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COMPARISON OF FATTY ACID CONTENT IN SHEEP'S MILK AND ITS FREEZE-DRIED FORM

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Abstract

Sheep's milk is a natural product rich in bioactive substances that can be used in the prevention of lifestyle diseases. The fatty acid content, especially CLA, may be of particular importance. The aim of the study was to compare the fatty acid content in raw sheep's milk and its freeze-dried form. The study was conducted on 15 Polish Mountain Sheep ewes matched for age and body weight. After collection, the milk was frozen at –80°C and then freeze-dried. Fatty acid content in the milk and freeze-dried product was determined by gas chromatography. The results were subjected to statistical analysis using Scheffé's test. The study showed that freeze-drying did not reduce fatty acid content, especially CLA, which has antioxidant properties.

Keywords: sheep milk, freeze-dried milk, fatty acids

Introduction

Milk is the first food consumed by mammals after birth. In humans, the consumption of animal milk began with the domestication of goats and sheep nearly 13,000 years ago, as a result of which milk of animal origin became an indispensable component of the human diet (Balthazar

et al., 2017). Milk is a secretion of the mammary gland and is formed through the synthesis and diffusion of blood-derived components during lactogenesis (Park and Haenlein, 2006; Balthazar et al., 2017). Sheep's milk contains approximately 18.3% dry matter, 6.0% fat, 5.2% protein and 4.9% lactose (Molik et al., 2021). Compared with cow's milk, it contains more minerals and vitamins, particularly water-soluble vitamins, and has a higher caloric value, with an energy value of 5932 kJ/kg (Danków and Pikul, 2011; Molik and Daniel, 2021).

Factors determining milk production include the short lactation period of sheep, their relatively low productivity and the seasonality of production. Demand for sheep's milk is expected to increase in the coming years, among other reasons because of its broad potential use as a functional food (Biegalski and Cais-Sokolińska, 2022). An important characteristic of sheep's milk is its distinctive lipid profile and fatty acid content (Gao et al., 2024). Milk fat occurs as globules (approximately 3.6 μm in diameter in sheep's milk) emulsified in the aqueous phase of milk (Balthazar et al., 2017; Moatsou and Sakkas, 2019). These globules are a key structural component of milk and are of major technological, nutritional and biological importance. A single fat globule contains a core of triacylglycerols, compounds composed of fatty acids of different chain lengths and physical properties. In sheep's milk, fatty acids containing 4, 16 and 18 carbon atoms and one double bond are among the most common (Moatsou and Sakkas, 2019). Milk fat globules are surrounded by a protective membrane rich in proteins, glycolipids, phospholipids, carotenoids, cholesterol and fat-soluble vitamins (Gantner et al., 2015; Moatsou and Sakkas, 2019). This membrane stabilizes the fat globule in emulsion, protects globules from aggregation and coalescence, and serves as a physical barrier preventing triacylglycerol hydrolysis by lipolytic enzymes (Gantner et al., 2015). During milk processing, the structure of the fat globule membrane is damaged. In addition, centrifugation, heating and cooling lead to losses of phospholipids, which are an important component of this membrane (Mohamed et al., 2022). The small size of fat globules in sheep's milk contributes to greater membrane stability and resistance to deformation and provides more efficient metabolism and higher lipid digestibility (Gantner et al., 2015; Mohapatra et al., 2019).

A fatty acid is an organic compound composed of a hydrocarbon chain terminated by a carboxyl group (Hames and Hooper, 2007). Naturally occurring fatty acids generally contain an even number of carbon atoms and range from 4 to 26 carbon atoms in chain length. Based on their structure, they can be classified as saturated, monounsaturated or polyunsaturated fatty acids. Lipogenesis of fatty acids synthesized de novo in animal tissues occurs, among other sites, in the mammary gland, and acetyl-CoA derived from carbohydrate and protein metabolism is a substrate for this process (Jelińska, 2005). Fatty acids are essential for normal functioning of the human body and have a significant impact on cognitive, social and emotional functions. Importantly, they are fundamental structural components of the brain, nervous system and every cell in the body because they form part of cell membranes (Wilczyńska, 2012).

Studies have shown that raw sheep's milk is characterized by high levels of short- and medium-chain fatty acids, and the contents of metabolically valuable fatty acids such as caproic, caprylic, capric and lauric acids are considerably higher than in cow's milk. Long-chain saturated fatty acids account for 20–45% of the fatty acid composition of sheep's milk and are components of lipids, phospholipids, glycoproteins and lipoproteins in the body (Molik and Kordeczka, 2020). Sheep's milk is also characterized by higher concentrations of butyric acid, omega-3 fatty acids and conjugated linoleic acid (CLA) than milk from other ruminants, as well as a high level of vaccenic acid, the physiological precursor of CLA (Balthazar et al., 2017; Mohapatra et al., 2019). Butyric acid can induce the expression of anti-inflammatory molecules in macrophages; moreover, in a mouse model of induced colitis it was shown to markedly inhibit neutrophil production of pro-inflammatory cytokines and chemokines (Du et al., 2024). Appropriate proportions of vaccenic acid isomers reduce adipose tissue levels and the rate of fat deposition while increasing the use of glucose substrates in lipolysis. A study in obese rats

demonstrated that vaccenic acid reduced total body fat and adipocyte size (Rutkowska et al., 2015). In sheep-milk yoghurt, the predominant fatty acids were palmitic, myristic and oleic acids, the latter having LDL-cholesterol-lowering properties (Balthazar et al., 2017).

Conjugated linoleic acid (CLA) is a group of positional and geometric isomers of linoleic acid C18:2 (Cichosz and Czeczot, 2012). CLA is synthesized mainly by acidophilic bacteria and bifidobacteria inhabiting the rumen through the isomerization and biohydrogenation of linoleic and α-linolenic acids (Danków and Pikul, 2011; Rutkowska et al., 2015). CLA may be one of the most potent naturally occurring anticarcinogenic compounds, inhibiting tumour development at the stages of initiation, promotion and progression. It has been shown to inhibit proliferation of melanoma, colorectal, prostate, ovarian and mammary cancer cells (Cichosz and Czeczot, 2012; Rutkowska et al., 2015). Further studies showed that CLA reduced the area of atherosclerotic lesions in mice fed a CLA-supplemented diet for two months (Janczy, 2012). A CLA-rich diet reduces adipose tissue and increases muscle mass by modulating adipokines, cytokines and TNF-alpha and by enhancing β-oxidation in skeletal muscle (Karwat et al., 2013). CLA prevents immune suppression in animals and has beneficial effects on the human immune system by increasing levels of T lymphocytes, IL-1, IL-10 and immunoglobulins A and M, which may potentially be important during recovery from COVID-19 (Rutkowska et al., 2015; Flis et al., 2022).

The most common method of milk processing is powder production; however, this method causes undesirable nutrient losses due to high temperatures. An alternative method is freeze-drying, which dehydrates a frozen product by sublimation. The processing conditions used in this method minimize damage to the final product, making it suitable for heat-sensitive substances such as milk. Freeze-dried products can also be readily restored to their original form by rehydration (Kim et al., 2010; Martysiak-Żurowska et al., 2020). Zhang et al. (2022) compared the lipid profile of freeze-dried and spray-dried goat milk. The freeze-dried product contained approximately 6% more monounsaturated fatty acids and 50% more long-chain omega-6 polyunsaturated fatty acids, including more than four times as much arachidonic acid (Zhang et al., 2022). Cavazos-Garduño et al. (2016), comparing the lipid profile of freeze-dried and spray-dried human milk, found higher concentrations of lauric, myristic, palmitic, stearic, oleic and linoleic acids in the freeze-dried product than in fresh milk. Compared with powdered milk, the freeze-dried product contained higher concentrations of all these fatty acids except oleic and linoleic acids (Cavazos-Garduño et al., 2016). Freeze-drying also does not cause substantial losses of fatty acids in cow's milk, as shown by Yao et al. (2016). Therefore, the aim of the present study was to compare the fatty acid content of sheep's milk and its freeze-dried form.

Material and methods

The study involved 15 Polish Mountain Sheep ewes aged 4–5 years and weighing 45±5 kg. Mating was carried out from 15 to 20 October. The ewes lambed between 20 and 25 March. The ewes included in the experiment reared single lambs. During the experiment, the sheep were fed using a traditional system in accordance with the standards developed by the National Research Institute of Animal Production (1993). The animals received haylage, hay and concentrate feed (ground cereal grain). Individual milk samples were collected three times. The first collection was performed on day 10 after lambing, followed by collections on days 20 and 30 of lactation. After collection, the milk was frozen at –80°C in preparation for freeze-drying and fatty acid analyses.

The frozen milk samples were placed in a Telstar LyoQuest -85 Plus laboratory freeze-dryer operating at a pressure below 10 Pa. The process was carried out for 7 days until a freeze-dried milk product was obtained. Fatty acid content in the milk and freeze-dried product was

determined by gas chromatography using a TRACE GC Ultra chromatograph (Thermo Electron, Milan, Italy) equipped with a SUPELCOWAX 10 column, 30 m × 0.53 mm, 1.0 µm (fused-silica capillary), according to the method described by Mann J. (1964). The analyses were performed at the laboratory of the Department of Genetics, Animal Breeding and Ethology, University of Agriculture in Krakow. The results were statistically analysed using SAS v. 9.3, one-way analysis of variance and Scheffé's test.

Results

Changes in the content of saturated fatty acids in sheep's milk and its freeze-dried form are presented in Table 1.

Table 1. Comparison of saturated fatty acid content in sheep's milk and freeze-dried milk

Fatty acids (g/100 g fatty acids)	Sheep milk	Freeze-dried milk
Butyric acid	2,837±0,3	2,957±0,4
Caproic acid	2,371±0,3	2,513±0,3
Caprylic acid	2,469±0,3	2,506±0,3
Capric acid	6,891±0,9	6,888±1,2
Lauric acid	3,529±0,6	3,549±0,06
Myristic acid	8,699±1,39	8,554±1,38
Pentadecanoic acid	1,049±0,02	1,145±0,02
Palmitic acid	23,761±8,74	23,281±8,66
Margaric acid	1,006±0,23	1,042±0,21
Stearic acid	11,368±2,36	11,658±2,44
Arachidic acid	0,208±0,04	0,213±0,04

The study showed that both sheep's milk and its freeze-dried form had the highest content of palmitic acid (C16:0). Stearic acid (C18:0) was the second most abundant saturated fatty acid in both milk and the freeze-dried product. The mean content of myristic acid (C14:0) was similar in milk and the freeze-dried product, amounting to 8.699±1.39 and 8.554±1.38 g/100 g, respectively. Freeze-dried sheep's milk had favourable contents of butyric acid (C4:0) and caproic acid (C6:0). No changes were found in the contents of the remaining fatty acids, particularly capric acid (C10:0), lauric acid (C12:0) and caprylic acid (C8:0), which have valuable properties in skin regeneration. No significant differences were found in the content of saturated fatty acids between milk and the freeze-dried product. The results concerning unsaturated fatty acid content are presented in Table 2.

Table 2. Comparison of unsaturated fatty acid content in sheep's milk and freeze-dried milk

Fatty acids (g/100 g fatty acids)	Sheep milk	Freeze-dried milk
Decenoic acid	1,55±0,01	1,57±0,01
Myristoleic acid	0,104±0,01	0,102±0,01

Palmitoleic acid (n-9)	0,357±0,01	0,382±0,01
Palmitoleic acid (n-7)	0,939±0,02	0,983±0,02
Heptadecenoic acid	0,484±0,01	0,472±0,01
Oleic acid (n-9)	25,104±4,32	24,924±5,37
Vaccenic acid (n-7)	2,806±0,04	2,972±0,03
Gadoleic acid	0,090±0,01	0,85±0,01

The study showed similar levels of oleic acid (C18:1 n-9) in milk (25.104±4.32 g/100 g) and the freeze-dried product (24.924±5.37 g/100 g). No differences were found in the content of vaccenic acid C18:1 (n-7). Analysis of unsaturated fatty acids in milk and the freeze-dried product showed that the contents of C10:1 and C16:1 (n-7) were similar. No differences were found in the contents of heptadecenoic acid (C17:1), palmitoleic acid C16:1 (n-9), myristoleic acid (C14:1) or gadoleic acid (C20:1). Changes in polyunsaturated fatty acid content are presented in Table 3.

Table 3. Comparison of polyunsaturated fatty acid content in sheep's milk and freeze-dried milk

Fatty acids (g/100 g fatty acids)	Sheep milk	Freeze-dried milk
Linoleic acid	2,759±0,04	2,646±0,02
γ-linolenic acid	0,119±0,01	0,116±0,01
α-linolenic acid	1,610±0,01	1,579±0,01
Conjugated linoleic acid	1,191±0,01	1,215±0,01

In the analysed sheep's milk, linoleic acid C18:2 (n-6) content was 2.759±0.04 g/100 g, compared with 2.646±0.02 g/100 g in the freeze-dried product. No differences were found in the contents of α-linolenic acid C18:3 (n-3) or γ-linolenic acid C18:3 (n-6) between milk and the freeze-dried product. CLA content was higher in the freeze-dried product (1.215±0.01 g/100 g) than in raw milk (1.191±0.01 g/100 g). The analysis of polyunsaturated fatty acids revealed no statistically significant differences between milk and the freeze-dried product.

Discussion

The study showed that freeze-drying did not reduce fatty acid content. In the case of unsaturated fatty acids, the concentrations observed in the freeze-dried product may have been affected by the volatility of short-chain fatty acids, which are more readily degraded. Freeze-drying involves sublimation, which may lead to losses of volatile compounds. Long-chain saturated fatty acids such as myristic and palmitic acids have greater thermal stability; nevertheless, slight losses may occur during freeze-drying, for example because of oxidation. Most unsaturated fatty acids showed an upward tendency in the freeze-dried product because they retain their stability under low temperature and pressure. Their stability is also favoured by the removal of water during freeze-drying and the resulting concentration of components in dry matter. The analysis showed an increase in CLA content in the freeze-dried product, which may have resulted from isomerization of linoleic acid under freeze-drying conditions and the formation of

conjugated dienes. The decrease in the other polyunsaturated fatty acids determined in the freeze-dried product may have resulted from oxidation, enzymatic hydrolysis or chemical degradation.

The results showed that freeze-drying preserves fatty acid content at a level comparable to that of raw milk. Freeze-drying is a more effective method of milk preservation than powder production. Ibrahim and Khalifa (2015) reported that in camel milk the fatty acid content per 100 g was 97.96 g in fresh milk and 98.1 g in the freeze-dried product. Further studies by Friend et al. (1983) and Martysiak-Żurowska et al. (2020) on the fatty acid profile of human milk and its freeze-dried form showed that freeze-drying does not cause significant changes in milk fatty acid profiles. Freeze-drying preserves bioactive milk components and facilitates their use in medicine and pharmacy. Freeze-dried milk is easier to store and transport. In addition, long-term storage does not adversely affect its composition and does not require special conditions. Freeze-dried milk also contains more nutrients than milk powder, as demonstrated in goat milk by Zhang et al. (2022).

Freeze-dried sheep's milk, particularly the bioactive substances it contains, may be used in the production of dietary supplements for the prevention of many diseases. The increasing prevalence of lifestyle diseases and population ageing create a need to identify natural biologically active substances that support treatment without placing additional burden on the body. Important biologically active lipids in sheep's milk include butyric acid, CLA and sphingolipids, which are components of fat globule membranes. The most biologically active CLA isomer is cis-9, trans-11, which inhibits the occurrence and development of skin cancer (Wang and Jones, 2004). Park et al. (2010) showed that CLA supplementation accelerates skin wound healing by regulating antioxidant and anti-inflammatory functions. This creates an opportunity to investigate the topical effects of CLA on wound healing. Hydrogel biomaterials enriched with bioactive substances from sheep's milk may therefore be of particular importance. Incorporating natural bioactive substances into hydrogel dressings may accelerate wound healing. Mansour et al. (2015) showed that increasing the fat content of topical preparations accelerated burn healing, reduced inflammation, enhanced wound contraction, minimized scarring and improved treatment of burn wounds. Freeze-drying of sheep's milk preserves its chemical and biological properties, making it an attractive raw material for the design of hydrogel biomedical materials that may improve treatment of hard-to-heal wounds.

The study showed that freeze-drying preserves valuable bioactive substances in milk. In sheep's milk, CLA is particularly important because of its antioxidant and anticarcinogenic activity. Freeze-dried sheep's milk is therefore a natural product that may be incorporated into hydrogel biomedical materials used for skin regeneration and to support wound healing.

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SUMMARY

Sheep's milk is a natural product rich in bioactive substances that can be used in the prevention of lifestyle diseases. The fatty acid content, especially CLA, may be of particular importance. The aim of the study was to compare the fatty acid content in raw sheep's milk and its freeze-dried form. The study was conducted on 15 Polish Mountain Sheep ewes, matched for age and body weight. After collection, the milk was frozen at –80°C and then freeze-dried. The fatty acid content of the milk and freeze-dried product was determined by gas chromatography. The results were analysed statistically using Scheffe's test. The studies showed that the freeze-drying process did not contribute to a reduction in fatty acid content, especially CLA, which has antioxidant properties.

Keywords: sheep milk, freeze-dried milk, fatty acids