INFLUENCE OF FREEZE-DRYING ON THE PHYSICOCHEMICAL COMPOSITION, FATTY ACID PROFILE, AND ANTIOXIDANT ACTIVITY OF DONKEY COLOSTRUM

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ABSTRACT

The present study provides comparative data on the physicochemical characteristics, antioxidant activity, and fatty acid composition of native and freeze-dried donkey colostrum. The results demonstrated that freezing, freeze-drying, and subsequent rehydration did not induce substantial changes in the overall physicochemical characteristics and fatty acid profile. The physicochemical characteristics and fatty acid profiles of the two matrices exhibited a high degree of similarity. Differences in the content of individual fatty acids between native and freeze-dried colostrum were below 30.0 g kg^{-1} of fat, while differences in the respective fatty acid groups and subgroups did not exceed 50.0 g kg^{-1} of fat. Elevated levels of polyunsaturated fatty acids (270.3 and 238.4 g kg^{-1} of fat), omega-3 fatty acids (184.6 and 162.0 g kg^{-1} of fat), and α-linolenic acid (183.9 and 159.8 g kg^{-1} of fat) were observed compared with the reference values reported in the literature for raw donkey colostrum. The omega-6/omega-3 ratio in the investigated samples was considerably lower than previously reported, indicating their high biological and nutritional value. Antioxidant activity, determined by the DPPH assay, showed comparable values for native (13.4 %) and rehydrated colostrum (14.5 %), while a similar trend was observed using the ABTS assay (79.9 % and 78.8 %, respectively). To the best of our knowledge, limited information is currently available on the physicochemical characteristics, fatty acid profile, and antioxidant activity of freeze-dried and subsequently rehydrated donkey colostrum.

Keywords: freeze-drying, donkey colostrum, fatty acid composition, antioxidant activity.

INTRODUCTION

In recent years, growing scientific interest has been directed toward the investigation of unconventional biological materials with potential applications in functional nutrition and preventive medicine. Donkey colostrum, both in its native and freeze-dried forms, has emerged as a promising subject of research in this field. Colostrum is the initial mammary secretion produced during the first days after parturition and serves as the first source of nutrition for newborn mammals. Its high biological value is attributed to its complex composition, which includes essential nutrients, growth factors, immunoglobulins, and anti-inflammatory components that provide passive immunity and support physiological development [1 - 3]. Compared with mature milk, colostrum contains higher concentrations of total solids, proteins, fats, vitamins, and minerals, while its lactose content is lower. It also stimulates the development of the digestive system and facilitates the excretion of meconium [2, 4, 5].

Due to its abundance of bioactive constituents, donkey colostrum plays an essential role in the growth and immune protection of newborns [6]. It differs from mature donkey milk in its higher concentrations of immunoglobulins (IgG and IgA), fats, vitamins, and proteins, as well as its lower lactose content [7, 8]. The composition of donkey colostrum is like that of human

colostrum, and Marchis et al. [1] suggested that donkey colostrum could serve as a suitable alternative to human colostrum [9].

In addition to its nutritional and immunological constituents, colostrum contains antioxidant systems that protect against reactive oxygen species. Antioxidants exert beneficial effects on human health by protecting the body against oxidative stress, which is associated with numerous diseases, including cardiovascular disorders [10, 11].

One of the biologically valuable components present in both milk and colostrum is α-linolenic acid, an omega-3 polyunsaturated fatty acid. Since α-linolenic acid cannot be synthesized by the human body, it must be obtained from dietary sources. It has been demonstrated to increase the levels of brain-derived neurotrophic factor (BDNF), a widely distributed protein that performs multiple essential functions in the brain [12, 13]. Furthermore, α-linolenic acid is converted into oxylipins, which contribute to vascular health by reducing inflammation and improving blood pressure regulation [14, 15]. Omega-3 fatty acids are essential for maintaining normal health, particularly for brain development and function [12].

Various preservation methods have been developed to extend the shelf life of food products, biological fluids, fruits, vegetables, microbial suspensions, and other biological materials over prolonged periods. One such method is freeze-drying (lyophilization), which combines freezing with dehydration under vacuum conditions. Freeze-drying effectively preserves sensory characteristics, including taste, color, and aroma, as well as the nutritional and biological value of the products. As a result, freeze-dried materials and samples can be stored for extended periods, typically 3 - 5 years, under ambient conditions (18 - 20°C) [3, 16, 17]. For the assessment of food products as healthy regarding their lipid content, the term “atherogenic index” is used. It was proposed by Ulbricht et al. [18] as a nutritional indicator associated with the risk of cardiovascular diseases, dyslipidemia, and other related disorders. The American Heart Association (AHA) recommends limiting the dietary intake of saturated fatty acids as part of a dietary strategy for reducing cardiovascular disease risk [19]. Among saturated fatty acids, lauric, myristic, and palmitic acids are particularly relevant because of their effects on blood cholesterol levels and their association with cardiovascular risk [20, 21]. Although the biological value of colostrum from commonly farmed ruminant species has been extensively studied, knowledge of the chemical composition and fatty acid profile of native donkey colostrum remains very limited [1, 4, 22 - 25]. Scientific publications concerning the effect of freeze-drying on this matrix are also scarce. The processing and application of freeze-dried colostrum from buffaloes, horses, goats, and cows have been the subject of several studies [3, 26 - 29].

Regarding donkey milk, only Polidori et al. [30] and Garhwal et al. [31] have investigated the physicochemical composition of freeze-dried donkey milk.

Similarly, antioxidant capacity, determined using the DPPH and ABTS assays, has been investigated mainly in freeze-dried colostrum from cows, sheep, goats, buffaloes, and camels [3, 11]. To date, published data on the physicochemical characteristics, fatty acid composition, and antioxidant activity of freeze-dried and subsequently rehydrated donkey colostrum remain very limited.

Due to this lack of information, the aim of the present study was to perform a comparative analysis of the physicochemical characteristics, fatty acid profile, and antioxidant activity of native and rehydrated freeze-dried donkey colostrum in order to determine the extent to which the freeze-drying process affects the stability of its biochemical matrix.

EXPERIMENTAL

The raw material, pooled donkey colostrum, was obtained from G&G Farm (Zhelyava village, Sofia Region, Bulgaria). The colostrum was collected within the first 24 to 48 h after parturition. Immediately after collection, the colostrum samples were homogenized and cooled to a working temperature of 4°C. Transportation and storage were carried out in specialized refrigerated containers. The samples were delivered in May 2025 to the Institute of Cryobiology and Food Technologies at the Agricultural Academy, Sofia, Bulgaria. Throughout the entire process, optimal conditions were maintained to preserve the physicochemical composition and microbiological stability of the biological material under investigation.

Physicochemical analyses

The moisture content of the freeze-dried donkey colostrum was evaluated using a thermo - balance moisture analyzer (DBS 60 - 3, KERN & Sohn GmbH, Balingen, Germany), and the dry matter content was calculated by subtracting the moisture percentage from 100 %. Water activity (*a*w) was measured at 25°C using a temperature - controlled water activity meter (LabTouch - aw, CM - 2/CM - 3 sensor version, Novasina AG, Lachen, Switzerland). The insolubility index (solubility/rehydration capacity) of the freeze - dried samples was determined according to ISO 8156:2005 [32].

The total protein content was quantified automatically with a Kjeldahl protein determination apparatus UDK - 129 Distillation Unit (VELP Scientifica) according to ISO 1871:2009 and the manufacturer's instructions [33]. Casein content was determined using the direct precipitation method according to ISO 17997 - 2:2004 [37]. Briefly, 10 % acetic acid was added to 100 mL of donkey colostrum to coagulate the casein by adjusting the pH to 4.6. The sample was subsequently filtered to separate the precipitated casein from whey proteins. The nitrogen content of the casein precipitate was then determined by the Kjeldahl method and converted to casein content. Whey protein content was calculated as the difference between the total protein and casein contents.

Total fat content was determined by extraction with hexane in a Soxtec Avanti 2055 apparatus (Foss Tecator, Denmark) according to EN ISO 659:2009 and the manufacturer's instructions [34]. Ash content was measured by sample mineralization in a muffle furnace according to ISO 936:1998 [35]. Lactose content was measured iodometrically according to the Luff - Schoorl method [36]. Finally, the energy value was calculated per 100 mL of product (kcal) based on its chemical composition according to Fusch et al. [38].

Biochemical analyses

Fatty acid composition of native and rehydrated freeze-dried donkey colostrum

Fatty acid composition of native and rehydrated freeze-dried donkey colostrum Milk fat was extracted using the Roese-Gottlieb method, and the fatty acids were methylated with sodium methoxide. Fatty acid methyl esters were analyzed by gas chromatography with a flame ionization detector using a Shimadzu 2000 gas chromatograph (Kyoto, Japan) equipped with an automatic injection system. The results were expressed as g kg^{-1} of fat.

Determination of the atherogenic index of native and rehydrated freeze-dried donkey colostrum

The atherogenic index (AI) of donkey colostrum was calculated according to Eq. (1) proposed by Ulbricht et al. [18]:

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / (MUFA + PUFA) \quad (1)$$

where: C12:0 - lauric acid (g kg^{-1} of fat); C14:0 - myristic acid (g kg^{-1} of fat); C16:0 - palmitic acid (g kg^{-1} of fat); MUFA - monounsaturated fatty acids, g kg^{-1} of fat; PUFA - polyunsaturated fatty acids, g kg^{-1} of fat.

Antioxidant activity analysis of native and rehydrated freeze-dried donkey colostrum

Sample preparation

The freeze-dried donkey colostrum was rehydrated to its native state based on the dry matter content. For the analysis, the samples were diluted fourfold - twice with distilled water and twice with methanol (MeOH). After the initial dilution, the samples were centrifuged for 10 min at 10°C (Beckman J2-21M System, CA, USA).

DPPH radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined as described by Brand-Williams et al. [39], with slight modifications. Trolox solutions in methanol at different concentrations (1.0 - 15 μg mL^{-1}) were used to construct the standard curve. To 1.2 mL of a methanolic DPPH solution (0.2 mM), 1.8 mL of pure methanol and 1.0 mL of the supernatant of the respective sample were added. After homogenization, the mixture was incubated in the dark for 60 min at room temperature, and the absorbance was measured spectrophotometrically at a wavelength of λ = 517 nm against methanol using a Libra S22 UV-Vis spectrophotometer (Biochrom, USA). In the control sample, the colostrum was replaced with 80 % methanol. The percentage of DPPH radical inhibition was calculated using Eq. (2):

DPPH radical scavenging activity (%) = [(Acontrol - Asample) / Acontrol] × 100 (2)

where Acontrol and Asample represent the absorbance of the control and analyzed samples, respectively.

ABTS radical scavenging activity

ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity was determined according to the method of Re et al., with modifications [40]. To generate the ABTS radical cation, equal volumes of a 7 mM ABTS solution in 0.1 M acetate buffer (pH 4.5) and 2.45 mM potassium persulfate in distilled water were mixed. The resulting solution was homogenized and kept in the dark for 12 - 16 h. To prepare the working solution, 4.0 mL of the generated ABTS radical solution was diluted with methanol to an absorbance of 0.700 - 0.900 at a wavelength of λ = 734 nm. Subsequently, 150 µL of the respective sample was added to 2.85 mL of the ABTS working solution. The solution was homogenized and kept in the dark for 60 min. Trolox solutions at concentrations ranging from 6.25 to 125.0 µg mL^{-1} were used to construct the standard curve. The percentage of ABTS radical inhibition was calculated using Eq. (3):

ABTS radical scavenging activity (%) = [(Acontrol - Asample) / Acontrol] × 100 (3)

where Acontrol and Asample represent the absorbance of the control and analyzed samples, respectively.

Technological experiments

Freeze-drying

The freeze-drying process was carried out using a Sublimator 30 EKS freeze-dryer (Zirbus technology GmbH, Germany). The samples were frozen directly in the freeze-dryer to -45°C. The secondary drying temperature ranged from 25°C to 30°C, ensuring final products with a low residual moisture content of 2 - 5 %, in accordance with the quality requirements for freeze-dried biological materials.

Statistical analysis

The analyses were performed in triplicate. The data are presented as mean ± standard deviation (SD). Differences between native and rehydrated freeze-dried donkey colostrum were evaluated using Student's t-test. Statistical significance was considered at $P \leq 0.05$, with significance levels indicated as follows: *$P \leq 0.05$, **$P \leq 0.01$, and ***$P \leq 0.001$; ns indicates a non-significant difference ($P > 0.05$). Statistical analyses were performed using Microsoft Excel 2016.

RESULTS AND DISCUSSION

The results for the physicochemical characteristics of freeze-dried donkey colostrum are presented in Table 1. The residual moisture content after freeze-drying was 2.38 %, which is below 5 %, while the dry matter content was 97.62 %. This indicates that the freeze-drying process was successfully completed. The total protein content was 45.52 %. Cingoz and Yorükoglu reported a similar concentration of 42.43 % in freeze-dried buffalo colostrum [3]. However, the authors found considerably lower total protein contents in freeze-dried colostrum samples from cows (34.87 %), sheep (38.07 %), and goats (28.29 %).

The fat content of the investigated freeze-dried donkey colostrum was similar to that reported by Cingoz and Yorükoglu for freeze-dried sheep colostrum (35.78 %) but was higher than the concentrations reported for freeze-dried bovine colostrum (16.69 %) and goat colostrum (29.20 %), and lower than the fat content of freeze-dried buffalo colostrum (40.21 %) [3].

The ash content was 6.39 %. Considerably lower values for this parameter were reported by Cingoz and Yorükoglu for freeze-dried colostrum from cows (0.95 %), sheep (1.03 %), goats (0.92 %), and buffaloes (0.98 %) [3]. The concentrations of casein and whey proteins in the investigated freeze-dried donkey colostrum were high,

Table 1. Physicochemical characteristics of freeze-dried donkey colostrum (n = 3) ($\bar{x}$ ± SD).

Parameter	Freeze-dried donkey colostrum
Moisture content, %	2.38 ± 0.34
Total protein, %	45.52 ± 0.80
Fat, %	37.30 ± 0.82
Lactose, %	6.87 ± 0.28
Dry matter, %	97.62 ± 0.34
Ash, %	6.39 ± 0.18
Water activity, *a*w	0.044 ± 0.0012
Casein, %	29.80 ± 0.45
Whey proteins, %	15.72 ± 0.37

amounting to 29.80 % and 15.72 %, respectively. This is attributable to the high total protein content.

Table 2 presents the physicochemical characteristics of the native and rehydrated freeze-dried donkey colostrum samples investigated.

The analyzed native and rehydrated donkey colostrum samples showed almost identical concentrations of moisture, total protein, dry matter, lactose, fat, ash, casein, and whey proteins. The calculated energy values of the two colostrum samples were also very similar. These results indicate that the freeze-drying process effectively preserved the physicochemical composition of donkey colostrum, as demonstrated by the high degree of similarity between the native and rehydrated freeze-dried samples. The slightly lower energy value of the rehydrated colostrum corresponded to its slightly lower fat content.

Martini et al. investigated the physicochemical characteristics of raw donkey colostrum, including total protein, fat, lactose, and ash, during the period from 0 to 48 h after the onset of lactation [4]. The fat content of the native colostrum analyzed in the present study was higher than the values reported by Martini et al., whereas the lactose concentration was lower than those reported by the same authors [4]. The total protein content of the analyzed raw colostrum was very similar to that determined by Martini et al. at 6 h after the onset of lactation (6.91 %) [4]. The ash content was within a range like that reported by Martini et al., who found values ranging from 0.52 to 0.82 % [4].

To the best of our knowledge, published data on the physicochemical characteristics of rehydrated freeze-dried donkey colostrum remain very limited.

Tables 3 - 7 present the fatty acid composition of native and rehydrated freeze-dried donkey colostrum. Table 3 shows the saturated fatty acid content, Table 4 the monounsaturated fatty acid content, Table 5 the polyunsaturated fatty acid content, Table 6 the branched-chain fatty acid content, and Table 7 the contents of the respective fatty acid groups and subgroups.

The results presented in Tables 3 and 4 show that there were no large differences in the concentrations of the individual saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) between native and rehydrated freeze-dried donkey colostrum. In the rehydrated colostrum, the concentration of lauric acid (C12:0) decreased slightly by 5.2 g kg^{-1} of fat, whereas that of myristic acid (C14:0) increased slightly by 9.5 g kg^{-1} of fat compared with their concentrations in the native colostrum. The palmitic acid (C16:0) content in the rehydrated colostrum increased by 21.1 g kg^{-1} of fat, with a highly significant difference ($P \leq 0.001$). Martini et al. reported palmitic acid concentrations in raw donkey colostrum ranging from 191.3 to 242.3 g kg^{-1} of fat, which were higher than the concentration determined in the native donkey colostrum investigated in the present study [4]. A highly significant increase ($P \leq 0.001$) was also observed for stearic acid (C18:0), the concentration of which increased by 4.4 g kg^{-1} of fat in the rehydrated colostrum. According to Martini et al., the stearic acid content of native donkey colostrum ranged from 12.14 to 16.48 g kg^{-1} of fat, which was slightly lower than that determined in the raw colostrum investigated in the present study [4].

Table 2. Physicochemical characteristics of native and rehydrated freeze-dried donkey colostrum (n = 3) ($\bar{x} \pm SD$).

Parameter	Native colostrum	Rehydrated freeze-dried colostrum	Statistical significance
Moisture content, %	87.08 ± 0.47	87.13 ± 0.58	ns*
Total protein, %	6.55 ± 0.05	6.57 ± 0.10	ns
Fat, %	4.51 ± 0.10	4.47 ± 0.09	ns
Lactose, %	1.07 ± 0.07	1.10 ± 0.05	ns
Dry matter, %	12.92 ± 0.47	12.87 ± 0.58	ns
Ash, %	0.79 ± 0.08	0.73 ± 0.06	ns
Casein, %	3.52 ± 0.06	3.60 ± 0.05	ns
Whey proteins, %	3.03 ± 0.06	2.97 ± 0.05	ns
Energy value, kcal $(100\ mL)^{-1}$	71.07 ± 1.34	70.91 ± 1.42	ns

ns - not significant (P > 0.05).

Table 3. Saturated fatty acids, g kg^{-1} of fat, in native and rehydrated freeze-dried donkey colostrum (n = 3) ($\bar{x} \pm SD$).

Saturated fatty acid	Native colostrum	Rehydrated freeze-dried colostrum	Statistical significance
C8:0	38.7 ± 0.4	37.2 ± 0.3	ns
C10:0	94.2 ± 1.0	89.1 ± 0.8	***
C11:0	0.5 ± 0.0	0.1 ± 0.0	*
C12:0	69.1 ± 0.8	63.9 ± 0.6	ns
C13:0	2.9 ± 0.0	11.7 ± 0.1	***
C14:0	50.2 ± 0.6	59.7 ± 0.5	ns
C15:0	0.5 ± 0.0	1.6 ± 0.0	ns
C16:0	169.2 ± 1.9	190.3 ± 1.7	***
C17:0	0.0 ± 0.0	3.0 ± 0.0	***
C18:0	17.3 ± 0.2	21.7 ± 0.2	***
C20:0	0.3 ± 0.0	2.0 ± 0.0	***
C22:0	0.5 ± 0.0	0.3 ± 0.0	ns
C23:0	0.1 ± 0.0	2.6 ± 0.0	***
C24:0	0.0 ± 0.0	0.8 ± 0.0	ns
C26:0	0.7 ± 0.0	0.4 ± 0.0	*

*ns - not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.*

Table 4. Monounsaturated fatty acids, g kg^{-1} of fat, in native and rehydrated freeze-dried donkey colostrum (n = 3) ($\bar{x} \pm SD$).

Monounsaturated fatty acid	Native colostrum	Rehydrated freeze-dried colostrum	Statistical significance
C10:1	16.5 ± 0.2	15.6 ± 0.1	*
C12:1 n-1	0.4 ± 0.0	2.3 ± 0.0	***
C14:1 n-5	3.6 ± 0.0	1.6 ± 0.0	***
C15:1 n-5	0.1 ± 0.0	1.3 ± 0.0	***
C16:1 *trans*-9	27.4 ± 0.3	30.2 ± 0.3	*
C16:1 n-7	36.5 ± 0.4	24.0 ± 0.2	***
C17:1 n-7	1.7 ± 0.0	3.3 ± 0.0	***
C18:1 *cis*-9	200.2 ± 2.2	195.5 ± 1.8	ns
C22:1 n-9	0.2 ± 0.0	1.1 ± 0.0	***

*ns - not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.*

Table 5. Polyunsaturated fatty acids, g kg^{-1} of fat, in native and rehydrated freeze-dried donkey colostrum (n = 3) ($\bar{x} \pm SD$).

Polyunsaturated fatty acid	Native colostrum	Rehydrated freeze-dried colostrum	Statistical significance
C18:2 *cis*-9,12	79.6 ± 0.9	72.8 ± 0.7	ns
γ-C18:3 n-6	0.5 ± 0.0	0.4 ± 0.0	***
α-C18:3 n-3	183.9 ± 2.0	159.8 ± 1.5	ns
C20:2 n-6	1.2 ± 0.0	1.1 ± 0.0	ns
C20:3 n-6	3.1 ± 0.0	1.1 ± 0.0	ns
C20:4 n-6	1.2 ± 0.0	1.5 ± 0.0	**
C20:5 n-3	0.1 ± 0.0	0.5 ± 0.0	***
C22:2 n-6	0.2 ± 0.0	0.5 ± 0.0	***
C22:6 n-3	0.5 ± 0.0	0.7 ± 0.0	ns

*ns - not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.*

Table 6. Branched-chain fatty acids, g kg^{-1} of fat, in native and rehydrated freeze-dried donkey colostrum (n = 3) ($\bar{x} \pm SD$).

Branched-chain fatty acid	Native colostrum	Rehydrated freeze-dried colostrum	Statistical significance
iso-C13:0	2.1 ± 0.0	2.4 ± 0.0	ns
anteiso-C13:0	2.0 ± 0.0	1.6 ± 0.0	ns
iso-C14:0	0.4 ± 0.0	0.1 ± 0.0	***
iso-C15:0	0.2 ± 0.0	0.9 ± 0.0	**
anteiso-C15:0	0.3 ± 0.0	1.3 ± 0.0	ns
iso-C16:0	0.4 ± 0.0	1.4 ± 0.0	ns
iso-C17:0	0.2 ± 0.0	0.4 ± 0.0	***
anteiso-C17:0	0.1 ± 0.0	0.5 ± 0.0	ns
iso-C18:0	0.1 ± 0.0	0.8 ± 0.0	***

*ns - not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.*

Table 7. Contents of fatty acid groups and subgroups, g kg^{-1} of fat, in native and rehydrated freeze-dried donkey colostrum (n = 3) ($\bar{x} \pm SD$).

Fatty acid group/subgroup	Native colostrum	Rehydrated freeze-dried colostrum
Σ C18:1 *cis*-FA	200.2 ± 2.2	195.5 ± 1.8
SFA	444.0 ± 4.9	484.7 ± 4.5
MUFA	286.6 ± 3.2	274.9 ± 2.6
PUFA	270.3 ± 3.0	238.4 ± 2.2
Σ n-3	184.6 ± 2.0	162.0 ± 1.5
Σ n-6	85.7 ± 0.9	77.3 ± 0.7
Σ n-6 / Σ n-3	0.46 ± 0.00	0.48 ± 0.00
Branched FA	5.5 ± 0.1	9.3 ± 0.1

The oleic acid (C18:1 *cis*-9) content decreased only slightly in the rehydrated freeze-dried colostrum, by 4.7 g kg^{-1} of fat. According to Martini et al., the oleic acid content of native donkey colostrum ranged from 296.1 to 334.3 g kg^{-1} of fat, which was considerably higher than the concentration determined in the raw colostrum investigated in the present study [4]. Highly significant increases ($P \leq 0.001$) in the concentrations of C12:1 n-1, C15:1 n-5, C17:1 n-7, and C22:1 n-9 were observed in the rehydrated donkey colostrum compared with the native colostrum.

The results presented in Table 5 show that there were no large differences in the concentrations of the individual polyunsaturated fatty acids (PUFA) between the two types of colostrum, with the exception of alpha-linolenic acid (α-C18:3 n-3), which decreased by 24.1 g kg^{-1} of fat in the rehydrated colostrum. Martini et al. reported alpha-linolenic acid concentrations in native donkey colostrum ranging from 27.82 to 38.06 g kg^{-1} of fat, which were considerably lower than the content determined in the native colostrum investigated in the present study [4]. Linoleic acid (C18:2 *cis*-9,12) decreased only slightly in the investigated rehydrated colostrum, by 6.8 g kg^{-1} of fat. According to Martini et al., the linoleic acid content of native donkey colostrum ranged from 92.77 to 105.5 g kg^{-1} of fat, which was higher than the content determined in the raw colostrum investigated in the present study [4]. Based on the PUFA results obtained for native and rehydrated freeze-dried donkey colostrum, the alpha-linolenic acid content can be considered high in both samples and remained at a relatively similar level in the rehydrated freeze-dried colostrum after freeze-drying and subsequent rehydration.

The contents of the individual branched-chain fatty acids in both native and rehydrated colostrum were below 3.0 g kg^{-1} of fat (Table 6), with very similar concentrations of the individual fatty acids observed in the two types of colostrum.

The results presented in Table 7 show that the SFA content differed by 40.7 g kg^{-1} of fat between native and rehydrated colostrum, with a higher concentration in the rehydrated colostrum. According to Martini et al., the SFA content of native donkey colostrum ranged from 417.1 to 476.5 g kg^{-1} of fat, which was like that determined in the native colostrum investigated in the present study [4].

The difference in MUFA concentration between the two types of colostrum was considerably smaller, amounting to 11.7 g kg^{-1} of fat, with a higher content in the native colostrum. According to Martini et al., the MUFA content of raw donkey colostrum ranged from 384.8 to 438.6 g kg^{-1} of fat, which was higher than that determined in the analyzed native colostrum (286.6 g kg^{-1} of fat) [4].

The PUFA content was lower in the rehydrated freeze-dried colostrum, with a difference of 31.9 g kg^{-1} of fat between the two types of colostrum. Martini et al. reported PUFA concentrations ranging from 133.9 to 144.3 g kg^{-1} of fat in native donkey colostrum during the first 0 - 48 h of lactation [4]. These values were considerably lower than the PUFA contents determined in both the native and rehydrated freeze-dried donkey colostrum investigated in the present study.

The Σ C18:1 cis-FA subgroup showed a small difference of 4.7 g kg^{-1} of fat between the two types of colostrum, with a lower content in the rehydrated freeze-dried colostrum. Branched-chain fatty acids also showed a small difference between the two samples, amounting to 3.8 g kg^{-1} of fat, with a higher concentration in the rehydrated colostrum.

The omega-3 fatty acid subgroup (Σ n-3) differed by 22.6 g kg^{-1} of fat between the two types of colostrum, with a higher content in the native colostrum. According to Martini et al., the omega-3 fatty acid content of native donkey colostrum ranged from 29.68 to 40.01 g kg^{-1} of fat, which was considerably lower than that determined in both the native colostrum (184.6 g kg^{-1} of fat) and the rehydrated freeze-dried colostrum (162.0 g kg^{-1} of fat) investigated in the present study [4]. The difference in omega-6 fatty acid (Σ n-6) concentrations between the two types of colostrum was small, amounting to 8.4 g kg^{-1} of fat. The content of this subgroup was lower in the rehydrated freeze-dried colostrum. According to Martini et al., the omega-6 fatty acid content of raw donkey colostrum ranged from 94.67 to 107.5 g kg^{-1} of fat, which was slightly higher than that determined in the native colostrum investigated in the present study [4].

The omega-6 / omega-3 fatty acid ratios (Σ n-6 / Σ n-3) of the native and rehydrated colostrum were very similar, differing by only 0.02. The omega-6 / omega-3 ratios of both colostrum samples were below 5.00, which is considered indicative of a favorable lipid profile in terms of human health [41]. Martini et al. reported omega-6 / omega-3 ratios ranging from 2.522 to 3.379 in native donkey colostrum during the first 0 - 48 h of lactation [4]. The ratios determined in the present study were considerably lower. A notable feature of the fatty acid composition of both colostrum samples was the substantially higher content of PUFA and omega-3 fatty acids compared with the values reported for native donkey colostrum in other scientific studies.

The calculated atherogenic index was 0.79 for native donkey colostrum and slightly higher, 0.96, for rehydrated freeze-dried donkey colostrum. This difference can be attributed mainly to the higher contents of myristic and palmitic acids, together with the slightly lower MUFA and PUFA contents of the rehydrated colostrum. The SFA content of both colostrum samples was below 500.0 g kg^{-1} of fat, while their PUFA content was high, indicating a favorable lipid composition of both native and rehydrated freeze-dried donkey colostrum.

The results for the antioxidant activity of native and rehydrated freeze-dried donkey colostrum, determined by DPPH and ABTS radical-scavenging assays, are presented in Table 8.

The obtained results indicate that donkey colostrum could donate hydrogen atoms or electrons that can react with free radicals, thereby converting them into more stable products. Compounds such as proteins, vitamins, enzymes, and polyphenols are considered to contribute to the antioxidant activity of milk and colostrum [11, 42]. Antioxidant activity determined by the DPPH assay was considerably lower (13.4 % and 14.5 %) than that determined by the ABTS assay (79.9 % and 78.8 %, respectively). The results showed comparable antioxidant activity in native and rehydrated freeze-dried colostrum, indicating that the freeze-drying process preserved the biologically active substances contributing to antioxidant activity.

Table 8. DPPH and ABTS radical-scavenging activity, %, of native and rehydrated freeze-dried donkey colostrum (n = 3) ($\bar{x} \pm SD$).

Assay	Native colostrum, %	Rehydrated freeze-dried colostrum, %	Statistical significance
DPPH	13.416 ± 0.149	14.455 ± 0.647	ns
ABTS	79.912 ± 0.405	78.808 ± 0.132	ns

ns - not significant (P > 0.05).

CONCLUSIONS

- The results of the present study indicate that freezing followed by freeze-drying (lyophilization) does not substantially alter the biochemical matrix of donkey colostrum. Native and rehydrated freeze-dried colostrum exhibited a very high degree of similarity in terms of their physicochemical characteristics and calculated energy values, while a difference was observed in their calculated atherogenic indices.
- The overall lipid profile was largely preserved, with differences in the contents of individual fatty acids below 30.0 g kg^{-1} of fat and differences in their major groups and subgroups below 50.0 g kg^{-1} of fat. Both samples were characterized by higher contents of polyunsaturated fatty acids (270.3 and 238.4 g kg^{-1} of fat, respectively), omega-3 fatty acids (184.6 and 162.0 g kg^{-1} of fat, respectively), and particularly α-linolenic acid (183.9 and 159.8 g kg^{-1} of fat, respectively), compared with the available reference data in the literature.
- Similarly, the antioxidant activity determined by the DPPH (13.4 % and 14.5 %) and ABTS (79.9 % and 78.8 %) assays was largely preserved, with only minimal variations between the native and rehydrated freeze-dried colostrum.
- From a practical perspective, the obtained results indicate that freeze-drying is a highly suitable and gentle technological approach for the preservation of donkey colostrum. This process enables the production of a shelf-stable product while largely preserving its nutritional value and functional properties.
- The present findings also contribute to the scientific knowledge in this field, given the limited number of published studies investigating the complex composition of donkey colostrum following freeze-drying and rehydration.

Authors' contributions

P.M. performed the freeze-drying experiments and prepared the original draft of the manuscript. K.L. and E.K. performed the physicochemical analyses, and K.L. conducted the antioxidant activity analyses. S.I. and A.V. performed the fatty acid composition analyses. M.D. contributed to the data analysis, interpretation, and synthesis of the results. I.N. supervised the study. All authors contributed to the review of the manuscript and read and approved the final version.

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