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RESEARCH ARTICLE

Free Fatty Acid Profile of Köy Cheese Consumed in Erzurum and Its Region

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ABSTRACT

Köy cheese is a type of cheese with white interior, slightly yellowish exterior, a slightly firm texture, and a mild salty taste. It is produced and consumed in some regions in the Marmara and Aegean regions of Turkey, as well as in Bayburt, Yusufeli and Erzurum regions. The present study was conducted to determine the free fatty acid profile of Köy cheeses consumed in Erzurum and its surrounding areas. Analyzes were carried out by GC-MS using 10 cheese samples, and as a result, the presence of 25 free fatty acids was determined. Among these, short-chain fatty acids accounted for 2%, medium-chain fatty acids for 7%, and long-chain fatty acids for 91%. Palmitic acid (36.3563±5.4150%), oleic acid (22.0077±5.3136%), myristic acid (12.3104±2.1054%) and stearic acid (11.9837±3.1320%) were the prominent fatty acids among total free fatty acids. This study is considered to be significant in terms of providing data about the free fatty acid profile of Köy cheese produced and consumed widely in Türkiye.

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1. Introduction

There are numerous types of cheese worldwide, each with distinct flavors and tastes. The diversity of cheeses varies based on the vegetation, cultural practices, types of milk-producing animals, and production methods of a country (Kubícková & Grosch, 1998). Köy cheese, which is the subject of our research, is a type of cheese that is slightly hard, white on the inside, slightly yellowish on the outside, slightly salty, and is generally not over-ripened and consumed fresh. In Türkiye, it is produced and consumed in various regions, including Mengen, Bolu, and Assos in Marmara region, as well as Bayındır, Ödemiş, Tire, Seferihisar, Söke, and Torbalı in and around Izmir. Additionally, it is produced and consumed in Yusufeli, Bayburt, and Erzurum region. Köy cheese is primarily made from cow, sheep, or goat milk, or a combination of these in certain proportions. It contains 16-22% milk fat (Kesenkaş et al., 2012).

Free fatty acids are components released as a result of lipolysis during ripening in cheese. The enzymatic hydrolysis of milk glycerides into free fatty acids is essential for flavor development in cheese (Thierry et al., 2017). In particular, short and medium-chain fatty acids directly contribute to the aroma of the cheese. They also serve as precursor molecules for a series of catabolic reactions leading to the production of taste and aroma compounds, such as lactones, esters, methyl ketones, alkanes, and secondary alcohols (Woo et al., 1984; Collins et al., 2003).

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As a result of the literature review, it was seen that although Köy cheese is produced and consumed in a wide area in Turkey, scientific research on it is insufficient. This study was conducted to obtain data on the free fatty acid profile of Köy cheese consumed in Erzurum and its surrounding areas.

2. Materials and Methods

2.1. Köy Cheese Production

According to the information obtained from manufacturers and literature, this cheese is produced as follows. Köy cheese is primarily made from cow, sheep, or goat milk, or a combination of these in certain proportions, depending on the characteristics of the region where it is produced. In Erzurum and its surrounding areas, it is possible to use raw or pasteurized milk in the production of Köy cheese. The milk brought to the facility for cheese production undergoes heat treatment at 85 °C for 15-20 minutes and is then cooled to 45-50 °C. Starter culture is not used in the production of Köy cheese. After the addition of $CaCl_2$, rennet is added in a specific amount that allows coagulation within 45-60 minutes. Following coagulation, the curd is broken, drained, and pressed (pressed in fine-pored cloths at 5-6 kg pressure) before being salted in brine for one night (15-degree Brix salt concentration for 10 hours). The salted cheeses are then filled into plastic buckets, brine is added on top, and they are transferred to storage rooms. They are stored at 4 °C in these rooms. Köy cheese is a cheese consumed fresh without over-ripening (Kesenkaş et al., 2012; Çayır & Güzeler, 2020). However, producers report that brine-salted Köy cheeses can last approximately one year (Kesenkaş et al., 2012).

Cheese samples obtained from 10 different sales points were used to determine the total free fatty acid profile of Köy cheese consumed in Erzurum and its region.

2.2. Free Fatty Acid Analysis

The method provided by Durna (2019) was modified and utilized for the analysis. The composition of free fatty acids in cheese samples was determined as fatty acid methyl esters (FAME). For the extraction of fat, approximately 3 g of cheese sample was weighed into a 50 ml Falcon tube, and 25 ml of a chloroform-methanol mixture (2:1, v:v) and 20 ml of a 10% NaCl solution were added. The mixture was vortexed for 15 minutes. Subsequently, it was centrifuged at 10,000 rpm for 20 minutes at 4 °C, and the chloroform+fat mixture collected in the lower phase was carefully pipetted. It was then filtered through filter paper containing Na_2SO_4 as a moisture-absorbing substance. The chloroform was removed using a rotary evaporator to obtain pure fat. For esterification, 0.2 g of fat was mixed with 4 ml of hexane and 0.4 ml of 2 N methanol KOH, vortexed for 2 minutes, and then centrifuged at 5,000 rpm for 5 minutes. A clear phase (1.5-2 ml) from the top was injected into a Shimadzu-GC 2010 Ultra series gas chromatograph equipped with an automatic sample injector and a flame ionization detector. Separation was performed on an Agilent J&W DB-Fast FAME, 30 m x 0.25 mm, df 0.25 fused-silica capillary column. The injector temperature was maintained at 250 °C, and the detector temperature at 260 °C. The oven program was initiated at 50 °C for 0.5 minutes, increased to 194 °C at a rate of 30 °C/min for 3.5 minutes, and then raised to 240 °C at a rate of 5 °C/min for 2 minutes. Helium was used as the carrier gas. Before analyzing the samples, a fatty acid standard (Supelco 37 Component FAME Mix, Supelco Inc., USA) was analyzed to determine the peak times for the fatty acids in the samples. Fatty acid methyl esters were expressed as a percentage (%) (Kocaman, 2017).

2.3. Statistical Analysis

The obtained data were analyzed statistically using the SPSS 22.0 software package (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

Table 1 presents the free fatty acids and their proportions detected in the Köy cheese samples. The classification of fatty acids based on chain lengths was performed based on Huang et al. (2011) and Running and Mattes (2014). A total of 25 free fatty acids were identified. It seems there are no studies that focus on fatty acid profile of Köy cheese. Therefore, the results obtained from this research will be interpreted taking into account studies conducted on other cheese types.

As short and medium chain fatty acids in cheese samples; butyric acid was detected at the rate of 0.8776 ± 0.3740%, caproic acid at the rate of 1.1540 ± 0.3366%, caprylic acid at the rate of 1.1520 ± 0.5767%, capric acid at the rate of 3.6310 ± 2.5917%, undecanoic acid at the rate of 0.0361 ± 0.0184% and lauric acid at the rate of 2.6375 ± 1.2367%. Şengül et al. (2011) determined the percentages of butyric acid as 1.823%, caproic acid as 1.243%, caprylic acid as 0.618%, capric acid as 1.217%, and lauric acid as 1.529%. The ratio of butyric and caproic acid was higher than the values the present study obtained, while the ratio of caprylic, capric, and lauric acid was observed to be low. While Köy cheese is a cheese that is consumed mostly fresh without over-ripening, Karın Kaymağı cheese is a cheese that is consumed after being ripened for 2-3 months at an average temperature of 5-10 °C and 70-80% relative humidity (Şengül et al., 2011). In studies on free fatty acids in cheeses, it has been reported by various researchers that the butyric acid (C4:0) ratio increases during ripening (Georgala et al., 2005; Katsiari et al., 2000; Oluk & Güven, 2015).

Table 1. Free fatty acids and their percentages (%) detected in Köy cheeses consumed in Erzurum and its region.

	Köy Cheese Samples												
Short-chain fatty acids	1	2	3	4	5	6	7	8	9	10	Min	Max	Mean (x ± Sx)
Butyric Acid (C4:0)	1.1064	1.0807	0.0605	0.8790	0.7253	0.6497	0.9038	1.0586	1.4835	1.0398	0.0605	1.4835	0.8776±0.3740
Caproic Acid (C6:0)	1.5039	1.1404	0.4269	1.0660	0.9947	1.6015	1.4517	1.0810	1.3730	1.1805	0.4269	1.6015	1.1540±0.3366
Medium-chain fatty acids													
Caprylic Acid (C8:0)	1.1981	0.7989	0.6818	0.7941	0.7191	2.5909	1.2922	0.7345	0.8915	0.8497	0.6818	2.5909	1.1520±0.5767
Capric Acid (C10:0)	3.1503	2.2028	2.6751	2.0379	1.7979	10.3497	3.4611	1.6283	2.1642	2.1264	1.6283	10.3497	3.6310±2.5917
Undecanoic Acid (C11:0	0.0538	0.0233	0.0271	0.0290	0.0233	0.0721	0.0531	0.0174	0.0234	0.0216	0.0174	0.0721	0.0361±0.0184
Lauric Acid (C12:0)	0.0000	2.7658	4.1757	2.8443	2.3333	4.1501	3.9106	1.9325	2.7020	2.6596	0.0000	4.1757	2.6375±1.2367
Long-chain fatty acids													
Tridecanoic Acid (C13:0)	0.1174	0.0884	0.0869	0.0926	0.1048	0.0750	0.0929	0.0735	0.1037	0.0880	0.0735	0.1174	0.0928±0.0135
Myristic Acid (C14:0)	14.9848	12.1103	15.9735	12.6268	11.2943	10.1412	13.5422	9.0623	11.5229	11.4307	9.0623	15.9735	12.3104±2.1054
Myristoleic Acid (C14:1)	1.4442	0.8828	1.7776	1.3393	0.8146	0.0718	0.8876	0.3872	1.1025	0.8555	0.0718	1.7776	0.9510±0.4975
Pentadecanoic Acid (C15)	1.6446	1.7762	1.6821	1.8743	1.9326	0.9206	1.4262	1.5732	1.8766	1.9493	0.9206	1.9493	1.6271±0.3116
Palmitic Acid (C16:0)	42.3424	33.4262	45.8247	40.3936	37.0843	31.5876	31.6372	28.5372	37.2424	33.8380	28.5372	45.8247	36.3563±5.4150
Palmitoleic Acid (C16:1)	1.9255	1.3801	1.5397	2.3198	1.2471	0.3039	0.8797	1.0048	1.5839	1.3703	0.3039	2.3198	1.3482±0.5586
Heptadecanoic Acid (C17:0)	0.8502	1.1912	0.7667	1.0410	1.0782	0.6996	0.4685	1.3914	1.1833	1.3579	0.4685	1.3914	0.9907±0.2998
Stearic Acid (C18:0)	7.7292	12.7447	8.3345	8.8121	11.9448	15.6818	16.1641	13.7050	9.9710	14.8234	7.7292	16.1641	11.9837±3.1320
Oleic Acid (C18:1n9c)	18.7491	24.3688	13.5282	20.2383	24.2692	17.3643	19.1754	33.2088	23.7240	22.7291	13.5282	33.2088	22.0077±5.3136
Linoleic Acid (C18:2n6c)	1.7572	1.9733	1.1460	1.5759	1.7392	1.7213	2.4305	2.1867	1.2885	1.5839	1.1460	2.4305	1.7483±0.3858
Alfa-Linolenic Acid (C18:3n3)	0.6834	0.8793	0.6383	1.0133	0.7568	1.0561	1.2173	1.0925	0.4221	0.9186	0.4221	1.2173	0.8598±0.2426
Arachidic Acid (C20:0)	0.2505	0.3528	0.1818	0.2381	0.4118	0.3734	0.3985	0.4429	0.4509	0.3675	0.1818	0.4509	0.3417±0.0922
Henicosanoic Acid (C21:0)	0.0630	0.0850	0.0556	0.0751	0.0941	0.1035	0.0710	0.1094	0.0971	0.1228	0.0556	0.1228	0.0879±0.0215
cis-8,11,14-Eicosatrienoic Acid (20:5n-3)	0.0470	0.0576	0.0226	0.0573	0.0437	0.0106	0.0376	0.0449	0.0548	0.0390	0.0106	0.0576	0.0403±0.0152
Arachidonic Acid (C20:4n6)	0.1353	0.1411	0.0879	0.1229	0.1040	0.0879	0.0820	0.1537	0.1338	0.0850	0.0820	0.1537	0.1141±0.0270
Behenic Acid (C:22:0)	0.1031	0.2073	0.1184	0.2075	0.2158	0.1650	0.1696	0.2298	0.2544	0.2526	0.1031	0.2544	0.1901±0.0523
cis-5,8,11,14,17-Eicosapentaenoic acid (20:5n-3)	0.0591	0.0882	0.0414	0.0845	0.0495	0.0596	0.0844	0.0931	0.0735	0.0654	0.0414	0.0931	0.0694±0.0176
Tricosanoic Acid (C23:0)	0.0401	0.1053	0.0614	0.1159	0.0870	0.0718	0.0768	0.1013	0.1035	0.1102	0.0401	0.1159	0.0858±0.0244
Lignoceric Acid (C24:0)	0.0614	0.1296	0.0853	0.1215	0.1339	0.0909	0.0859	0.1501	0.1735	0.1350	0.0614	0.1735	0.1168±0.0347

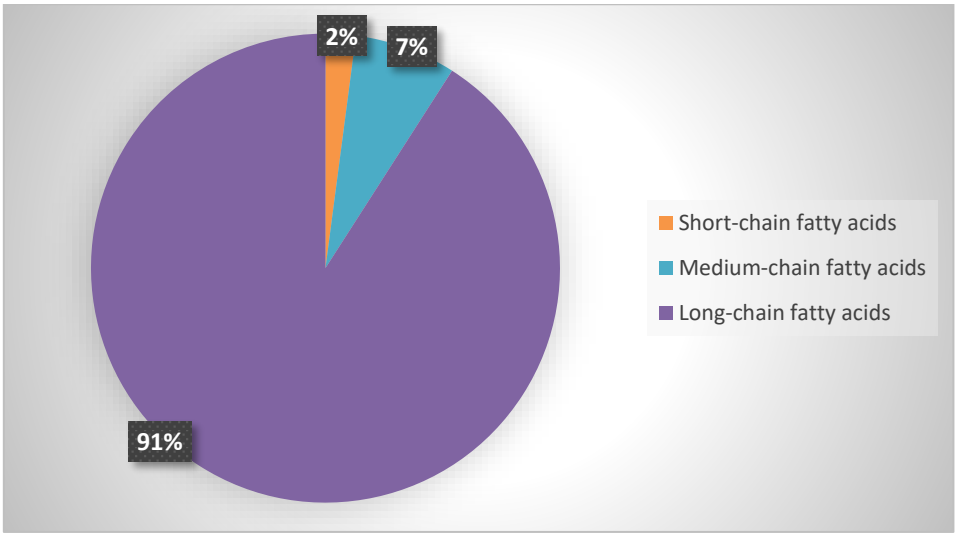

Figure 1. Distribution of free fatty acids identified in Köy cheese based on chain lengths.

Butyric acid (C4:0) is characterized by a rancid and cheesy taste and odor (Durna, 2019). The highest butyric acid rate was detected in sample number 9, and sample number 9 may be the most ripened among the cheese samples. Additionally, cheeses produced using traditional methods have been reported to contain significantly higher amounts of butyric acid compared to industrially produced cheeses (Georgala et al., 2005).

Released through the action of lipases on milk fat, free fatty acids contribute to the flavor of matured cheese, with certain fatty acids, notably caproic acid (C6:0), considered as an index for measuring cheese lipolysis (Güler, 2005; Durna, 2019). Atasoy and Türkoğlu (2008) found that the volatile free fatty acid (C4:0–C10:0) content of raw milk cheese was significantly higher than that of cheeses heat-treated at 65 °C and 72 °C, also reported that there were differences in butyric, caproic, caprylic and capric acid levels between raw milk cheese and low and high heat treated cheeses on the 90th day. In a study evaluating lipolysis development in 11 different cheese varieties sold in Türkiye, Hayaloglu and Karabulut (2013) determined the profiles of free fatty acids in cheese samples. They reported that, except for Çanak cheese, concentrations of short-chain fatty acids (C4 and C6 free fatty acids) were similar among all cheeses.

The highest levels of caproic (1.6015%), caprylic (2.5909%), capric (10.3497%), and undecanoic (0.0721%) acids in the cheese samples were detected in sample number 6, indicating that this sample may be produced from either sheep or goat milk. The type of milk has a significant impact on the formation of free fatty acids, with sheep and goat milk fats containing higher amounts of caproic, caprylic, and capric acids. These fatty acids contribute to a pleasant bitterness and a peppery taste in cheeses (Abd El-Salam et al., 1987; Durna, 2019).

Short and medium-chain free fatty acids have relatively low detection thresholds and directly contribute to cheese aroma (Güler, 2005). The short and medium-chain fatty acids identified in Köy cheese constitute 9% of the total free fatty acids (Figure 1). Atasoy and Türkoğlu (2008) determined these rates in Urfa cheese as 7-8% and 20-23%, respectively. It is thought that these values are higher than ours due to the use of starter culture and the maturation period. Georgala et al. (2005) detected the percentages of short-chain fatty acids in total free fatty acids as 44% and 33% in samples of Feta cheese made with traditional rennet from sheep and goat abomasum (Sample A) and in samples made by blending this rennet with a certain amount of commercial rennet made from calf abomasum (Sample B) after a 120-day storage period, respectively. Enzymes responsible for lipolysis in cheese include lipoprotein lipase naturally found in raw milk, pregastric esterase in cheeses made with rennet, and those originating from the starter or non-starter microbiota (Georgala et al., 2005; Erdoğan et al., 2012; Thierry et al., 2017). It is thought that this difference with Georgala et al. (2005) is mainly due to the ripening time and the lipolytic activity of rennet.

In the cheese samples, palmitic acid (36.3563 ± 5.4150%), oleic acid (22.0077 ± 5.3136%), myristic acid (12.3104 ± 2.1054%), and stearic acid (11.9837 ± 3.1320%) were prominent in terms of long-chain fatty acids. Güler (2005) identified the dominant free fatty acids in Kaşar cheese as palmitic, oleic, myristic, and stearic acids, while Hayaloglu and Karabulut (2013) reported the prominent free fatty acids in 11 different cheese varieties as palmitic (C16) and oleic (C18:1) acids. Şengül et al. (2011) reported average palmitic acid content as 24.639%, oleic acid as 9.384%, myristic acid as 7.346%, and stearic acid as 2.028%. These values appear to be lower than the results the present study revealed. It is thought that this may be due to the use of milk from different animal breeds in cheese production, the cheeses being at different maturity levels or different ripening conditions (Temiz et al., 2009; Hayaloglu & Karabulut, 2013). In addition, Karın

Kaymağı cheese is a ripened cheese and during this process, the breakdown of fatty acids into their sub-components may occur. Hayaloglu and Karabulut (2013) reported significant differences among samples for other free fatty acids, including C8 and C18:2, in various cheese varieties. The concentration of salt in the cheese also influences the formation of free fatty acids, with lipolysis levels decreasing as salt concentration increases. This effect is attributed to the inhibitory impact of salt on bacterial growth and enzymatic activity (Abd El-Salam et al., 1987; Katsiari et al., 2000).

Sample 3 was remarkable for its high levels of palmitic and myristic acids, sample 8 for oleic acid, and sample 7 for stearic acid. These variations could be attributed to the use of different animal milks or their blends in cheese production. The variation in fatty acid profile of milk depends on various factors, including dietary fatty acids, the species of the animal, season, lactation stage, lactation number, age, and the biohydrogenation process observed in the rumen (Djordjevic et al., 2019). Durna (2019) reported the descending order of palmitic acid levels as cow-goat-sheep milk, myristic acid levels as goat-cow-sheep milk, oleic acid levels as goat-sheep-cow milk, and stearic acid levels as sheep-cow-goat milk.

Pentadecanoic acid was determined to have an average ratio of 1.6271 ± 0.3116%, palmitoleic acid at 1.3482 ± 0.5586%, and linoleic acid at 1.7483 ± 0.3858% in the cheese samples. In addition, 12 minor long-chain free fatty acids were identified. Long-chain fatty acids constituted 91% of the total free fatty acids. Atasoy and Türkoğlu (2008) determined this rate as 70-72% in Urfa cheese. In Van Otlu cheese, although most short-chain fatty acids (C3, C4, C5, C6, C7, and C8) had a significant increase during maturation, their proportions in both fresh and mature samples were lower than those of medium and long-chain fatty acids (Ocak et al., 2015). While long-chain free fatty acids make up a significant proportion of the total free fatty acids, they have no substantial impact on cheese aroma (Attaie & Richter, 1996; Freitas & Malcata, 1998).

4. Conclusion

In conclusion; with this study, the free fatty acid profile of milk fat in Köy cheese, which increases production and consumption day by day, was determined and distribution rates were determined according to chain lengths. It was observed that the ratio of short and medium chain fatty acids, which have a significant effect on the taste and aroma of cheese, in total free fatty acids was quite low compared to cheeses ripened or produced using starter culture. In general, not many studies on Köy cheese have been found in the research. This study is important in terms of revealing the free fatty acid composition of Köy cheese and providing data for future studies.

Conflict of Interest

The author has no conflict of interest to declare.

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