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Fatty acid profile of artisanal hard cheeses made from raw goat milk during the ripening process

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Hard cheeses are an important source of nutrients and biologically active substances, particularly fatty acids, in the human diet. Among the most valuable and attractive hard cheeses to consumers are those produced on small-scale farms from raw goat milk. The unique microbiome of raw goat milk creates distinctive taste and aroma characteristics of the cheeses and significantly influences the fatty acid composition and quality of milk fat. The determination of the fatty acid composition of Caciotta and Canestrato hard cheeses made from raw goat milk during the ripening period of 24 and 12 months, respectively, was conducted four times using gas chromatography. The primary saturated fatty acids in Caciotta and Canestrato cheeses are palmitic, stearic, capric, and myristic acids, comprising over 55% of the total fatty acid content. Among the unsaturated fatty acids in Caciotta and Canestrato cheeses, the sum of oleic and linoleic acids exceeds 25% of the total fatty acids. There were no significant trends in the dynamics of short-chain, medium-chain, and long-chain fatty acid content in Caciotta and Canestrato cheeses during the ripening period. In both cheeses, the main ω3 polyunsaturated fatty acid is linolenic acid, and the main ω6 polyunsaturated fatty acid is linoleic acid. Their ratio in the cheeses fluctuated within the range of 2.8:1–4.6:1 throughout the ripening period. The accumulation of polyunsaturated fatty acids in Caciotta cheese increased during its ripening period, accompanied by a decrease in the delta-9 desaturase index (C_{14}). In Canestrato cheese, the delta-9 desaturase index (C_{14}) shows a direct correlation with its age. During the ripening period, the atherogenic index of Canestrato cheese fat decreased, while the hypocholesterolemic/hypercholesterolemic acid ratio increased, whereas this pattern was not observed in Caciotta cheese. The research results can be used as criteria for selecting healthy nutrition, assessing the naturalness, and authenticity of artisanal hard cheeses made from goat milk.

Keywords: Caciotta; Canestrato; saturated fatty acids; unsaturated fatty acids; atherogenic index; desaturase index; lipid quality indicators.

Introduction

Cheese production is one of the oldest methods of milk processing, which has been continuously refined by humans, increasing the variety of dairy products available on the market. Cheeses are made using different milk sources, processing methods, cultures, coagulants, and maturation conditions, resulting in a wide range of textures, flavors, and forms (Tekin & Hayaloglu, 2023). Although goat cheeses account for only about 2% of all cheeses produced worldwide, there has been a recent increase in their production in Asia, Africa, and Europe (Kahi & Wasike, 2019; Miller & Lu, 2019). In Ukraine, the production of goat milk cheeses is becoming increasingly common among small-scale farms that use modern goat breeds and practice grazing on natural pastures. The production of such artisanal cheeses generally involves minimal milk processing or its use in raw form, which ensures the creation of unique taste and aroma nuances, as well as their authenticity (Penna et al., 2021; Smaoui et al., 2023).

Consumers prefer products made from goat milk due to their better digestibility compared to cow's milk (Miller & Lu, 2019). Goat cheeses are consumed both young (unaged) and mature. Among the nutrients found in cheeses, protein and fat are particularly important (Popović-Vranješ et al., 2017). The fat composition of goat milk determines not only its technological characteristics and nutritional quality but also the taste, softness, and melting properties of the cheese (Popović-Vranješ et al., 2017). The originality of the flavor and aroma of these cheeses intensifies with extended aging, which increases consumer demand. During the ripening process of hard cheeses, changes in pH occur, and proteins and lipids break down (Hayaloglu et al., 2013). Lipolysis causes the release of fatty acids, especially short- and medium-chain ones, which directly influence the formation of a specific cheese flavor. Additionally, free fatty acids serve as substrates for further enzymatic reactions that lead to the formation of compounds contributing to the aroma of cheeses (Zheng et al., 2021).

Lipids in cheeses carry natural fat-soluble vitamins (A, D, E, and K), as well as β-carotene and other carotenoids, which contribute to their health benefits. The main lipids in milk fat are triacylglycerols, which make up over 98%, while the remainder consists of diacylglycerols, monoacylglycerols, free fatty acids, phospholipids, sterols, and hydrocarbons. The composition of milk fat triacylglycerols is extremely complex, as it includes over 400 different fatty acids that can be esterified in the three positions: sn-1, sn-2, and sn-3 of the glycerol molecule (Gómez-Cortés et al., 2018).

Saturated fatty acids are the main fatty acids in milk fat. They include short-chain, medium-chain, and long-chain fatty acids, as well as odd-chain and branched-chain fatty acids (Mansson, 2008; Danchuk et al., 2021). Depending on their molecular structure, fatty acids have different effects on human health (Bernard et al., 2018). Saturated fatty acids, particularly lauric ($C_{12:0}$), myristic ($C_{14:0}$), and palmitic ($C_{16:0}$) acids, increase blood cholesterol levels while simultaneously raising the anti-atherogenic levels of high-density lipoprotein cholesterol and have beneficial properties for health (Parodi, 2009). Fatty acids with odd and branched chains have anticancer effects (Mao et al., 2023), can reduce the incidence of necrotizing enterocolitis, and are considered biomarkers of dairy fat consumption in humans (Adamska & Rutkowska, 2014).

Monounsaturated and polyunsaturated fatty acids are structural components of many biologically active substances that are beneficial to human health. Some of the best and most accessible natural trans fatty acids in milk fat are vaccenic acid (trans-11 $C_{18:1}$) and conjugated linoleic acid (cis-9, trans-11 $C_{18:2}$) (Summer et al., 2017; Serrapica et al., 2020). ω_3 polyunsaturated fatty acids (PUFAs) found in milk fat help prevent heart disease and improve the body's immune response. Consumption of ω_3 PUFAs and ω6 PUFAs positively affects human health by providing regenerative and preventive functions. Milk fat is also a rich source of butyric acid ($C_{4:0}$), which promotes the functional stability of the gastrointestinal tract and reduces the intensity of inflammatory processes (Gómez-Cortés et al., 2018).

The quantitative composition of fatty acids in milk fat changes under the influence of various factors such as animal feed (Frétin et al., 2019), breed, lactation period, individual characteristics, climatic conditions, health status, age (Abdoul-Aziz et al., 2021; Kupczyński et al., 2024), as well as the season of milk production (Paszczyk, 2022). Among these factors, animal feed, including the use of pasture, consumption of green grass or hay, and concentrated feed, has the greatest impact on the fatty acid composition of milk and its products (Cabiddu et al., 2019; Delgadillo-Puga & Cuchillo-Hilario, 2021). The chemical and fatty acid composition of cheese also depends on the microbiological and chemical composition of the milk, production technology (Paszczyk et al., 2019; Uzun et al., 2020; Rosario et al., 2023), ripening period, and additives (Murtaza et al., 2014; Starkute et al., 2023).

Despite the significant popularity, undeniable benefits, and high demand for hard artisanal goat milk cheeses, the literature lacks data on the dynamics of their fatty acid composition and lipid quality during long-term aging – 12 and 24 months. Therefore, the aim of this research is to determine the fatty acid composition of artisanal hard cheeses Caciotta and Canestrato made from raw goat milk during the ripening process.

Material and methods

For the research, two batches of artisanal cheeses made from raw goat milk were produced: Caciotta and Canestrato, with 20 wheels each, weighing an average of 2.3–2.5 kg, under the conditions of the Eco Farm "Zhuravka" in the Kyiv region. The milk used for cheese production came from Anglo-Nubian goats.

Caciotta cheese was made using the starter culture of lactic acid bacteria MA 4001 (Danisco France SAS, France). The starter culture consisted of *Lactobacillus lactis*, *Lactococcus cremoris*, *Lactococcus diacetylactis*, and *Streptococcus thermophilus*. For Canestrato cheese, the starter culture TA 45 (Danisco France SAS, France) was used, which included *Streptococcus thermophilus*. During the production of Caciotta and Canestrato cheeses, liquid rennet Rennet Liquid 92/8 (Pamir Service, Kyiv, Ukraine) was added.

Table 1
Technological scheme for the production of Caciotta and Canestrato cheeses

Technological stage	Caciotta	Canestrato
1	Heating of raw goat milk to 37 °C and addition of the starter culture (45–60 minutes).	Heating raw goat milk to 32 °C and adding the starter culture
2	Milk coagulation with the addition of rennet (35–40 minutes).	Milk coagulation with the addition of rennet (45 minutes).
3	Cutting the curd and separating the whey.	Cutting the curd and separating the whey.
4	Ripening of the curd grains in a warm chamber at 32–38 °C (1.0–1.5 hours), during which thermophilic streptococci exhibit enzymatic activity.	Heating the curd grains to a temperature of 46 °C.
5	Cooling the cheese at room temperature (2–3 hours).	Separating the whey and pressing the curd (15 minutes).
6	Removal of remaining whey in the refrigerator on a draining mat (8–10 hours).	Heating the curd grains in whey at a temperature of 65–71 °C.
7	Salting the cheese.	Cooling the cheese at room temperature for 24 hours.
8	Ripening the cheese in a chamber at 12–15 °C with 85–90% humidity (24 months).	Removing the remaining whey in the refrigerator on a draining mat (8–10 hours).
9	–	Salting the cheese.
10	–	Ripening the cheese in a chamber at 12–15 °C with 80–85% humidity (12 months).

Note: the stages mentioned are not included in the cheese production technology.

Starting from the 6-month ripening period, the wheels of Caciotta and Canestrato cheeses were washed with artesian water, taking into account the intensity of infestation by the mite *Acarus siro* (Linnaeus, 1758), genus *Acarus* (Linnaeus, 1758), family Acaridae (Latreille, 1802), once every 2–3 months.

For the study of fatty acid composition, average samples of 200 g each were taken from 5 wheels of Caciotta cheese at the ripening stages of 10 days, 1 month, 12 months, and 24 months, and from Canestrato cheese at the stages of 10 days, 3 months, 6 months, and 12 months.

Lipid extraction from the cheeses was performed using the Folch et al. (1957) method. Hydrolysis and methylation of fatty acids from the lipid samples were carried out according to Christie (1982). The analysis of fatty acid methyl esters was performed on a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Waltham, USA, 2008) with a flame-ionization detector and a temperature-programmed injector on an SPTM-2560 high-polar capillary column (Supelco, St. Louis, USA, 2018), 100 m long, with an inner diameter of 0.25 mm and a stationary phase thickness of 0.20 µm. Chromatography conditions: column temperature 140–240 ºC, detector temperature 260 ºC. The sample was injected into the chromatograph using a TriPlus autosampler (Thermo Fisher Scientific, Waltham, USA, 2008) in a volume of 1 µL. The analysis duration was 65 minutes.

Identification of fatty acids was carried out using the Supelco 37 Component FAME Mix standard (Supelco, St. Louis, USA, 2023). The quantitative assessment of the fatty acid spectrum in the lipids of the cheeses was performed using the internal normalization method, determining their content as a percentage. The studies were conducted in triplicate. The following fatty acids were identified in the cheeses: butyric ($C_{4:0}$), caproic ($C_{6:0}$), caprylic ($C_{8:0}$), capric ($C_{10:0}$), hendecanoic ($C_{11:0}$), lauric ($C_{12:0}$), tridecanoic ($C_{13:0}$), myristic ($C_{14:0}$), myristoleic ($C_{14:1}$), pentadecanoic ($C_{15:0}$), pentadecenoic ($C_{15:1}$), palmitic ($C_{16:0}$), palmitoleic ($C_{16:1}$), heptadecanoic ($C_{17:0}$), cis-10-heptadecenoic ($C_{17:1}$), stearic ($C_{18:0}$), oleic ($C_{18:1}\omega_9$), linoleic ($C_{18:2}\omega_6$), linolenic ($C_{18:3}\omega_3$), arachidic ($C_{20:0}$), cis-11-eicosenoic ($C_{20:1}\omega_9$), eicosatrienoic ($C_{20:3}\omega_3$). To assess the quality of lipids in the cheeses, the Δ_9 desaturase index (C_{14}) was calculated using the formula: Δ_9 desaturase index (C_{14}) = $C_{14:1}/(C_{14:1} + C_{14:0})$ (Burgos et al., 2021). This index is the best indicator of this activity, as all $C_{14:0}$ in dairy fat depends on de novo synthesis in the mammary gland.

Another parameter of biological interest is the atherogenic index (AI), which characterizes the atherogenicity of dietary fats. The formula used was: $AI = [C_{12:0} + (4 \times C_{14:0}) + C_{16:0}]/(MUFA + PUFA)$ (Ulbricht & Southgate, 1991; Osmari et al., 2011). The content of hypocholesterolemic fatty acids (DFA) in the cheeses was calculated using the formula: $DFA = MUFA + PUFA + C_{18:0}$ (Medeiros et al., 2014). For calculating the content of hypercholesterolemic fatty acids (OFA), the formula used was: $OFA = C_{12:0} + C_{14:0} + C_{16:0}$ (Medeiros et al., 2014). The hypocholesterolemic/hypercholesterolemic ratio (H/H) was calculated according to Ivanova & Hadzhinikolova (2015): $H/H = (C_{18:1n-9} + C_{18:2n-6} + C_{18:3n-3})/(C_{12:0} + C_{14:0} + C_{16:0})$.

Statistical analysis of the research results was conducted using regression and correlation analyses. The data in the tables are presented as x ± SD (mean ± standard deviation). Differences between groups were considered significant using the Tukey test at $P < 0.05$ (taking into account the Bonferroni correction).

Results

Low molecular weight fatty acids present in the Caciotta cheese included butyric, caproic, and caprylic acids. The content of caproic acid remained unchanged during the ripening of the cheese, while the proportions of butyric and caprylic acids in the fat of Caciotta cheese increased by the 24th month of ripening compared to the young cheese (Table 2), which corresponds to the peak of their total content during this period (Table 3). Among the medium-chain fatty acids in Caciotta cheese fat, the main ones were palmitic, myristic, and capric acids, which together account for over 40% of the fatty acids in the cheese. The levels of capric and myristic acids showed the greatest fluctuations during the ripening period, while by the end – at the 24th month – they returned to their initial values. An increase in the content of hendecanoic and lauric acids has been noted against the background of decreasing proportions of myristoleic, pentadecanoic, and palmitic acids by the end of the ripening period of Caciotta cheese. Throughout the entire ripening period, the concentrations of tridecanoic and pentadecanoic acids did not undergo significant changes. Such fluctuations in the content of medium-chain fatty acids in Caciotta cheese fat contribute to their peak at the 12th month of ripening (Table 3).

The main long-chain fatty acids in Caciotta cheese fat were oleic and stearic acids, which together accounted for over 35% of the total fatty acid content. The highest content of long-chain fatty acids in Caciotta cheese was recorded at the 1st month of ripening, primarily due to cis-10-heptadecenoic, stearic, oleic, and cis-10-eicosenoic acids. The minimum content of long-chain fatty acids in Caciotta cheese fat was found at the 12th month of ripening, which was facilitated by a decrease in the proportions of stearic acid and the disappearance of cis-11-eicosenoic and eicosatrienoic acids in the fat. By the 24th month of ripening, the level of long-chain fatty acids in Caciotta cheese fat had equalized with the initial value at the 10th day.

Table 2
Content of fatty acids in Caciotta cheese during the ripening period (% of the total fatty acid content, x ± SD, n = 5)

Acid	Term of cheese maturation			
	10 days	1 month	12 months	24 months
Butyric, $C_{4:0}$	2.65 ± 0.06^{ab}	2.58 ± 0.13^{a}	2.86 ± 0.08^{b}	3.51 ± 0.16^{c}
Caproic, $C_{6:0}$	3.15 ± 0.05^{a}	2.83 ± 0.08^{a}	3.04 ± 0.02^{a}	3.32 ± 0.12^{a}
Caprylic, $C_{8:0}$	3.19 ± 0.03^{ab}	2.79 ± 0.12^{a}	3.07 ± 0.04^{ab}	3.39 ± 0.11^{b}
Capric, $C_{10:0}$	10.01 ± 0.16^{a}	8.13 ± 0.13^{b}	11.20 ± 0.12^{c}	10.67 ± 0.51^{ac}
Hendecanoic, $C_{11:0}$	0.11 ± 0.01^{a}	0.15 ± 0.02^{a}	0.23 ± 0.03^{b}	0.39 ± 0.05^{c}
Lauric, $C_{12:0}$	3.80 ± 0.11^{a}	2.81 ± 0.07^{b}	3.99 ± 0.23^{a}	4.80 ± 0.34^{c}
Tridecanoic, $C_{13:0}$	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}	0.00 ± 0.00^{b}	0.06 ± 0.01^{a}
Myristic, $C_{14:0}$	8.47 ± 0.19^{a}	6.79 ± 0.06^{b}	9.51 ± 0.17^{c}	8.69 ± 0.18^{a}
Myristoleic, $C_{14:1}$	0.39 ± 0.01^{a}	0.30 ± 0.03^{a}	0.19 ± 0.02^{b}	0.11 ± 0.01^{c}
Pentadecanoic, $C_{15:0}$	0.77 ± 0.02^{a}	0.61 ± 0.01^{b}	0.78 ± 0.06^{a}	0.84 ± 0.05^{a}
Pentadecenoic, $C_{15:1}$	0.19 ± 0.01^{a}	0.18 ± 0.03^{a}	0.09 ± 0.02^{b}	0.05 ± 0.01^{b}
Palmitic, $C_{16:0}$	21.77 ± 0.23^{a}	20.69 ± 0.29^{b}	22.54 ± 0.21^{c}	18.27 ± 0.18^{d}
Palmitoleic, $C_{16:1}$	0.49 ± 0.02^{a}	0.52 ± 0.04^{a}	0.97 ± 0.06^{b}	2.07 ± 0.12^{c}
Heptadecanoic, $C_{17:0}$	0.52 ± 0.03^{a}	0.55 ± 0.05^{a}	0.80 ± 0.07^{b}	0.85 ± 0.03^{b}
Cis-10-heptadecenoic, $C_{17:1}$	0.19 ± 0.02^{a}	0.27 ± 0.03^{b}	0.53 ± 0.05^{c}	0.66 ± 0.04^{d}
Stearic, $C_{18:0}$	16.85 ± 0.15^{a}	18.61 ± 0.19^{b}	13.58 ± 0.65^{c}	14.14 ± 0.78^{c}
Oleic, $C_{18:1n9c}$	22.28 ± 0.17^{a}	27.13 ± 0.41^{b}	21.86 ± 0.34^{a}	22.07 ± 0.18^{a}
Linoleic, $C_{18:2n6c}$	3.18 ± 0.06^{a}	3.17 ± 0.03^{a}	3.69 ± 0.09^{b}	4.29 ± 0.19^{c}
Arachidic, $C_{20:0}$	0.24 ± 0.02^{a}	0.20 ± 0.03^{a}	0.31 ± 0.03^{b}	0.29 ± 0.02^{b}
Cis-11-eicosenoic, $C_{20:1n9}$	0.90 ± 0.02^{a}	0.68 ± 0.03^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Linolenic, $C_{18:3n3}$	0.71 ± 0.03^{a}	0.79 ± 0.04^{a}	0.81 ± 0.09^{a}	1.54 ± 0.07^{b}
Eicosatrienoic, $C_{20:3n3}$	0.13 ± 0.02^{a}	0.18 ± 0.04^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

Note: different superscript letters indicate values that significantly differ in the same row of the table ($P < 0.05$) based on comparisons using Tukey's test with Bonferroni correction.

Saturated fatty acids in Caciotta cheese are represented by palmitic, stearic, capric, and myristic acids, which together account for over 55% of the total fatty acid content. The decrease in the proportion of capric, myristic, lauric, pentadecanoic, and palmitic acids at 1 month of Caciotta cheese maturation resulted in the minimum value of total saturated fatty acids in the cheese fat. During the subsequent maturation period, fluctuations in the content of individual saturated fatty acids did not affect their proportion in the fat structure (Table 3). The main unsaturated fatty acids in Caciotta cheese were oleic and linoleic acids, whose sum reached over 25% of all fatty acids in the cheese. In the fat of Caciotta cheese, there was an increase in the proportion of unsaturated fatty acids over the 1-month maturation period, mainly due to monounsaturated fatty acids, particularly oleic acid, which reaches its peak during this time.

It is noteworthy that the highest content of polyunsaturated fatty acids in Caciotta cheese is recorded at 24 months of maturation, occurring due to both ω_3 PUFA and ω_6 PUFA, represented respectively by linoleic and linolenic acids.

Table 3
Quality indicators of lipids in Caciotta goat cheese during maturation (% of the total fatty acid content, x ± SD, n = 5)

Indicator	Term of cheese maturation			
	10 days	1 month	12 months	24 months
Σ SFA	71.56 ± 0.12^{a}	66.77 ± 0.40^{b}	71.87 ± 0.47^{a}	69.21 ± 0.42^{a}
Σ UFA	28.44 ± 0.11^{a}	33.23 ± 0.41^{b}	28.13 ± 0.44^{a}	30.79 ± 0.39^{a}
Σ MUFA	24.43 ± 0.14^{a}	29.08 ± 0.39^{b}	23.63 ± 0.36^{a}	24.97 ± 0.33^{a}
Σ PUFA	4.02 ± 0.06^{a}	4.15 ± 0.07^{a}	4.50 ± 0.07^{b}	5.83 ± 0.21^{c}
Σ ω_3 PUFA	0.84 ± 0.03^{a}	0.97 ± 0.05^{a}	0.81 ± 0.09^{a}	1.54 ± 0.07^{b}
Σ ω_6 PUFA	3.18 ± 0.06^{a}	3.17 ± 0.03^{a}	3.69 ± 0.09^{b}	4.29 ± 0.19^{c}
ω_3/ω_6 PUFA	3.78 ± 0.15^{a}	3.29 ± 0.16^{a}	4.60 ± 0.51^{b}	2.80 ± 0.17^{c}
Σ SCFA (4–8)	8.98 ± 0.11^{a}	8.20 ± 0.32^{a}	8.96 ± 0.12^{a}	10.22 ± 0.22^{b}
Σ MCFA (10–16)	46.03 ± 0.16^{a}	40.21 ± 0.23^{b}	49.49 ± 0.38^{c}	45.94 ± 0.98^{a}
Σ LCFA (17–20)	44.99 ± 0.27^{a}	51.60 ± 0.46^{b}	41.55 ± 0.31^{c}	43.85 ± 0.90^{a}
DFA	45.30 ± 0.25^{a}	51.84 ± 0.43^{b}	41.71 ± 0.27^{c}	44.93 ± 0.88^{a}
OFA	34.04 ± 0.07^{a}	30.29 ± 0.16^{b}	36.04 ± 0.49^{c}	31.76 ± 0.63^{d}
Δ^9 desaturase index (C_{14})	0.043 ± 0.002^{a}	0.043 ± 0.002^{a}	0.019 ± 0.001^{b}	0.013 ± 0.001^{c}
Atherogenicity index (AI)	2.09 ± 0.03^{a}	1.52 ± 0.04^{b}	2.30 ± 0.03^{c}	1.88 ± 0.04^{d}
Hypocholesterolaemic/hypercholesterolaemic ratio (H/H)	0.77 ± 0.01^{a}	1.03 ± 0.02^{b}	0.73 ± 0.02^{a}	0.88 ± 0.01^{c}

Note: see Table 2; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; UFA – unsaturated fatty acids; SCFA – short-chain fatty acids; MCFA – medium-chain fatty acids; LCFA – long-chain fatty acids; DFA – hypocholesterolaemic fatty acids; OFA – hypercholesterolaemic fatty acids; H/H – hypocholesterolaemic/hypercholesterolaemic ratio.

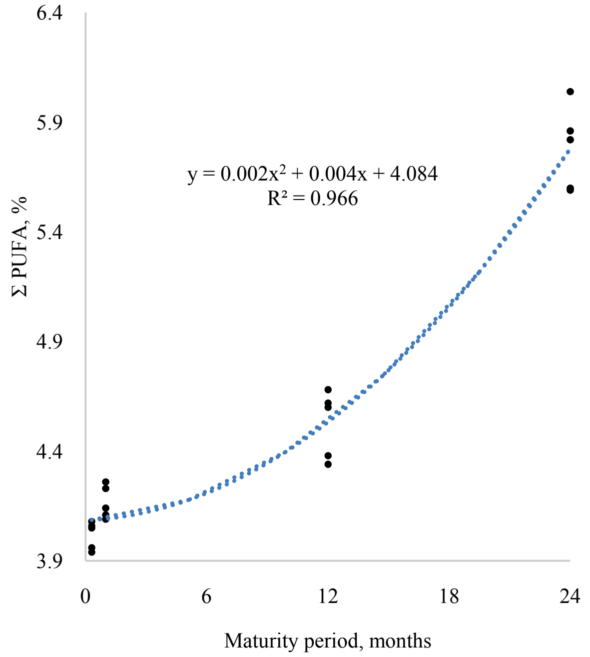

Fig. 1. Dependence between Σ PUFA and maturation time of Caciotta cheese, N = 20

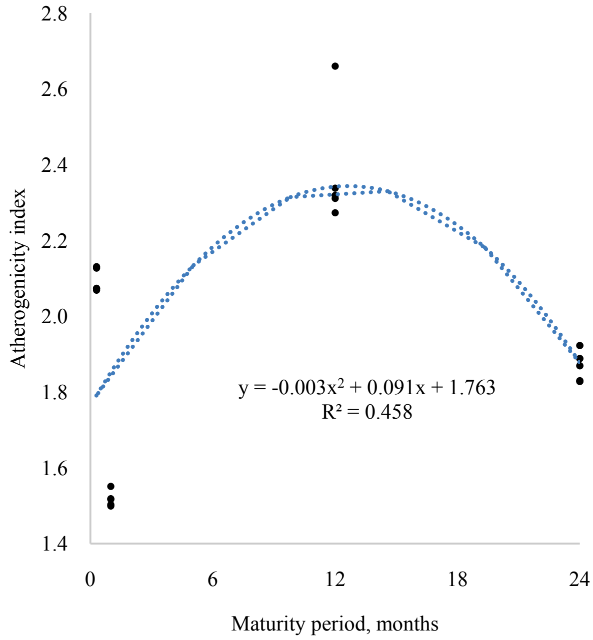

Fig. 3. Dependence of the atherogenicity index on the maturation time of Caciotta cheese, N = 20

A strong direct correlation was found between Σ PUFA and the maturation time of Caciotta cheese (r = 0.951 ± 0.096, P < 0.01). The regression line illustrates the dependence between Σ PUFA and the maturation time of Caciotta cheese, described as a second-degree polynomial (Fig. 1).

The desaturase index decreases with the aging of Caciotta cheese fat: the highest value was recorded during the period from 10 days to 1 month of aging, while the lowest was observed at 24 months. Correlation analysis indicates a strong inverse dependence between Δ_9 desaturase index (C_{14}) and the maturation time of Caciotta cheese (r = –0.957 ± 0.101, P < 0.01). The regression line confirms that there is an inverse dependence between Δ_9 desaturase index (C_{14}) and the maturation time of Caciotta cheese, characterized by a second-degree polynomial (Fig. 2).

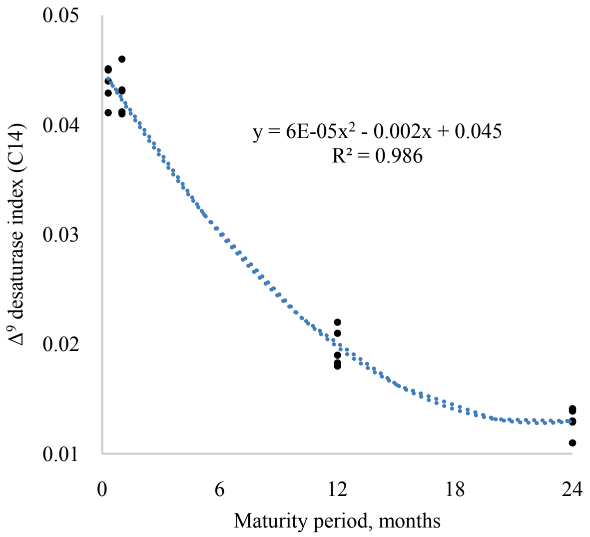

Fig. 2. Dependence of Δ_9 desaturase index (C_{14}) on the maturation time of Caciotta cheese, N = 20

The atherogenic index of the fat in Caciotta cheese shows significant fluctuations throughout the maturation period, reaching its maximum on the 10th day and at 12 months, and decreasing at 1 month and 24 months of maturation (Table 3). Regression analysis indicated that this dependence of the atherogenic index on the maturation time of Caciotta cheese is not linear and is best expressed by a polynomial of the second-degree (Fig. 3).

The dynamics of the atherogenic index in Caciotta cheese are related to changes in the concentration of OFA in the cheese (Table 3). The regression line that describes the dependence of OFA content in the fat of Caciotta cheese during the maturation period also resembles a polynomial of the second-degree (Fig. 4).

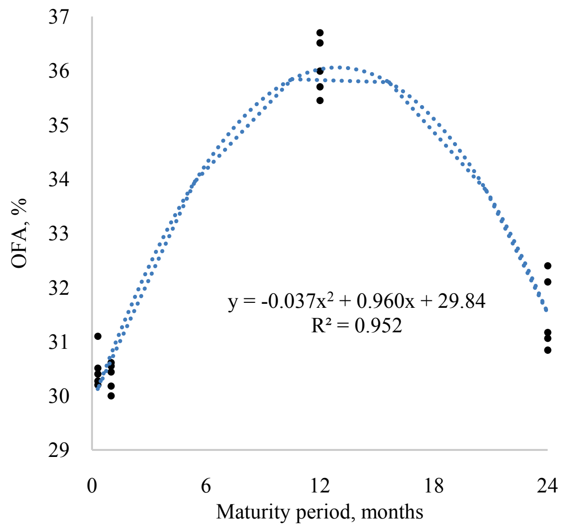

Fig. 4. Dependence of OFA on the maturation time of Caciotta cheese, N = 20

The proportion of desirable fatty acids with hypocholesterolemic effects in the fat of Caciotta cheese reaches its maximum level at 1 month and its minimum at 12 months of maturation, which corresponds with Σ UFA and the content of stearic acid in the fat of the cheese during this period. The lowest H/H ratio of Caciotta cheese fat is observed at 10 days and 12 months of maturation, while it significantly increases at 1 and 24 months.

The maturation period of Canestrato cheese does not significantly affect the levels of short-chain fatty acids in the fat, specifically butyric, caproic, and caprylic acids (Tables 4, 5). The main medium-chain fatty acids in the fat of Canestrato cheese, as in Caciotta cheese, are palmitic, capric, and myristic acids, which reach their peak total at 10 days of maturation. With an increase in maturation time to 12 months, a decrease in the concentration of capric, lauric, myristic, pentadecenoic, palmitoleic acids, and even a complete disappearance of tridecanoic acid in the fat of Canestrato cheese is observed. In contrast, throughout the maturation period, the content of myristoleic acid in the fat of Canestrato cheese increased, reaching its highest level at 12 months.

The main long-chain fatty acids in Canestrato cheese are oleic and stearic acids. The proportion of stearic acid decreased with the increase in maturation time, while the proportions of oleic, linoleic, and cis-11-eicosenoic acids increased. In the fat of Canestrato cheese, unlike in Caciotta cheese, eicosatrienoic acid was not detected (Table 4). The content of long-chain fatty acids in Canestrato cheese increased throughout the entire maturation period compared to the initial value at 10 days of age (Table 5).

Table 4
Content of fatty acids in Canestrato goat cheese during the maturation period (% of the total fatty acid content, x ± SD, n = 5)

Acid	Term of cheese maturation			
	10 days	1 month	12 months	24 months
Butyric, $C_{4:0}$	2.89 ± 0.07^{ab}	2.59 ± 0.11^{a}	3.00 ± 0.26^{ab}	3.22 ± 0.37^{b}
Caproic, $C_{6:0}$	3.08 ± 0.03^{a}	2.61 ± 0.06^{b}	2.86 ± 0.10^{ab}	3.06 ± 0.03^{a}
Caprylic, $C_{8:0}$	2.84 ± 0.07^{ab}	2.70 ± 0.08^{ab}	2.57 ± 0.07^{b}	2.88 ± 0.09^{a}
Capric, $C_{10:0}$	10.22 ± 0.14^{a}	8.35 ± 0.23^{b}	8.78 ± 0.26^{b}	8.75 ± 0.49^{b}
Hendecanoic, $C_{11:0}$	0.19 ± 0.02^{a}	0.16 ± 0.01^{a}	0.20 ± 0.03^{a}	0.16 ± 0.02^{a}
Lauric, $C_{12:0}$	3.95 ± 0.04^{a}	3.48 ± 0.28^{a}	3.68 ± 0.09^{a}	2.79 ± 0.10^{b}
Tridecanoic, $C_{13:0}$	0.08 ± 0.02^{a}	0.04 ± 0.01^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Myristic, $C_{14:0}$	8.36 ± 0.07^{a}	8.13 ± 0.19^{a}	8.73 ± 0.38^{a}	7.25 ± 0.04^{b}
Myristoleic, $C_{14:1}$	0.23 ± 0.02^{a}	0.22 ± 0.03^{a}	0.42 ± 0.05^{b}	0.37 ± 0.04^{b}
Pentadecanoic, $C_{15:0}$	0.73 ± 0.03^{a}	0.66 ± 0.02^{b}	0.81 ± 0.03^{c}	0.72 ± 0.04^{a}
Pentadecenoic, $C_{15:1}$	0.19 ± 0.02^{a}	0.09 ± 0.01^{b}	0.08 ± 0.02^{b}	0.11 ± 0.03^{b}
Palmitic, $C_{16:0}$	22.44 ± 0.18^{a}	20.48 ± 0.14^{b}	22.22 ± 0.21^{a}	21.96 ± 0.54^{a}
Palmitoleic, $C_{16:1}$	0.52 ± 0.03^{a}	0.42 ± 0.02^{b}	0.41 ± 0.02^{b}	0.42 ± 0.03^{b}
Heptadecanoic, $C_{17:0}$	0.52 ± 0.02^{a}	0.43 ± 0.03^{b}	0.41 ± 0.02^{b}	0.43 ± 0.01^{b}
Cis-10-heptadecenoic, $C_{17:1}$	0.12 ± 0.04^{a}	0.15 ± 0.03^{a}	0.22 ± 0.02^{b}	0.16 ± 0.02^{a}
Stearic, $C_{18:0}$	16.92 ± 0.36^{a}	17.32 ± 0.11^{a}	13.38 ± 0.21^{b}	15.79 ± 0.14^{c}
Oleic, $C_{18:1n9c}$	22.51 ± 0.18^{a}	27.46 ± 0.31^{b}	25.60 ± 0.38^{c}	25.78 ± 0.19^{c}
Linoleic, $C_{18:2n6c}$	3.14 ± 0.06^{a}	3.41 ± 0.26^{a}	4.35 ± 0.21^{b}	4.27 ± 0.12^{b}
Arachidic, $C_{20:0}$	0.19 ± 0.01^{a}	0.25 ± 0.02^{a}	0.13 ± 0.02^{b}	0.20 ± 0.03^{a}
Cis-11-eicosenoic, $C_{20:1n9}$	0.05 ± 0.01^{a}	0.25 ± 0.03^{b}	0.66 ± 0.04^{c}	0.74 ± 0.03^{d}
Linolenic, $C_{18:3n3}$	0.86 ± 0.02^{a}	0.81 ± 0.04^{a}	1.51 ± 0.09^{b}	0.94 ± 0.08^{a}
Eicosatrienoic, $C_{20:3n3}$	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Note: see Table 2.

Table 5
Quality indicators of lipids in Canestrato goat cheese during the maturation period (% of the total fatty acid content, x ± SD, n = 5)

Indicator	Term of cheese maturation			
	10 days	1 month	12 months	24 months
Σ SFA	73.39 ± 0.17^{a}	67.20 ± 0.46^{b}	66.76 ± 0.65^{b}	67.21 ± 0.17^{b}
Σ UFA	27.61 ± 0.18^{a}	32.80 ± 0.45^{b}	33.24 ± 0.67^{b}	32.79 ± 0.16^{b}
Σ MUFA	23.61 ± 0.21^{a}	28.59 ± 0.32^{b}	27.39 ± 0.44^{b}	27.59 ± 0.07^{b}
Σ PUFA	4.01 ± 0.06^{a}	4.22 ± 0.24^{a}	5.86 ± 0.23^{b}	5.21 ± 0.17^{b}
Σ ω_3 PUFA	0.86 ± 0.02^{a}	0.81 ± 0.04^{a}	1.51 ± 0.09^{b}	0.94 ± 0.08^{a}
Σ ω_6 PUFA	3.14 ± 0.06^{a}	3.41 ± 0.26^{a}	4.35 ± 0.21^{b}	4.27 ± 0.12^{b}
ω_3/ω_6 PUFA	3.63 ± 0.11^{a}	4.22 ± 0.42^{b}	2.89 ± 0.17^{c}	4.59 ± 0.27^{b}
Σ SCFA (4–8)	8.81 ± 0.09^{a}	7.90 ± 0.15^{b}	8.43 ± 0.37^{ab}	9.16 ± 0.35^{a}
Σ MCFA (10–16)	46.89 ± 0.17^{a}	42.04 ± 0.49^{b}	45.32 ± 0.66^{a}	42.53 ± 0.13^{b}
Σ LCFA (17–20)	44.30 ± 0.22^{a}	50.07 ± 0.51^{b}	46.25 ± 0.55^{c}	48.31 ± 0.26^{d}
DFA	44.52 ± 0.22^{a}	50.12 ± 0.48^{b}	46.62 ± 0.57^{a}	48.58 ± 0.30^{b}
OFA	34.74 ± 0.19^{a}	32.09 ± 0.28^{b}	34.63 ± 0.70^{a}	32.00 ± 0.60^{b}
Δ^9 desaturase index (C_{14})	0.027 ± 0.002^{a}	0.026 ± 0.002^{a}	0.046 ± 0.006^{b}	0.049 ± 0.001^{b}
Atherogenicity index (AI)	2.17 ± 0.01^{a}	1.72 ± 0.04^{bc}	1.83 ± 0.08^{b}	1.64 ± 0.01^{c}
Hypocholesterolaemic/hypercholesterolaemic ratio (H/H)	0.76 ± 0.01^{a}	0.99 ± 0.02^{b}	0.91 ± 0.03^{b}	0.97 ± 0.01^{b}

Note: see Table 2, 3.

The main saturated fatty acids in Canestrato cheese are palmitic, stearic, caprylic, and myristic acids, which, like in Caciotta cheese, account for over 55% of the total fatty acid content. The decrease in the total content of saturated fatty acids starting from the third month of maturation is associated with a reduction in the proportions of caproic, caprylic, pentadecanoic, palmitic, heptadecanoic acids, and the disappearance of tridecanoic acid in the cheese fat (Table 4, 5).

Corresponding to the dynamics of saturated fatty acids, changes in the content of unsaturated fatty acids in Canestrato cheese fat occur. During this period, the proportion of unsaturated fatty acids in Canestrato cheese increased due to myristoleic, oleic, linoleic, and cis-11-eicosenoic acids. Thus, the increase in the proportion of unsaturated fatty acids in Canestrato cheese during maturation is supported by both monounsaturated and polyunsaturated fatty acids.

Among the unsaturated fatty acids classified as ω3 PUFA, only linolenic acid is found in Canestrato cheese, and its content dynamics do not exhibit a characteristic dependency on the maturation time. The only acid in Canestrato cheese that belongs to ω6 PUFA is linoleic acid. Thus, the ω_3/ω_6 PUFA ratio in Canestrato cheese is calculated as the ratio of linoleic to linolenic acid content, similar to that in Caciotta cheese. This ratio does not show a significant dependency on the maturation period of the cheese.

Unlike Caciotta cheese, the Δ_9 desaturase index (C_{14}) in Canestrato cheese increased throughout the entire maturation period. The lowest value was recorded from the 10th day to the 3rd month, while at the 6th and 12th months of maturation, it reached its maximum. A strong direct correlation is found between the Δ_9 desaturase index (C_{14}) and the age of Canestrato cheese ($r = 0.842 \pm 0.096$, $P < 0.01$). The regression line indicates the relationship between the Δ_9 desaturase index (C_{14}) and the maturation period of Canestrato cheese, which is expressed as a second-degree polynomial (Fig. 5).

The atherogenicity index of Canestrato cheese fat decreased with increasing maturation time; however, no significant correlation between these indicators is observed. The proportion of desirable fatty acids, classified as hypocholesterolemic, fluctuated throughout the maturation period of Canestrato cheese, reaching its maximum at the 3rd and 12th months compared to the data from the 10th day and the 6th month, while the dynamics of hypercholesterolemic acid content were opposite. The H/H ratio in Canestrato cheese increased starting from the 3rd month and remained stable until the 12th month of maturation.

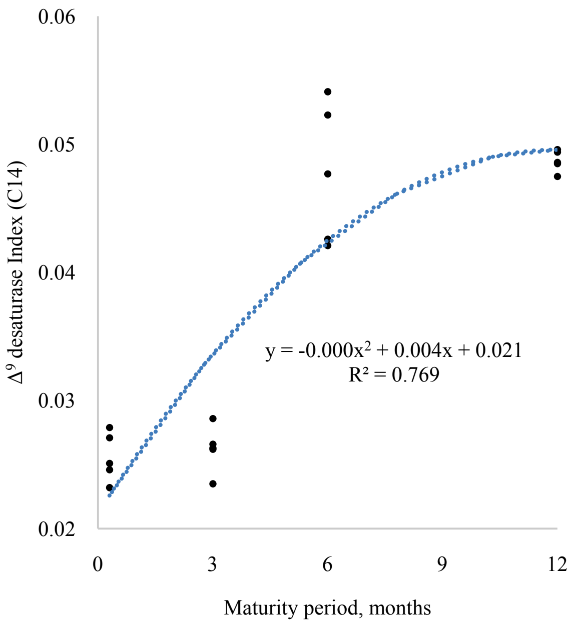

Fig. 5. Dependency of Δ9 desaturase index (C_{14}) on the maturation period of Canestrato cheese, N = 20

Discussion

Caciotta cheese belongs to semi-hard cheeses with a maturation period of 2 months, traditionally made in Italy from a mixture of unpasteurized raw sheep, goat, and cow milk (Fusco et al., 2019). Canestrato is classified as a hard cheese produced in Italy from a blend of sheep and goat milk under artisanal conditions. Its maturation period ranges from 2 to 12 months (Trani et al., 2016). Both cheeses enjoy significant consumer demand, and each region has its own modifications of the production technology. In our experiment, only one type of milk was used – unpasteurized goat milk – and the maturation period for Caciotta cheese was extended to 24 months, allowing for the development of both semi-hard and hard textures depending on its age.

According to the literature, the fatty acid content in goat cheeses varies within the following ranges: SFA – from 58% to 74%, SCFA – from 11.2% to 37.0%, MUFA – from 19.7% to 31.4%, PUFA, including ω_3 – from 0.3% to 1.3% and ω_6 – from 1.4% to 7.0%, and conjugated linolenic acid – from 0.1% to 1.0% (Vieitez et al., 2016; Szterk et al., 2022). Similar results were obtained by Manuelian et al. (2017) and Popović-Vranješ et al. (2017), who established that the most stable fatty acids with the least variability in goat cheese were capric ($C_{10:0}$), palmitic ($C_{16:0}$), and stearic ($C_{18:0}$) acids. In this study, goat cheese contains 15.1% short-chain fatty acids (SCFA), which are beneficial for human health, particularly caproic ($C_{6:0}$), caprylic ($C_{8:0}$), and capric ($C_{10:0}$) acids. These fatty acids are responsible for the unique sensory properties and better digestibility of goat milk fat (Bessa et al., 2016). Paszczyk & Łuczyńska (2020) found that sheep and goat cheeses differ little in their content of short-chain fatty acids (SCFA) (14.7 ± 2.5% and 14.8 ± 2.8%, respectively), while in cow milk cheeses, this content is 9.4 ± 0.9%.

The total content of SFA, MUFA, and PUFA in Caciotta and Canestrato cheeses throughout the maturation period did not exceed the mentioned ranges; however, the content of SCFA was lower than the indicated range for goat milk cheese and approached that of cow milk cheese. Regarding the SFA content in goat cheeses Caciotta and Canestrato, palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids were dominant, with somewhat lower levels of capric ($C_{10:0}$) and myristic ($C_{14:0}$) acids throughout the maturation period.

The fatty acid profile of milk and its products is related to the goats' feeding diet (Klir et al., 2012) and depends on the proportions of fatty acids in the dietary components and the microbial activity in the rumen. According to EU Commission Regulation (2010), food products are sources of ω_3 fatty acids only if they contain at least 0.3 g/100 g of alpha-linolenic acid, and a food product is considered high in ω_3 fatty acids if it contains at least 0.6 g/100 g of this acid. In Caciotta cheese, with fat content ranging from 27% to 36%, and in Canestrato cheese, from 31% to 35%, the proportion of alpha-linolenic acid varies from 0.2% to 0.5% and from 0.3% to 0.5%, respectively, indicating a high intensity of its synthesis, particularly due to lactic acid bacteria during the maturation of the cheeses (Iorizzo et al., 2024), and allows us to consider both cheeses as sources of this fatty acid.

The obtained values of ω_3 PUFA in goat cheeses Caciotta and Canestrato fall within the range recommended by FAO (2010), which is 0.25–2.00 g/day. The functions of ω6 fatty acids include reducing the cholesterol content of low-density lipoproteins in human blood, which helps control risk factors for cardiovascular diseases (Maki et al., 2018). The content of ω6 PUFA in Caciotta cheese (0.8–1.6 g/100 g) and Canestrato cheese (1.0–1.5 g/100 g) is below the range recommended by FAO (2010), which is 4.4–6.7 g/day.

In human nutrition, an optimal ω_3/ω_6 PUFA ratio of 4:1 to 1:1 is considered ideal (Alagawany et al., 2019), and achieving this requires consideration of the ratios of individual food products (Shevchenko et al., 2020; Honchar et al., 2022). Analysis of this ratio in Caciotta and Canestrato cheeses showed that it is close to the ideal almost throughout their maturation period.

Our research aligns with data obtained by Paszczyk & Łuczyńska (2020), which confirm the high content of ω3 PUFA in goat cheeses alongside a low thrombogenic index compared to sheep and cow cheeses. This study notes that cow, sheep, and goat cheeses exhibit similar values of hypocholesterolemic/hypercholesterolemic (H/H) ratios. The features of changes in the atherogenic index (AI) and the H/H ratio vary depending on the type of milk fat (hard, soft cheeses, and butter) (Paszczyk, 2022) as well as the season of milk production (Paszczyk et al., 2022). It is important to note that the hypocholesterolemic/hypercholesterolemic fatty acid ratios in Caciotta and Canestrato cheeses are lower than in women's breast milk (Purkiewicz & Pietrzak-Fiećko, 2024), indicating their high dietary characteristics.

It is believed that the intensity of lipolysis is higher during the maturation of cheeses made from raw milk compared to pasteurized milk (Ioannidou et al., 2022). Lipolysis occurs due to the metabolism of microbiota through the esterases/lipases of lactic and propionic acid bacteria, non-starter lactic acid bacteria, surface microorganisms, yeasts, and molds. Esterases from starter and non-starter microorganisms catalyze the release of short-chain fatty acids from milk fat and the synthesis of short-chain ethyl esters. Additionally, during lipolysis, some bioactive lipids, such as conjugated linoleic and linolenic acids, may be produced by enzymes from the isomerase-desaturase complex (Taboada et al., 2015).

The atherogenic index in cow's milk cheeses depends on the production season (Hirigoyen et al., 2018). For animal-derived lipids, the recommended atherogenic index is between 0.5 and 1.0 (Senso et al., 2007). Regarding the AI of the fats in Caciotta and Canestrato cheeses, it depends on their maturation period and exceeds the specified value throughout the entire maturation process in both cheeses. Atherogenic and thrombogenic indices characterize the impact of dietary fats on the risk of cardiovascular diseases. The higher the values of these indices, the greater the risk of developing and manifesting heart pathology. Dairy products with lower AI values can reduce total cholesterol and low-density lipoprotein cholesterol levels in human plasma (Fehily et al., 1994). In our case, the AI is lower in Caciotta cheese than in Canestrato cheese, which may be considered when selecting a healthy diet for individuals prone to hypercholesterolemia.

The Δ_9 desaturase index (C_{14}) of Caciotta and Canestrato cheeses depends on their production technology and age. The Δ_9 desaturase index (C_{14}) of Caciotta cheese decreases during maturation, while that of Canestrato cheese, conversely, increases. This is related to changes in their chemical and microbial composition as well as the processes of proteolysis and lipolysis. Low activity levels of this enzyme may be caused by changes in the cheese's microenvironment (such as moisture or pH) and the presence of antibacterial components (Bayer et al., 2017a, 2017b).

It is believed that the content of desirable hypocholesterolemic fatty acids (DFA) and hypercholesterolemic fatty acids (OFA) in milk fat depends on the season of its production (Kolarič et al., 2022). In the fat of Caciotta and Canestrato cheeses, the levels of these fatty acids fluctuate during their maturation, and the patterns of their dynamics differ.

Conclusions

The fatty acid profile of craft hard cheeses Caciotta and Canestrato made from raw goat's milk depends on their maturation period. The ratio of saturated to unsaturated fatty acids in Caciotta cheese does not show a characteristic dependence on its age, whereas in Canestrato cheese, the proportion of saturated fatty acids decreases by the third month of maturation against an increase in the proportion of unsaturated fatty acids, remaining at this level until the twelfth month. The main saturated fatty acids in both cheeses are palmitic, stearic, capric, and myristic acids, which together exceed 55% of all acids. The proportion of short-chain fatty acids in Caciotta and Canestrato cheeses ranged from 7.9% to 10.2%, medium-chain from 40.2% to 49.5%, while the remainder consisted of long-chain fatty acids. The unsaturated fatty acids in Caciotta and Canestrato cheeses were represented by oleic and linoleic acids, which together constitute over 25% of all fatty acids in the cheese. During the maturation of Caciotta cheese, there was an accumulation of Σ PUFA against a decrease in the Δ_9 desaturase index (C_{14}). The Δ_9 desaturase index (C_{14}) of Canestrato cheese increased throughout the entire maturation period. The maturation period of Caciotta and Canestrato cheeses significantly affects the dynamics of the atherogenic index and the ratio of hypocholesterolemic/hypercholesterolemic fatty acids. In both Caciotta and Canestrato cheeses, the main ω_3 PUFA is linolenic acid, while the main ω_6 PUFA is linoleic acid, with a ratio of ω_3 PUFA/ω_6 PUFA ranging from 2.8:1 to 4.6:1. The obtained research results can be used to assess the naturalness and authenticity of craft hard cheeses made from raw goat's milk.

The authors declare that they have no potential conflict of interest.

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