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Original Article/Özgün Araştırma

Unraveling the Unique Fatty Acid Signatures of Blended Butters: A Gas Chromatography Study

Karışım Tereyağlarının Benzersiz Yağ Asidi Profillerinin Belirlenmesi: Bir Gaz Kromatografi Çalışması

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Abstract

Objective: This study aimed to analyze the changes in the fatty acid profile of butter obtained by mixing goat butter and cow butter at different ratios (25%, 50%, 75% w/w) using gas chromatography and to identify the key fatty acids that can be used to detect goat butter adulterated with cow butter.

Materials and methods: The purity of the fats used was determined by Real-Time PCR. Fatty acid composition analyses were performed using a Gas Chromatograph (GC) device.

Discussion and results: The results showed that as the proportion of goat butter increased, the amount of capric acid increased significantly, and the total percentage of unsaturated fatty acids decreased. Capric acid was found to be dominant in goat butter, while the highest saturated fatty acid content was determined in cow butter. As a result of the study, it was determined that caproic acid (6:0), caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristoleic acid (14:1), linoleic acid (18:2), linolenic acid (18:3), and eicosenoic acid (20:1) content in goat and cow butter mixtures can be used as marker acids.

Keywords: Fatty Acid; butters; adulteration; GC-FID; RT-PCR

Öz

Amaç: Bu çalışmada, keçi tereyağı ile inek tereyağının farklı oranlarda (%25, 50, 75 w/w) karıştırılmasıyla elde edilen tereyağının yağ asidi profilindeki değişimlerin gaz kromatografisi kullanılarak analiz edilmesi ve inek tereyağı ile tağşiş edilmiş keçi tereyağlarının saptanmasında belirleyici yağ asitlerinin tespit edilmesi amaçlanmıştır.

Materyal ve yöntem: Kullanılan yağların saflığı Real-Time PCR ile belirlenmiştir. Yağ asitleri kompozisyonu analizleri Gaz Kromatografi (GC) cihazı ile yapılmıştır.

Tartışma ve sonuç: Çalışma sonucunda, keçi tereyağı oranı arttıkça kaprik asit miktarının önemli ölçüde arttığı ve doymamış yağ asitlerinin toplam yüzdesinin azaldığı gözlenmiştir. Kaprik asidin keçi tereyağında baskın olduğu, en yüksek doymuş yağ asidi içeriğinin inek tereyağında belirlendiği görülmüştür. Çalışma sonucunda keçi ve inek tereyağı karışımlarında kaproik asit (6:0), kaprilik asit (8:0), kaprik asit (10:0), laurik asit (12:0), miristoleik asit (14:1), linoleik asit (18:2), linolenik asit (18:3) ve eikosenoik asit (20:1) içeriğinin belirleyici asit olarak kullanılabileceği saptanmıştır.

Anahtar kelimeler: Yağ asitleri; tereyağı; tağşiş; GC-FID; RT-PCR

1. Introduction

Goat butter has been gaining increasing attention due to its nutritional value, digestibility, and positive health effects. Its high nutritional value stems from being a rich source of minerals and containing vitamins A, D, E, and K, which support the immune system, improve bone health, and maintain skin health. Goat butter is rich in essential minerals such as calcium and phosphorus. Goat milk and butter contain smaller fat globules compared to cow milk, which facilitates digestion and absorption. Goat milk has a lower lactose content than cow milk. This means that individuals with lactose intolerance experience fewer digestive problems. While cow milk contains approximately 4.8% lactose, goat milk contains approximately 4.1% lactose. This difference helps many lactose intolerant individuals tolerate goat milk better (Haenlein, 2004; Mora Garcia & Clark, 2017).

Short-chain fatty acids in goat butter support gut health and reduce inflammation. Monounsaturated fatty acids improve heart health and lower levels of bad cholesterol (Mansour & Sinclair, 1993). Goat butter contains natural anti-inflammatory compounds, making it a valuable aid in managing inflammatory conditions such as joint pain and arthritis. Its unique flavor profile makes goat butter a preferred choice in gourmet cooking and baking. With a lower melting point, it imparts a creamier texture to dishes (Konar, 2001; Mora Garcia & Clark, 2017; He, et al., 2023).

The valuable properties and increasing market value of goat butter have made it a prime target for adulteration. Goat butter can be adulterated with cow milk or butter to reduce costs. This diminishes the unique flavor and nutritional value of goat butter, while posing health risks for individuals with lactose intolerance or cow milk allergy. Cheaper vegetable oils can be added to goat butter to reduce production costs. This negatively impacts the nutritional value and health benefits of butter, and the addition of vegetable oils, especially those containing trans fats, can have adverse effects on heart health. Colorants and artificial flavors are used to mimic the natural color and taste of goat butter. These additives mislead consumers and jeopardize the naturalness of butter (Gimonkar, Van Fleet, & Boys, 2021; Baptista, Cunha, & Domingues, 2021).

Various analytical methods are employed to detect adulteration in goat butter. Gas chromatography (GC) is a prominent technique among these methods. GC is a widely used method to detect adulteration based on the fatty acid profile of butter. Since the fatty acid profiles of goat and other milks differ, GC analyses can aid in the detection of adulteration. This method is carried out by converting fatty acids into their methyl esters and separating them using a GC instrument. Goat butter contains saturated fatty acids (palmitic acid, myristic acid, stearic acid), monounsaturated fatty acids (oleic acid, palmitoleic acid), and polyunsaturated fatty acids (linoleic acid, linolenic acid). GC analysis can determine the profile of these fatty acids, providing insights into the purity of goat butter. It also provides valuable information for adulteration detection and quality control. This method, with technical details such as column selection, temperature program, and standard use, offers accurate and reliable results (Dıraman, 2006; Chen, et al., 2023).

Apart from GC, methods such as mass spectrometry, HPLC, and NMR spectroscopy are employed to identify specific components in goat butter and detect adulteration. In addition to these chromatography-based analyses, DNA-based analytical methods, which have become widespread in recent years for various food analyses, are also available. Real-Time PCR (RT-PCR) methods are at the forefront of these (Bansal, et al., 2017; Artuvan & Aksay, 2022).

The risk of adulteration in goat butter is a significant concern for consumer safety and food quality. Adulteration methods include mixing with cow milk or butter, adding vegetable oils, and using artificial additives. Strategies such as regulatory controls, producer certification, and consumer awareness can be implemented to prevent adulteration. These measures ensure the purity and quality of goat butter, providing consumers with safe and healthy products (Gimonkar, Van Fleet, & Boys, 2021; Baptista, Cunha, & Domingues, 2021; Sassi, Arena, & Scaloni, 2015).

The aim of this study was to investigate the changes in fatty acid profile of goat butter adulterated with different proportions of cow butter and to propose an alternative method for detecting adulteration with cow butter. During the study, the purity of butter samples was determined using RT-PCR, a DNA-based analytical method, effectively certifying the commercial butters obtained from the market.

2. Materials and methods

2.1. Materials

The goat and cow butter used in this study were procured from the Mersin/Türkiye Market. A Maxwell® RSC PureFood GMO and

Authentication Kit (Promega Global, 2018) was utilized for nucleic acid isolation. For species identification analyses on the RT-PCR device, the following kits were procured from SNP Biotechnology: "Goat Species Identification Real-Time PCR Kit for Dairy Products," "Bovine Species Identification Real-Time PCR Kit for Dairy Products," "Ovine Species Identification Real-Time PCR Kit for Dairy Products," and "Buffalo Species Identification Real-Time PCR Kit for Dairy Products" (SNP Biyoteknoloji, 2019). KOH and Methanol, used in the GC analysis of fatty acid profile, were obtained from Sigma-Aldrich (Merck)

2.2. Methods

2.2.1. Butter blending processes

Pure cow butter and pure goat butter samples were subjected to a melting process at a controlled temperature of 40°C. The melted fats were then blended to create mixtures with 25%, 50%, and 75% (w/w, goat butter/cow butter) fat ratios.

2.2.2. RT-PCR based species identification analysis

To perform species identification analyses on an RT-PCR device, DNA was extracted from 1 g samples of suspected 100% cow and goat butter using the Maxwell® RSC PureFood GMO and Authentication Kit (Promega Global, 2018). The extraction protocol involved an incubation step at 85°C for 120 minutes.

The concentration (ng/µl) and purity of the isolated DNA were determined spectrophotometrically using a Shimadzu Biospec-Nano device (Manchester, 1996). The DNA extracted from the mixed butter samples was subjected to RT-PCR analysis using a species identification kit provided by SNP Biotechnology, following the thermal cycling conditions outlined in Table 1.

Table 1. RT-PCR Thermal Profile

Step	Stage		T (°C)	t (s)	Measurement	Cycle
1	Taq® Polymerase Activation		95	600	No	1
2	Amplification	Denaturation	95	15	No	50
		Annealing & Extension	60	60	Yes	

2.2.3. Fatty acid profile analyses

To determine the fatty acid profile of the mixed butter samples, an analytical method based on the formation of fatty acid methyl esters and their subsequent analysis by GC, as described by the International Olive Council, was employed (International Olive Oil Council, 2001). The method was applied as follows:

- 11.2 g of KOH were dissolved in 100 ml of methanol to prepare a 2 N methanolic KOH solution.
- 0.1 g sample of the mixed fat was transferred to a 5 ml screw-cap vial.
- 2 mL of chromatographic grade hexane was added and the mixture was thoroughly mixed.
- 0.2 mL of 2 N methanolic KOH solution was added, and the vial was tightly capped and vortexed for 30 seconds.
- This mixture was allowed to stand for approximately 30 minutes until a clear phase formed at the top.

The hexane phase was collected and analyzed using an Agilent 6890 GC. The GC conditions are presented in Table 2.

Table 2. GC Conditions for Fatty Acid Profile Analysis (IOOC, 2001)

Descriptions	Conditions
Detector	FID (Flame Ionization Detector)
Column	DB-23 (50%-Cyanopropyl)-methylpolysiloxane; (60m×0,25mm×0.25µm)
Thermal gradient	100 °C/5 min 5°C/min – 180°C 2°C/min – 200°C – 30 min (61-minute programmable)
Injection volume	0,2 µl
Inlet temperature	220°C
FID Temperature	280°C
Carrier gas flow rate	1 ml/min
Split	1/25

2.2.4. Statistical analysis

Statistical analyses of fatty acid profile data were performed using IBM SPSS Statistics 20.0. Descriptive statistics (mean, standard deviation) were calculated. Normality tests were performed, followed by analysis of variance to compare groups and post-hoc tests Tukey HSD and Duncan Tests were applied.

3. Results and discussion

3.1. RT-PCR based species identification analysis

A search for national and internationally certified reference materials (CRMs) of 100% goat butter and 100% cow butter yielded no results. The survey revealed that the animal products produced by these certified manufacturers were primarily ground meat products. Due to the unavailability of certified products for the production of the mixed butter used in the study, goat and cow butters were procured from the Mersin, Türkiye market. To verify that these butters were indeed 100% goat and 100% cow butter, Real-Time PCR analyses were employed. For these analyses, DNA isolations were performed in triplicate. The quantity and quality of the extracted DNA are presented in Table 3.

Table 3. DNA Isolation Results from Pure Samples of Cow and Goat Butter

Butter Sample	DNA Concentration (ng/µl)	DNA Quality (OD260/280)
100% Goat Butter	20,22±0,58	2,05±0,07
100% Cow Butter	111,50±11,26	1,89±0,01

Isolated DNA was analyzed by RT-PCR to detect the presence of adulterants in goat and cow butter samples. All samples were screened for buffalo, sheep, cow, and goat DNA. Triplicate PCR reactions were performed. Amplification curves for buffalo and sheep are depicted in Figure 1.

Amplification curves for cow and goat gene screening are presented in Figure 2. Analysis of Figure 1 revealed amplification only in positive control reactions for buffalo and sheep, with no amplification observed in butter samples or negative controls. In contrast, Figure 2 demonstrates that cow butter samples amplified only for cow genes and not for goat genes, while goat butter samples amplified only for goat genes and not for cow genes. These results indicate that no adulteration with milk from other sources was detected in the purchased cow and goat butter samples.

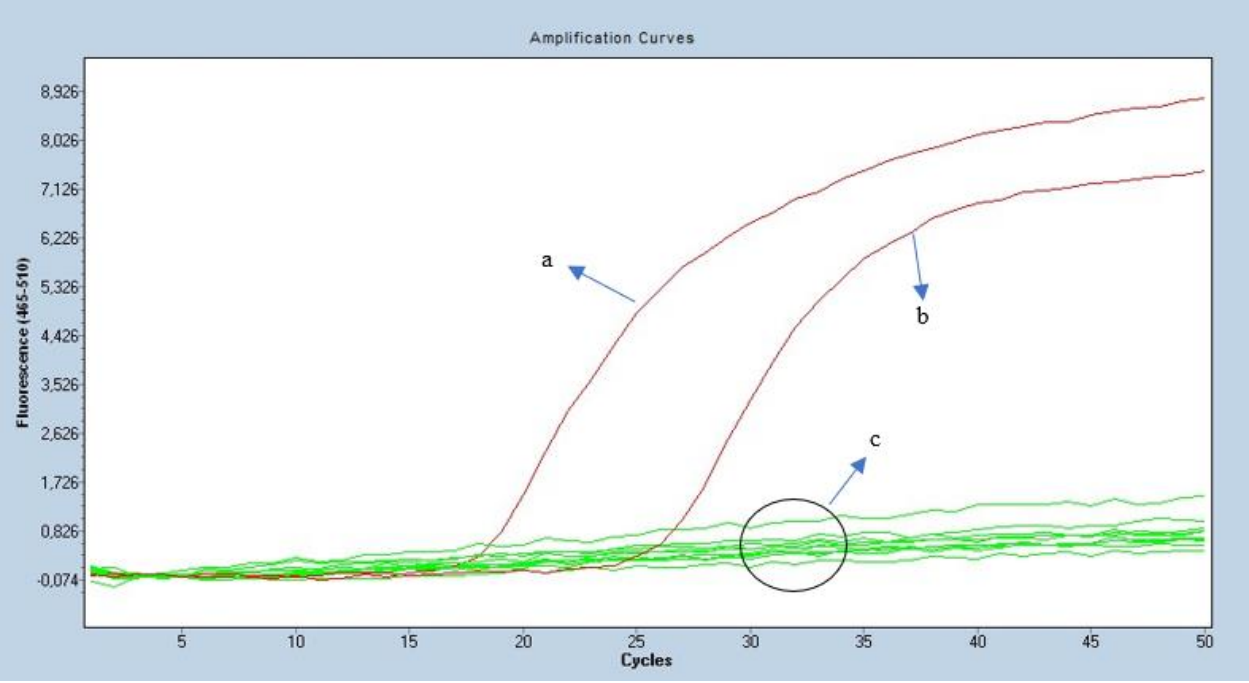

Figure 1. RT-PCR amplification curves for screening buffalo and sheep genes in samples (a: Sheep positive control curve, b: Buffalo positive control curve, c: Butter samples and negative control curves)

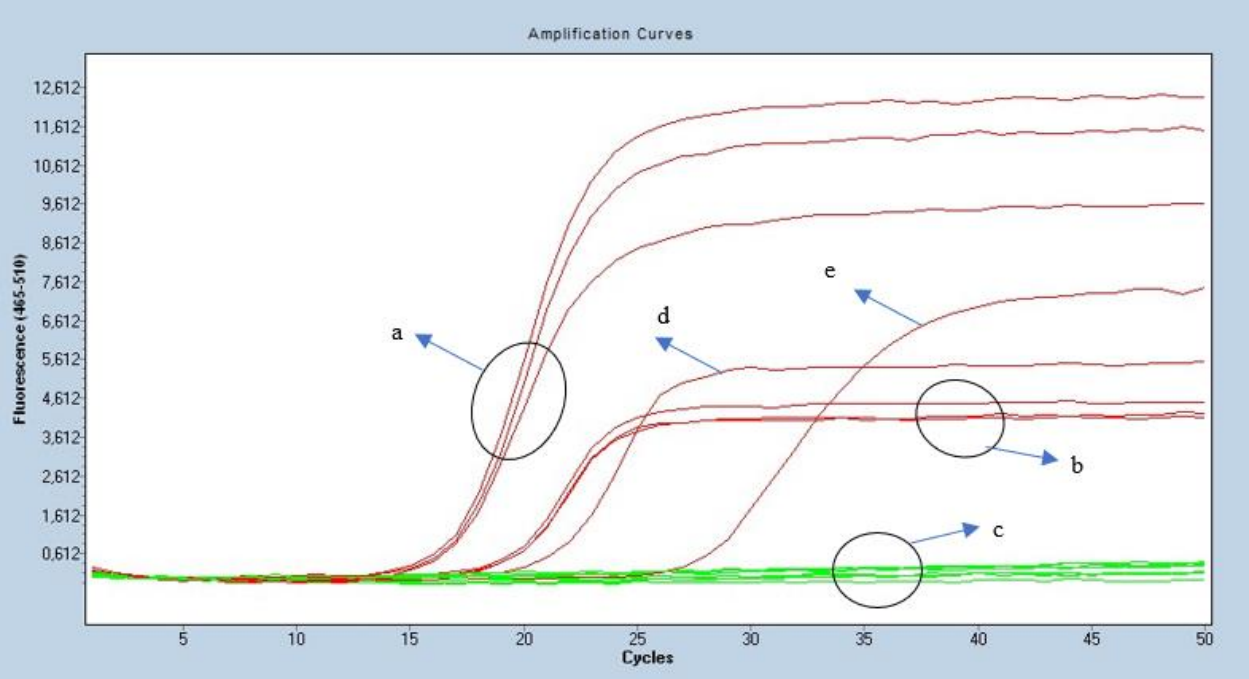

Figure 2. RT-PCR analysis amplification curves for Cow and Goat gene screening (a: cow gene amplification curves for cow butter, b: Goat gene amplification curves of goat butter, c: cow gene for goat butter, goat gene for cow butter and negative control amplification curves, d: Positive control amplification curve for bovine genes, e: Positive control amplification curve for caprine genes)

4. Fatty acid profile analyses

Fatty acid profile analyses of the mixed butter samples were performed in triplicate using GC. Fatty acid profile analysis is a widely used method, especially for vegetable oils, to detect potential adulteration with other oils (Dıraman, 2006). In this context, GC-based fatty acid profile analysis has become a well-established and reliable method. Therefore, it was aimed to monitor changes in fatty acid profile after the mixing process in butter products.

Fatty acid profiling revealed a saturated fatty acid content of 68.87% in goat butter and 63.53% in cow butter. Unsaturated fatty acid content was correspondingly lower in goat butter (31.13%) compared to cow butter (36.40%).

When the total saturated and unsaturated fatty acid contents of cow and goat butter were compared, it was found that goat butter had a higher proportion of saturated fatty acids and a lower proportion of unsaturated fatty acids. In another study by Sağdıç et al. investigating the fatty acid profile of 100% goat and cow butter produced traditionally from cow, sheep, and goat milk, it was determined that cow milk had 6% less saturated fatty acids and 3% more unsaturated fatty acids compared to goat milk (Sagdic, Dönmez, & Demirci, 2004).

Fatty acid profiles of the mixed butter samples are summarized in Table 4. As the proportion of goat butter increased, there was a significant increase in short- and medium-chain saturated fatty acids and linoleic acid ($p<0.05$), while long-chain fatty acids decreased significantly ($p<0.05$). These results support previous findings suggesting a higher omega-6 fatty acid content in goat butter, which may have implications for cardiovascular health (Gonzales-Martin, et al., 2020; Sagdic, Dönmez, & Demirci, 2004).

Figure 3 shows a significant increase in capric acid (10:0) with increasing goat butter proportion. Conversely, total unsaturated fatty acid content decreased. Paszczyk and Luczynska's findings corroborate these results, indicating high levels of capric acid in goat cheese and lower unsaturated fatty acid content in sheep cheese (Paszczyk & Łuczynska, 2020).

Trans fatty acids are formed in dairy products, especially those from ruminants, as a result of biochemical hydrogenation carried out by rumen bacteria in the rumen (Çakmakçı & Tahmas-Kahyaoglu, 2012). An increase in the percentage of cow butter in goat butter blends was associated with an increase in saturated fatty acids content. A study conducted by Aro et al. in 1998 provides a good example of this. In their study, the fatty acid profile of cheese and butter products made from

cow, goat, and sheep milk in 14 European countries was examined. The results showed that trans-fat content was significantly different among dairy products from these three species (Aro, et al., 1998). Goat butter, with its elevated linoleic acid content, is a prominent source for acquiring conjugated linoleic acid (Gürsoy, et al., 2021).

Table 4. Results of fatty acid profile analysis

Butter Sample (Goat/Cow, w/w)	0%	25%	50%	75%	100%
Butyric Acid (4:0)	2,19 ± 0,07	2,05 ± 0,06	1,89 ± 0,03	1,68 ± 0,05	1,59 ± 0,03
Caproic Acid (6:0)	1,43 ± 0,12	1,64 ± 0,01	1,80 ± 0,01	1,99 ± 0,04	2,21 ± 0,12
Caprylic Acid (8:0)	0,84 ± 0,01	1,30 ± 0,01	1,79 ± 0,01	2,24 ± 0,01	2,66 ± 0,01
Capric Acid (10:0)	1,80 ± 0,00	3,69 ± 0,02	5,70 ± 0,03	7,61 ± 0,02	9,24 ± 0,06
Lauric Acid (12:0)	2,17 ± 0,01	2,65 ± 0,01	3,15 ± 0,01	3,64 ± 0,01	4,11 ± 0,03
Myristic Acid (14:0)	9,76 ± 0,04	9,71 ± 0,02	9,75 ± 0,03	9,75 ± 0,03	9,77 ± 0,06
Myristoleic Acid (14:1)	1,59 ± 0,01	1,30 ± 0,00	1,01 ± 0,00	0,72 ± 0,00	0,46 ± 0,00
Pentadecanoic Acid (15:0)	1,47 ± 0,01	1,32 ± 0,00	1,18 ± 0,01	1,04 ± 0,00	0,94 ± 0,00
1-Pentadecanoic Acid (15:1)	0,42 ± 0,01	0,37 ± 0,00	0,40 ± 0,09	0,31 ± 0,03	0,23 ± 0,21
Palmitic Acid (16:0)	30,02 ± 0,06	29,17 ± 0,18	28,50 ± 0,07	27,73 ± 0,06	27,21 ± 0,17
Palmitoleic Acid (16:1)	2,24 ± 0,15	2,51 ± 0,22	2,23 ± 0,04	2,10 ± 0,13	1,81 ± 0,12
Stearic acid (18:0)	12,39 ± 0,05	11,83 ± 0,02	11,26 ± 0,02	10,57 ± 0,01	10,10 ± 0,07
Oleic Acid (18:1)	26,66 ± 0,02	25,70 ± 0,05	24,78 ± 0,04	24,10 ± 0,02	23,23 ± 0,15
Linoleic Acid (18:2)	2,28 ± 0,03	2,63 ± 0,26	3,35 ± 0,03	3,70 ± 0,03	4,06 ± 0,02
Arachidic Acid (20:0)	0,29 ± 0,04	0,26 ± 0,02	0,28 ± 0,02	0,26 ± 0,00	0,18 ± 0,16
Linolenic Acid (18:3)	0,82 ± 0,01	0,73 ± 0,01	0,58 ± 0,06	0,50 ± 0,00	0,41 ± 0,01
Eicosenoic Acid (20:1)	1,91 ± 0,02	1,67 ± 0,01	1,37 ± 0,06	1,24 ± 0,18	0,92 ± 0,00

Comparative studies on the fatty acid profiles of cow and goat butters reveal distinct compositional differences between the two types. For instance, the study conducted by Akgül et al. (2020) reported that goat butter is richer in saturated fatty acids (SFAs), whereas cow butter contains higher proportions of unsaturated fatty acids (UFAs). This finding aligns with the observed increase in SFA levels when goat butter is added to cow butter, suggesting consistency with existing literature (Akgül, Ceylan, & Atasoy, 2020). Similarly, research by Abdugamitova et al. (2024) showed an increase in SFA content in cheeses produced with cow and goat milk mixtures, while cheeses made with 100% goat milk displayed higher levels of oleic acid and polyunsaturated fatty acids. This outcome supports a similar trend of SFA enrichment upon the addition of goat butter to cow butter (Abdugamitova et al. 2024). Furthermore, Karaoğlu's (2024) study examined the effects of adding microencapsulated goat butter to cow butter on free fatty acid levels and other physicochemical properties. This research suggests that the inclusion of goat butter may significantly alter the fatty acid composition of the resulting product. Findings in the literature collectively indicate that incorporating goat butter into cow butter could enrich the fatty acid profile and increase the SFA content (Karaoğlu, 2024).

Figure 3 clearly demonstrates a distinct fatty acid profile for goat butter compared to cow butter. The most notable distinction is the nearly nine-fold increase in capric acid content in goat butter, which serves as a defining characteristic of this dairy product. While linoleic ($F= 117,297$; $p<0,05$), lauric ($F= 6740,930$; $p<0,05$), caprylic ($F= 23587,323$; $p<0,05$), and caproic ($F= 42,397$; $p<0,05$) acids also showed elevated levels, eicosenoic ($F= 61,040$; $p<0,05$), myristoleic ($F= 13402,85$; $p<0,05$), and linolenic ($F= 126,839$; $p<0,05$) acids were significantly lower in goat butter.

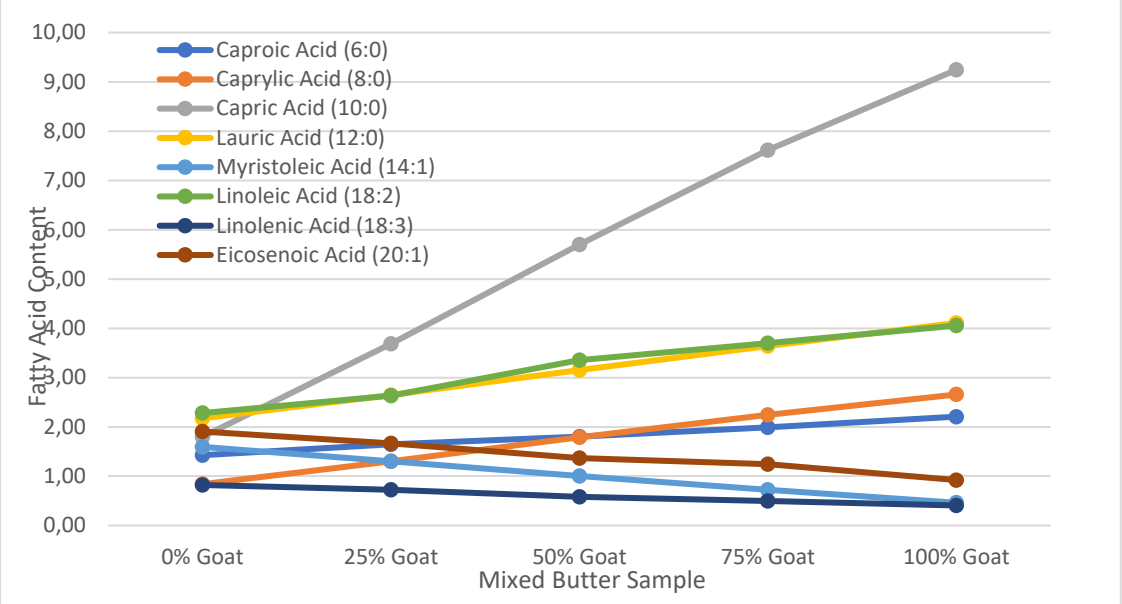

Figure 3. Changes in the percentage of signature fatty acids in goat and cow butter mixtures

In conclusion, GC analysis of the fatty acid profile in goat and cow butter blends revealed distinct fatty acid profiles for each butter type. Our study demonstrated that goat butter is rich in health-beneficial fatty acids such as linoleic acid and conjugated linoleic acid, while cow butter contains higher levels of saturated fatty acids. The analysis of the blends showed that the proportions of these fatty acids in butter vary, and the fatty acid profile of the blends presents a balanced profile combining the individual characteristics of both types. Notably, the RT-PCR method employed at the beginning of the study played a crucial role in determining the purity of the butters. The characteristic genetic profile of milk from different origins provided a sensitive indicator for assessing butter purity. These findings offer valuable tools for ensuring the purity and quality of butter products. Future studies investigating the effects of different feeding regimes and processing methods on fatty acid profile could provide deeper insights into this field and potentially enhance the nutritional value of butter products. Our study represents a significant step towards a better understanding of the diversity in the composition of goat and cow butter and the potential health benefits associated with this diversity.

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