in order to compare the chemical and nutritional properties of raw donkey milk and reconstituted milk samples made from freeze-dried donkey milk powder. Fresh donkey milk samples were collected from 18 Ragusana breed pluriparous donkeys. The freeze-drying process was performed on fresh milk using a lyophilizer programmed to operate for 1 h initial freezing at - 45°C followed by primary drying at 30 °C at 0.10 mbar pressure for 48 h and secondary drying at 5°C for 3 h at the same pressure. Reconstituted milk (90%, wb) was prepared dissolving 0.9 g of freeze-dried donkey milk in 10 ml of distilled water. Nutritional properties of freeze-dried donkey milk remained basically unchanged compared with fresh milk, obtaining very similar values as regard chemical composition, fatty acid profile and minerals content in both kind of milks. The results of the present study confirmed that freeze-drying is a relatively sparing method for preservation of short-lived products due to the fact that the main quality parameters of fresh milk are not significantly affected by the treatment, while the transport and the storage costs of freeze-dried milk are greatly reduced.

Keywords: Donkey Milk; Chemical Composition; Freeze-Drying; Fatty Acid Profile; Essential Fatty Acids; Mineral Content

Abbreviations: FDA: Food and Drug Administration; CRUA: Consorzio Di Ricerca Unico Di Abruzzo; TN: Total Nitrogen; BHT: Butylated Hydroxytoluene; PGE3: Prostaglandins; LTB5: Leukotrienes.

Introduction

Donkeys belong to the equine family and today's number in the entire world is over 44 million, mostly in developing countries [1]. There has recently been a steady increase in donkey populations despite very minimal investment in its production, research and management [2]. The consumption of donkey meat and milk is increasing in both developing and less developed countries although the donkey is not considered in many countries of the world as a food producing animal [3]. Donkey milk is also gaining prominence and has been shown to have some nutritional beneficial factors compared to cow's milk [4-6]. One of the most important nutritional characteristic of donkey milk is the similar chemical composition compared to human milk, considering especially lactose and mineral contents, fatty acid and protein profiles, which make it the most appropriate mammalian milk in those cases in which other milk types cannot be employed, such as in presence of cow milk protein allergies in children and also in adults [7-9].

Considering the hygienic characteristics, raw donkey milk shows, compared to ruminants milk, a lower total bacterial count, mainly represented by lactic bacteria [10]. The healthy donkey milk properties are related to the high content of some antimicrobial peptides, particularly lysozyme and lactoferrin [11-13], and also to the anatomy of the udder [14]. Donkey milk is not produced in large amount, therefore it is not always or easily available on the market [15]; the most common family practice for storing donkey milk at home is freezing the fresh milk at temperatures ranging between -15 and -20°C. Consumption of raw donkey milk must be avoided because this food can be contaminated with foodborne pathogens of animal and environmental origin [16,17] which may develop during milk storage at room temperature. Recently, also the European Food Safety Authority has been called upon to provide scientific opinion on the public health risks related to the consumption of raw drinking milk [18]. The main conclusion indicates that raw milk consumption represents a realistic threat for human health and a public risk, because it can act as a vector of pathogens and spoilage microorganisms. The hazards related to raw milk consumption are also well evidenced on the websites of authoritative institutions like the Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention [19].

For all the above-mentioned reasons, use of thermal treatments to reduce microbial content and to prolong donkey milk shelf life received interest by the scientific community; freezing [20,21], pasteurization [22,23], condensation [24], High Pressure Processing [25] and spray-drying [26,27] have been investigated. Freeze-drying (or lyophilisation) is another common method used for milk preservation; the effects freeze-drying on donkey milk volatile profile has been recently tested [28]. To fulfil the aim of extending donkey milk shelf life, the main targets of all the thermal treatments tested till now are to reduce the microbial population and minimize changes of chemical composition, in order to maintain the nutritional properties of donkey milk [29]. Freeze-drying is generally seen as a very expensive preservation method, but should not be considered as a prohibitively expensive process if it gives a reasonable added value to the product, or if it keeps its high-value, as compared to other preservation methods [30].

Freeze-drying is a thermal treatment which does not significantly affect donkey milk enzymatic activity, even if lysozyme concentration is slightly reduced [31], and does not affect the thermal behaviour of donkey milk whey proteins [28]. Donkey milk fatty acid profile has been previously investigated [32,33] but nothing is known about the changes that occur after donkey milk lyophilization. Minerals content in donkey milk is very similar to that one determined in human milk [9]; dietary trace element supplementation did not affect the donkey milk macro-mineral profile [34] but the effects of freeze drying on donkey milk minerals content are unknown. Milk freeze-drying treatment increases its safety for human consumption by killing pathogens and reducing microorganisms which may cause spoilage. The aim of the present study was to investigate the effects of freeze-drying treatment on donkey milk chemical composition, fatty acid profile and mineral content, in order to compare the nutritional properties of fresh and lyophilized donkey milk.

Materials and Methods

Animals and milk samples

The experimental protocol was approved by the Italian Ministry of University, and the study was conducted in compliance with the requirements of the Animal Ethics Committee of the University of Camerino. The bulk raw donkey milk samples were collected in June 2016 from the morning milking of 18 Ragusana breed pluriparous jennies (age ranging between 7 and 9 years) in midstage of lactation (90-110 days after foaling), with an average body weight of 289±24 kg. Donkeys were reared in a registered farm, in compliance with Regulations EC n. 853/2004, named "Ciucolandia" and located in Capestrano (AQ), Abruzzo Region, Italy. The farm followed the typical farming systems based on natural pasture integrated with ad libitum polyphite hay and an integration of 1 kg/head per day of concentrate. The jennies were daily machine milked; foals were physically separated from the dams 3 h before the milking. Milk samples were collected in duplicate, then were transported in a refrigerated bag (+4°C) to the laboratories for chemical analysis. The two aliquots collected were named, respectively: sample A, fresh donkey milk, and sample B, to be processed for obtaining freeze-dried donkey milk.

The freeze-drying process was performed by CRUA (Consorzio di Ricerca Unico di Abruzzo), Avezzano (AQ), Italy. A Beta 1-16 lyophilizer (Christ, Osterode am Harz, Germany), was used for whole raw donkey milk. The device was programmed to operate for 1 h initial freezing at – 45°C followed by primary drying at 30°C at 0.10 mbar pressures for 48 h and secondary drying at 5°C for 3 h at the same pressure [35]. During this process, the following parameters were continuously monitored: temperature of the dish, milk temperature and condenser temperature.

Microbiological and Chemical Analysis

The total solids in freeze-dried donkey milk were determined according to the AOAC procedure [36] for fat, ash and total protein contents; lactose determination in freeze-dried donkey milk samples was calculated according to the modified phenol-sulfuric acid procedures [35].

Total Bacterial Count in whole fresh milk at 32°C was determined using the culture method described by Sarno, et al. [14], while Somatic Cell Count was obtained in whole fresh milk, too, following the protocol indicated by Beghelli, et al. [37]. Fat and lactose were determined by infrared analysis [38]; total nitrogen (TN) in milk was determined by the Kjeldahl method, then total proteins (TN×6.38), were calculated [36]. Dry matter was determined on 10 g milk in a drying oven at a temperature of 102°C. Ash content was determined using the gravimetric method after calcination of the milk sample in a muffle furnace at 530°C using 20 g of donkey milk [39].

To prepare the reconstituted donkey milk samples with a moisture content of 90% (wb), 0.9 g of freeze-dried donkey's milk was completely dissolved in 10 ml of distilled water. Moisture, protein, fat and ash contents in reconstituted milk were determined using the AOAC method [36], while lactose content was evaluated using the method described by Kwak HS, et al. [40].

Gross energy content in fresh and reconstituted donkey milk was determined in excess oxygen by adiabatic bomb calorimeter (Mod. 700, IKA GmbH & Co., Staufen, Germany), using benzoic acid as a reference (26.454 MJ/kg), following the protocol [10].

Fatty acid composition was determined in fresh and in reconstituted donkey milk. Total lipids were extracted from the milk [41]; for the preparation of fatty acids methyl esters the lipid sample (20 mg) was dissolved in 0.1 ml of tetrahydrofuran in a test tube and 10% methanolic hydrogen chloride (2 ml) were added [42]. The fatty acids methyl esters were extracted with 2x2 ml of hexane and 1 µl was injected into a gas-chromatograph. Fatty acid analysis was performed on a Chrompack (model CP 9003, Agilent Technologies, California, USA) gas chromatograph with a flame ionization detector and a fused-silica capillary column, film thickness 0.2 µm, packed with CP Sil 88 (100 m x 0.25 mm i.d.). Helium was used as the carrier gas; fatty acid identification was made by comparing gas chromatographic retention times with the anti-oxidant standard butylated hydroxytoluene (BHT).

Minerals content were determined by using Graphite Furnace Atomic Absorption Spectrophotometer (Perkin Elmer AAnalyst 800, Shelton, CT, USA) for Calcium, Phosphorus, Potassium. Sodium and Magnesium, working on dry ash obtained in muffle furnace at 530°C and redissolved with HCl [39]. Chloride was determined using a potentiometric method [43].

Statistical Analysis

Data were analysed by the method of least squares using the general linear model procedures [44]. Significant differences between means were indicated when $P < 0.05$.

Results and Discussion

Chemical Composition of Freeze-Dried Donkey Milk

Chemical composition determined in lyophilized donkey milk powder is shown in (Table 1). The expected low moisture level determined in freeze-dried donkey milk (3.1±0.67 %) makes the lyophilized product much lighter than those dried using other drying methods, without the need of refrigeration during storage [35]. Moisture content determined in lyophilized donkey milk showed the same value (3.1%) obtained in freeze-dried goat milk [45]. Considering freeze-dried cow's milk, an experiment [46] found moisture content of 3.4% in cow's milk lyophilized samples.

Chemical composition of freeze-dried donkey milk powder (means±s.e.).	
Dry matter (%)	96.9±3.98
Moisture (%)	3.1±0.67
Lactose (%)	73.2±4.2
Proteins (%)	16.1±1.23
Fat (%)	4.48±0.75
Ash (%)	3.20±0.47

Table 1: Chemical composition of freeze-dried donkey milk powder (means±s.e.).

Milk components during freeze drying process are redistributed in the drying droplets and the powder composition is significantly different from that of the bulk [47]. In the present study (Table 1), chemical composition determined in freeze-dried donkey milk powder showed lactose as the most represented nutrient found in lyophilized donkey milk (73.2 %), followed by proteins content (16.1 %), then fat (4.48%) and finally ash (3.20%): the Total Solid content was 96.9 %. Previous studies in which lyophilized powders obtained by camel milk [35] and goat milk [45] were chemically analysed found lactose as the most represented nutrient, too, with Total Solid content, respectively, of 96.7 % in camel milk and 96.9 % in goat milk. Studies performed in order to determine chemical composition of industrial spray dried cow's milk powders [46,47] found higher amount of lactose compared to protein and fat, too, with a Total Solid content (96.7%) very similar to that one obtained in freeze-dried donkey milk.

Microbiological Characteristics and Chemical Composition

Table 2 shows the microbiological characteristics of raw donkey milk and the comparison of chemical composition determined in fresh donkey milk and in reconstituted samples made from freeze-dried donkey milk. In fresh donkey milk, Somatic Cells Count (SCC) were in the same range of values determined in a study performed using Martina Franca donkey breed [48-50], in which SCC were evaluated in different milkings per day. Another study [51] found similar values of SCC in milk produced by Ragusana jennies, too. Total Bacterial Count (TBC) showed an average value of 4.28±1.1 Log CFU/mL (Table 2); the possible reasons for low bacterial values could be [14] the high presence of natural concentration of antimicrobial compounds like lactoferrin and lysozyme which acts directly on bacteria [11]. In other studies, mesophilic bacteria range from 1 to 2.39 Log CFU/mL [16] and mean values of 4.46 Log CFU/mL were showed in a review [43]. Results obtained in this study showed that TBC values were in compliance with the stipulated standards values for raw milk from "other species" (Reg. EC 853/2004, amended by Reg. EC n.1662/06). However, despite no violations of current raw milk regulatory standards, these results do not ensure the complete safety of donkey milk, because in previous studies undesirable pathogens (such as *Streptococcus spp*., *Staphylococcus spp*., *Listeria spp*., *E. coli* strains) responsible for food-borne diseases harmful to humans have been detected in donkey milk [52].

The moisture, protein, fat, lactose, and ash contents of the reconstituted milk were not significantly different compared to the values obtained in fresh donkey milk (Table 2), confirming the results obtained in a study [46] in which raw and reconstituted lyophilized cow's milk were analysed. Both fresh and reconstituted donkey milk were characterized by a mean protein content close to 1.6 g/100 g and a mean fat content lower than 0.5 g/100 g. Lactose content was higher (7.23 ±0.46 g/100 g) in reconstituted donkey milk compared with fresh one (7.08±0.62), while ash content was higher in fresh donkey milk (0.42±0.07 g/100 g vs 0.37±0.11 g/100 g).

	Fresh milk (n=18)	Reconstituted milk (n=18)	*P value*
Dry matter (%)	9.56±0.38	9.69±0.23	n.s.
Moisture (%)	90.4±1.01	90.3±1.67	n.s.
Lactose (%)	7.08±0.62	7.23 ±0.46	n.s.
Proteins (%)	1.62±0.44	1.61±0.42	n.s.
Fat (%)	0.44±0.16	0.48±0.14	n.s.
Ash (%)	0.42±0.07	0.37±0.11	n.s.
Gross Energy (kcal/100 mL)	38.2±5.84	39.0±6.01	n.s.
Total Bacterial Count (log CFU/mL)	4.28±1.1		
Somatic Cell Count (SCC/mL)	29.65±2.55		

n.s.: not significant

Table 2: Chemical composition, energy content and microbiological characteristics of raw and reconstituted donkey milk (means±s.e.).

Energy content showed no significant differences between freeze-dried reconstituted and fresh donkey milk (Table 2), as determined also in a previous study in which freeze-dried and fresh camel milk gross energy contents were compared [35]. The low energy content in donkey milk is the main limit to its use in nutrition of children allergic to cow's milk protein, in their first year of life, since recommended dietary allowances may not be reached, unless adequate supplementation is provided [10]. On the other hand, this nutritional characteristic makes donkey milk a hypo-caloric and highly digestible food for consumers with specific dietary requirements, such as athletes and elderly people [53].

The results of the present study confirmed that freeze-drying of raw milk could be a very efficient technique for producing dried milk powder with little changes in most nutrient compounds, especially if compared with the spray-drying technique [28,46].

Fatty Acid Composition

Results obtained for fatty acid profile in fresh and reconstituted donkey milk are shown in (Table 3). Fatty acids content in fresh and in freeze-dried donkey milk did not show significant differences among the two kinds of milk used in this study. Fatty acid profile is characterised, both in fresh and in freeze-dried milk, by large amounts of polyunsaturated fatty acids, including essential fatty acids (n-6 and n-3) which counteracting with the oxidative stress, diminishing the inflammatory response in allergic disease [15]. Moreover, the low presence of short chained fatty acids renders it adequate (better tolerance) for infant diet [43]. Both fresh and lyophilized donkey milk show valid MUFA and PUFA content, with a ratio UFA/SFA respectively of 0.89 in fresh and 0.90 in freeze-dried milk. The most abundant fatty acids are: among the saturated fatty acids, palmitic acid (21.1% in fresh and 19.3% in lyophilized), among the monounsaturated fatty acids, oleic acid (26.8% in fresh and 28.3% in lyophilized) and among the polyunsaturated fatty acids, linoleic acid (9% in fresh and 8.2% in lyophilized). PUFA also showed small amounts of eicosapentaenoic (EPA, C20:5 n-3) and docosahexaenoic acids (DHA, C22:6 n-3) in both fresh (0.29 % EPA, 0.31 DHA) and freeze-dried (0.28 % EPA, 0.30% DHA) donkey milk.

Fatty acid	Fresh milk	Freeze-dried milk	P-value
C4:0	0.52±0.01	0.59±0.05	n.s.
C6:0	0.89±0.01	0.84±0.03	n.s.
C8:0	5.57±0.22	4.85±0.21	n.s.
C10:0	8.81±0.67	8.24±0.58	n.s.
C12:0	7.41±0.21	7.52±0.11	n.s.
C14:0	4.64±0.51	5.77±0.44	n.s.
C15:0	0.68±0.02	0.62±0.03	n.s.
C16:0	21.1±2.68	19.3±2.11	n.s.
C16:1 (n-7)	3.30±0.42	3.07±0.18	n.s.
C18:0	2.60±0.38	2.90±0.41	n.s.
C18:1 (n-9)	26.8±3.11	28.3±2.18	n.s.
C18:2 (n-6)	9.03±0.47	8.22±1.01	n.s.
C18:3 (n-3)	7.16±0.89	7.34±0.76	n.s.
C20:0	0.68±0.06	1.76±0.02	n.s.
C20:4 (n-6)	0.23±0.02	0.17±0.08	n.s.
C20:5 (n-3)	0.29±0.04	0.28±0.03	n.s.
C22:6 (n-3)	0.31±0.06	0.30±0.05	n.s.
SFA	52.9±1.99	52.4±1.51	n.s.
UFA	47.1±1.78	47.6±1.66	n.s.
UFA/SFA	0.89	0.9	n.s.
∑ n-3	7.76	7.92	n.s.
∑ n-6	9.57	8.39	n.s.
∑ n-6/∑ n-3	1.23	1.06	n.s.

n.s.: not significant
SFA: Saturated Fatty Acids, UFA: Unsaturated Fatty Acids
Table 3: Fatty acid composition (% total fatty acids) determined in fresh and in freeze-dried donkey milk (means±s.e.).

The considerable presence of unsaturated fatty acids together with the low atherogenic index and thrombogenic index [20] observed in donkey milk make it very interesting in the prevention of the cardiovascular, auto-immune and inflammatory diseases [54]. The high amount of n-3 essential fatty acids found in both fresh and freeze-dried donkey milk, higher than in human milk, makes donkey milk an adequate substitute for human milk, especially during the early lactation period. Moreover, the higher levels of n-3 polyunsaturated fatty acids content of donkey milk, characteristic constituents of the fish oils, can counteract the above-mentioned pathologies through the synthesis of anti-inflammatory, antiaggregant and non-immunosuppressant substances, like lipid mediators (eicosanoids), prostaglandins (PGE3) and leukotrienes (LTB5), and protein mediators (cytokines), interleukins (IL4, IL10, IL13, IL1ra), tumour necrosis factor, etc. [55].

Mineral Content

Mineral content of fresh and reconstituted donkey milk is shown in (Table 4). Minerals are extremely important components of milk, necessary for the vital activity of the suckling animals and for human nutrition. Minerals have a significant physiological importance for the young animals and participate in the synthesis of enzymes, vitamins and hormones of vital importance; without them the assimilation of the nutritional substances by the organism is impossible [34]. Donkey milk is characterised by a high concentration of calcium and phosphorus [9, 43]. Results obtained in this study showed the highest average concentration was determined for Ca, followed by P, K, Cl, Na and Mg (Table 4), remaining in the interval of results obtained in mare's milk and in donkey's milk in previous studies [9]. Minerals content in both fresh and freeze-dried donkey milk shows values lower compared to dairy cows milk but higher if compared to human milk [56]. A study performed on milk of small ruminants [57] determined that the levels of Ca, Cu, K, Na, Mg, and Zn in both goat and sheep milk were variable during the lactation period, confirming the results obtained in donkey milk [34].

Macroelement	Fresh donkey milk	Freeze-dried donkey milk	P value
Calcium	714.2±38.7	762.3±27.8	n.s.
Phosphorus	481.5±28.3	497.4±23.5	n.s.
Potassium	442.7±27.1	428.8±31.0	n.s.
Chloride	320.6±36.1	361.5 ±42.4	n.s.
Sodium	188.3±13.8	194.7±17.4	n.s.
Magnesium	25.8±3.87	28.6±2.76	n.s.

n.s.: not significant

Table 4: Mineral content (means±s.e.) in fresh and in freeze-dried donkey milk (mg kg-1 milk).

The main reasons for this variability during lactation may be related to the quality of feed and pasture, associated with fluctuations in vegetation and climate conditions. Further studies are necessary in order to better understand the effects of different diets on the variation of mineral content in donkey milk, in order to define the correct dietary requirements.

Conclusion

The therapeutic and nutritional value of donkey milk is a crucial and important landmark development for improving donkey husbandry, production and valorisation. Good agricultural practices and good animal husbandry practices at the farm level enable to obtain high quality raw donkey milk, which allows the application of less severe thermal treatments and thus the preservation of the primary quality of raw milk. Lyophilized donkey milk is easy to transport, requires no special conditions for prolonged storage, shows nutritional properties very similar compared with fresh donkey milk. Therefore, freeze-drying and can be a useful tool to supply donkey milk on the market all-over the year.

Further studies are necessary to better evaluate the effects of feeding and nutrition on donkey milk quality, considering the strong influence of diet on fatty acid composition of non-ruminant milk and also on mineral content. Given the low fat and the high n-3 essential fatty acids content, donkey milk has an additional important benefit: it may help prevent the onset of obesity and chronic diseases, with a consequent significant financial and social impact.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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