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Fatty Acid Analysis of Soft Cheese Using Gas Chromatography as A Prospective Method for the Detection of Cheese Adulterations with Vegetable Oil

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Abstract

This study aimed to detect the adulteration of different types of soft cheese with vegetable oils. Therefore, a total of 36 samples (6 for each) of Talaga, Kiri spread, Baramili spread, Baramili, and white and yellow feta cheese were collected from local markets in Damietta governorate, Egypt. The samples were evaluated for their chemical composition, assessed their fatty acid profile using gas chromatography, and estimated their peroxide value. The chemical evaluation revealed that all Talaga, Baramili, white and yellow feta cheese samples were matching the Egyptian standards while all Kiri and Baramili spread samples didn't match. By comparing the fatty acid profile of the control cow milk with that of the examined cheese samples, there were differences in certain FAs values demonstrating the fat impurity in the cheese samples. 83% of Talaga samples and all other cheese types were adulterated with vegetable oils. The saturated FA decreased, unsaturated FA increased and the peroxide value elevated, conversely from the situation in control milk, that happened in 50% of Talaga and white feta, and 100% of other types. In conclusion, FA profile estimation could be an efficient detection method for vegetable oil adulteration in dairy products.

KEYWORDS
Soft cheese, Vegetable oils, Fatty acid profile, Gas chromatography, Peroxide value

INTRODUCTION

Since more than 8000 years ago, dairy products, especially cheese, have been a main human daily food because they contain almost all the nutrients the human body needs. Egyptians are accustomed to hard and soft cheeses but soft cheeses are more common and produced from cow or buffalo milk or a combination of them (Nasr *et al*., 2022).

Due to rising demands, milk fat has become a target of illegal substitution with cheaper non-milk fats of plant and animal origin, which is not only an economic scam but may also threaten human health. There are several vegetable oils used in the production of imitation cheese or vegetable oil-based cheese. The most commonly used oil is Palm oil, which has expanded dramatically in recent years (Abd El-Aziz *et al*., 2013). Palm oil is an oil derived from palm trees growing in tropical areas and contains 50% palmitic acid, 40% oleic acid, and 10% linoleic acid. Palmitic acid is the main saturated fatty acid (SFA) naturally found in animal and vegetable fats, which raises LDL- cholesterol and pro-inflammatory factors and increases the incidence of cardiovascular disease besides a negative impact on insulin resistance. Furthermore, products containing vegetable oils are liable to be oxidized and rancid, producing unwanted chemicals such as aldehydes and peroxides, and partially hydrogenated vegetable oils contain trans-FA which their consumption on long-term increases the risk of heart disease, cancer, atherosclerosis, obesity, hypercholesteremia, and Alzheimer (El-Nabawy *et al*., 2023).

Numerous techniques for detecting milk fat adulteration have been developed including Gas chromatograph and HPLC. The most prevalent method is to determine the FA content of the suspected product depending on the fact that milk fat has short-chain FAs, but vegetable oils contain medium to long-chain FA in addition to the type of the FA and the proportion of butyric and oleic acids (Lotfy *et al*., 2023). Therefore, the aim of this study is to detect the adulteration of some types of soft cheese with vegetable oils depending on their chemical analysis and fatty acid profile assessment.

MATERIALS AND METHODS

A total of 36 samples of soft cheese samples, 6 for each Talaga, Kiri spread, Baramili spread, Baramili, white and yellow feta, were randomly collected in September 2022 from Damietta governorate, Egypt, and transferred to Food laboratories in National Research Center at Dokki for analysis.

Evaluation of chemical composition

The percentage of moisture, total solids, fat, Fat/TS, protein, carbohydrates, and ash were determined according to AOAC (2013).

Fatty acids profiles

Preparation of samples according to AOAC (2005)

The methyl ester of fatty acid was produced by mixing 30 ml of sulphuric acid/methanol combination (1:24 v/v) with 0.2 g of the cheese sample, then heated for 3 hours and extracted twice with petroleum ether (40-60°C), and washed several times with

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distilled water until the washings were phenolphthalein neutral. The layers of fatty acids methyl esters were dried and filtered over anhydrous sodium sulphate.

Identification and determination of fatty acids (Wirasnita et al., 2013)

The methyl ester of fatty acid standard substances was examined using a Perkin Elmer Auto System XL gas chromatography (GC) model 7890B.

Peroxide value was determined according to AOAC (2005), in brief, 5 g of cheese was dissolved in 30 ml of glacial acetic acid/chloroform (3:2) and 1 mL of saturated potassium iodide and 40 ml of distilled water were added and titrated with 0.1 N $Na_2S_2O_3$ until the yellow tint was gone. 0.5 ml of 1% starch solution was added, and the titration continued until the blue hue disappeared. PV was determined using the following formula: Peroxide value (mEq/kg sample) = S x N x l000/W

Where: S= ml of $Na_2S_2O_3$ solution required for test sample, N= = normality of $Na_2S_2O_3$ solution, W= weight of the sample

Table 1. Chemical composition of examined cheese samples in percentage.

		Moisture	TS	Protein	Fat	Carbohydrates	Fat\TS	Ash	Egyptian standards
Talaga	S1	58.7	41.3	8.4	23.5	6.2	56.9	3.2	Confirmed
	S2	58.3	41.7	8.4	28	2.2	67.1	3.1	
	S3	58.9	41	8.6	25	4.8	60.9	2.6	
	S4	52.5	47.5	9.2	26	9	54.7	3.4	
	S5	55.7	44.3	9.2	28	3.5	63.2	3.6	
	S6	52.5	47.5	9.6	26.5	8.4	55.8	2.98	
	Mean±SE	56.1±1.24	43.9±1.24	8.89±0.2	26.2±0.72	5.7±1.10	59.8±1.98	3.14 + 0.14	
Kiri spread	S1	61.2	38.76	4.3	21	10.3	54.7	3.1	Not Confirmed
	S2	62.1	37.9	3.6	22	9.3	58	2.9	
	S3	61.8	38.21	4	21	10.3	54.9	2.9	
	S4	62.8	37.2	3.2	23	7.8	61.8	3.1	
	S5	61.6	38.4	3.2	22	10.5	57.3	2.8	
	S6	63.2	36.84	3.4	22.5	8.4	61.1	2.6	
	Mean±SE	62.1±0.29	37.9±0.29	3.6±0.19	21.9±0.33	9.5±0.46	57.9±1.22	2.9±0.09	
Baramili spread	S1	58.65	41.35	10.6	23	2.89	54.17	4.86	Not Confirmed
	S2	61.32	38.68	9.43	22	2.6	58.04	4.65	
	S3	60.44	39.56	8.67	24	2	54.95	4.89	
	S4	59.11	40.89	8.9	23.5	3.94	61.82	4.55	
	S5	58.85	41.15	9.46	22	4.9	57.29	4.79	
	S6	60.8	39.2	9.14	23	2.45	61.06	4.61	
	Mean±SE	59.9±0.46	40.1±0.46	9.4±0.28	22.9±0.33	3.1±0.44	57.1±1.013	4.7±0.06	
Baramili	S1	49.56	50.44	4.33	33	10.23	65.42	2.88	Confirmed
	S2	51	49	4.57	30.5	11.23	62.24	2.7	
	S3	49.98	50.02	4.6	32	10.53	63.97	2.89	
	S4	50.72	49.28	4.51	31	10.84	62.9	2.93	
	S5	50.22	49.78	4.65	33.5	8.66	67.29	2.97	
	S6	49.32	50.68	4.62	33	10.12	65.11	2.94	
	Mean±SE	50.1±0.3	49.9±0.27	4. 6±0.05	32.2±0.49	10.3±0.36	64.5±0.75	2.9±0.04	
White feta	S1	56.61	43.39	5.85	28	6.6	64.53	2.94	Confirmed
	S2	57.21	42.79	5.83	30.5	3.7	71.27	2.76	
	S3	55	45	3.6	33	5.85	73.33	2.55	
	S4	56.56	43.44	5.7	28	6.77	64.45	2.97	
	S5	51.52	48.48	3.85	38.5	3.67	79.41	2.46	
	S6	53.98	46.02	3.39	32	8.58	69.53	2.05	
	Mean±SE	55.2±0.87	44.9±0.87	4.7±0.49	31.7±1.60	5.9±0.78	70.4±2.32	2.6±0.141	
Yellow feta	S1	55	45	5.1	28	8.2	62.22	3.7	Confirmed
	S2	51.72	48.28	4.92	30	10.13	62.13	3.23	
	S3	54.8	45.2	5.3	27	9.35	59.73	3.55	
	S4	52.5	47.5	5	31	8.02	65.26	3.48	
	S5	51.98	48.02	5.07	31	8.35	64.55	3.6	
	S6	55.72	44.28	5	27	8.87	60.97	3.41	
	Mean±SE	53.6±0.71	44.9±0.87	5.1±0.05	29.0±0.77	8.8±0.33	62.5±0.86	3.5±0.07	

RESULTS AND DISCUSSION

Chemical composition of the examined cheese samples

The chemical evaluation of the cheese samples is revealed in Table 1. The Egyptian Organization for Standards and Quality (ES:1008-1/2005) stated that the moisture content in Talaga cheese must be not more than 62% and Fat\TS% not less than 40% respectively. Consequently, all examined Talaga cheese samples confirmed the legal requirements. These results matched the finding showed by Saleh *et al.* (2020) and Hamad and El-din (2021), while lower percentage were recorded by Nasr *et al.* (2022) and Lotfy *et al.* (2023).

None of the examined Kiri and Baramili spread samples confirmed the Egyptian Organization for Standards and Quality (ES:1132/2013) which confirmed that the moisture% must be not more than 47% and Fat\TS% not less than 65%. Nearly similar results were documented by Ghita *et al.* (2017) and Abdel-Ghany *et al.* (2020). Regarding the Baramili cheese, all samples were validating the Egyptian Organization for Standards and Quality (ES: 8390/ 2020) which mentioned that the Fat\TS% must not be lower than 45% and the moisture% not higher than 48%. Nearly similar findings recorded by Jaoude *et al.* (2010). Considering white and yellow Feta cheese results, none of the examined samples failed to confirm the Egyptian Organization for Standards and Quality (ES: 1008-12/ 2020) that stated that the moisture% should not exceed 62% and Fat\TS% not below 40%. These results closely accepted with those recorded by Jalili (2016).

Fatty acids profile of control fresh raw cow milk sample

A cow milk sample was collected in September 2022 and analyzed using Gas chromatography. The findings represented in Tables 2.1 and 2.2, showed that it contains short chain FAs including Butyric acid (C4:0), Caprylic acid (C8:0) and Caproic acid (C6:0), medium chain FAs which are capric (C10:0) and lauric (C12:0) and long chain FAs which ranged from C14-C18. The most abundant FAs were the palmitic acid (16:0), Myristic acid (14:0), Oleic acid (C18:1n9c), and stearic acid (C18:0). These results agreed with findings obtained by Abbas *et al.* (2017) and Salem *et al.* (2019). Dawod *et al.* (2020) and Hassan *et al.* (2020) found that milks from cows feeding ration contains whole linseed or linseed oil, or soybean oil, decrease the concentrations of short and medium chain FA and SFA, and increased the concentrations of long chain FA and unsaturated FA (USFA) specially α linolenic acid. Abd El-Aziz *et al.* (2013) reported marked increase in the Palmitic acid (C16:0) in dairy cow's milk fed on diets containing palm oil. El-Tarabany *et al.* (2018) examined milks from different breeds of

Table 2.1. Fatty acid profile and peroxide valuesof a control cow milk sample and the examined cheese samples.

	Cow milk	Talaga							Kiri spread							Baramili spread						
		S1	S2	S3	S4	S5	S6		S1	S2	S3	S4	S5	S6		S1	S2	S3	S4	S5	S6	
Butyric acid (C4:0)	2.4	1.1	3.9	3.9	1.4	0.5	1.2		0.7	1.0		0.9	0.6	1.2		2.5	2.6	2.6	3.0	2.6	2.5	
Caproic acid (C6:0)	4.9	0.5	1.0	0.6			2.9		0.9	0.9	0.5	2.2				1.2		0.9			1.5	
Caprylic acid (C8:0)	1.7	4.2	3.7	3.4		0.1	0.9		0.8	0.8	0.7	1.3	1.1	1.5		0.6	0.8	0.7	0.8	0.7	0.8	
Capric acid (C10:0)	2.8	3.6	3.7	3.0		0.1	0.7		2.0	2.1	2.7	1.3	1.2	1.5		1.3	1.8	1.5	1.6	1.7	1.6	
Caproleic acid (C10:1)											0.3											
Lauric acid (C12:0)	3.9	37.1	35.8	33.2	0.9	0.6			2.6	2.6	3.0	17.8	17.3	18.7		3.5	3.3	3.4	3.1	4.5	4.5	
Myristic acid (C14:0)	11.9	11.8	13.0	12.8	2.6	2.0	1.3		10.1	10.0	10.0	6.3	6.5	6.7		8.3	9.3	9.0	9.5	9.5	8.8	
Myristoleic acid (14.1)	0.9	0.9							0.5	0.8	0.8	0.4				1.0	1.0	0.9	1.1	1.2	0.9	
Myristolinoleic (C14:2)	0.9								0.6	0.7	0.3					0.4		0.4		0.4		
Pentadecanoic acid (C15:0)	2.7								1.3	1.3	1.3					0.5		0.4		0.2	0.9	
Cis-10-Pentadecenoic (C15:1)									0.4	0.4	0.3											
Palmitic acid (C16:0)	30.6	11.2	11.6	13.3	48.9	61.1	41.9		33.5	33.3	33.7	29.0	31.1	30.1		33.0	34.9	34.1	34.0	32.9	33.3	
Palmitoleic acid (C16:1)	2.4	0.2			2.1	2.2	0.9		1.6	1.7	1.6	1.0				1.1	1.6		1.5	1.5	1.4	
Palmitoleic acid (C16:1, n7)	3.3								0.6	0.6	0.5					0.3		0.4				
Heptadecanoic acid (C17:0)									0.7	0.7	0.5					0.6	0.5	0.7	0.7	0.7	0.6	
Cis-10-Heptadecanoic acid (C17:1)	1.6								0.8		0.8											
Stearic acid (C18:0)	9.1	16.4	16.2	17.8	3.7	4.1	5.3		11.9	11.9	12.0	12.7	14.1	13.0		11.9	12.1	12.5	12.9	12.4	11.6	
Oleic acid (C18:1n9c)	20.1	10.8	10.6	11.4	31.5	23.7	36.0		27.7	28.0	26.9	22.3	23.8	23.1		27.9	28.1	28.5	28.0	28.1	27.1	
Linoleic acid (C18:2n6c)	1.1	0.4	0.4	0.5	7.7	5.8	9.1		2.6	2.5	2.4	4.2	4.4	4.4		4.5	4.5	3.2	4.0	3.0	4.6	
α- Linolenic acid (C18:3n3)	0.1				0.9																	
Arachidic acid (C20:0)		1.9		0.2	0.2				0.9	0.9	0.9	0.6										
Saturated fatty acids (SFA)	69.8	87.7	89.0	88.2	57.7	68.3	54.1		65.3	65.4	65.4	72.1	71.8	72.6		63.3	65.3	65.7	65.5	65.2	66.1	
Unsaturated fatty acids (UFA	30.3	12.3	11.0	11.8	42.3	31.7	45.9		34.7	34.5	34.0	28.0	28.2	27.4		35.2	35.2	33.4	34.6	34.2	33.9	
Peroxide value	1.2	1.0	0.9	1.1	2.6	1.9	2.2	1.6*	1.2	1.1	1.8	1.0	1.3	1.2	1.3*	1.3	1.1	1.5	1.5	1.9	1.9	1.5*

*Means of peroxide values of examined cheese samples (mEq/kg)

dairy cows and found difference in the FA profile based on the breed type. Moreover, the seasonal variations and technical concerns may influence the FA profile (Foltys and Kirchnerová, 2012).

Fatty acid profile of the examined cheese samples

The fatty acid profile obtained by GC fraction of each examined cheese samples was detailly revealed in Tables 2.1 and 2.2. In comparing with the control cow milk sample, the Talaga cheese results showed higher content of palmitic, oleic and linoleic acid in three samples out of the six examined samples (50%). Moreover, the Arachidic acid (C20:0), which is not present normally in milk fat and present in vegetable oils such as palm oil, was detected in 3 Talaga cheese samples, two of them were other than the previously mentioned suspected 3 samples. There was also a marked decrease in myristic acid content in 33.3% of Talaga cheese samples that indicates adulteration with vegetable oil which in turn dilutes the concentration of myristic acid in milk fat. Therefore, out of the examined 6 samples there were 5 samples (83%) containing vegetable oils. Those investigation are in agreement with the finding recorded by Kesenkaş *et al*. (2009) and Abd El-Aziz *et al*. (2013) who found that vegetable oil cheeses characterized by significant increase in palmitic, stearic, oleic and linolic acids. Mancini *et al*. (2015) and Montoya *et al*. (2014) recorded that the Arachidic FA was estimated in the FA profile of the palm oil. Abd El-Aziz *et al*. (2013) reported that the addition of palm oil to milk fat cause dilution in myristic acid concentration by the same degree of addition. Kim *et al*. (2016) mentioned that the linoleic acid content in vegetable oils is much higher than in milk fat, so even if the adulteration occurred with small amount of vegetable oil, it can be detected by linolic acid content.

All Kiri spread and Baramili spread cheese samples showed increase in the palmitic, stearic, oleic and linoleic acids, and decrease in butyric and myristic acids. There were marked increase in lauric acid in 3 samples presented by 17.84, 17.28 and 18.74% than in control milk. Moreover, the Arachidic acid and Heptadecanoic acid, which were absent in the control milk FA profile, were present in 4 and 3 samples out of the 6 examined Kiri samples, respectively. The presence of arachidic acid proved the adulteration of Kiri samples with palm oil which matching the results recorded by Mancini *et al*. (2015) and Montoya *et al*. (2014). Pospišil *et al*. (2007) mentioned that the heptadecanoic acid is found in vegetable oils. These finding also matched that stated by El-Nabawy *et al*. (2023) who found marked decrease in short-chain FA (C4-C10) and increase in palmitic and stearic in imitation palm oil cheeses.

All Baramili cheese samples had palmitic acid content around that in the control milk, while there were marked elevation in oleic and linoleic acid content and decrease in myristic and stearic acid. Maybe there were adulteration with vegetable oils but in

Table 2.2. Fatty acid profile and peroxide valuesof a control cow milk sample and the examined cheese samples.

	Baramili							White feta							Yellow feta						
	S1	S2	S3	S4	S5	S6		S1	S2	S3	S4	S5	S6		S1	S2	S3	S4	S5	S6	
Butyric acid (C4:0)	2.4	2.1	1.9	1.6	2.5	2.0		1.0	1.9	2.0	2.5	2.2	0.4		0.3		0.3	0.2		0.9	
Caproic acid (C6:0)	0.8	0.8	1.1	0.9	0.9	0.9		1.3	1.1	1.7	1.4	1.0	0.2		0.6	0.9	0.5			0.7	
Caprylic acid (C8:0)	1.7	2.3	1.9	2.0	1.9	2.4		1.9	2.6	1.7	2.1	1.1	0.1		0.1	0.3		0.1		0.3	
Capric acid (C10:0)	1.7	1.5	1.9	1.7	1.7	1.8		1.2	1.8	1.5	2.0	1.1	0.1		0.1		0.2		0.4	0.4	
Caproleic acid (C10:1)																					
Lauric acid (C12:0)	17.0	18.0	18.5	18.6	17.5	18.5		15.9	17.8	16.0	19.6	0.9	0.9		0.6	0.5	0.7	1.1	1.0	0.8	
Myristic acid (C14:0)	6.0	5.2	5.4	5.4	5.4	5.8		6.3	5.7	5.3	7.9	2.3	1.5		1.7	2.0	2.0	1.2	2.5	1.9	
Myristoleic acid (14.1)																					
Myristolinoleic (C14:2)																					
Pentadecanoic acid (C15:0)																					
Cis-10-Pentadecenoic (C15:1)																					
Palmitic acid (C16:0)	30.0	29.7	29.1	28.6	29.6	29.6		28.7	30.1	30.8	32.5	43.2	46.2		47.7	48.2	49.0	48.0	48.4	48.5	
Palmitoleic acid (C16:1)	0.2		0.5	0.7				1.4	0.7	0.6	0.7	0.2	0.2		0.1	0.2	0.2	0.2	0.2	0.2	
Palmitoleic acid (C16:1, n7)																					
Heptadecanoic acid (C17:0)																					
Cis-10-Heptadecanoic acid (C17:1)																					
Stearic acid (C18:0)	5.2	6.6	7.7	6.4	7.7	7.7		4.6	6.8	5.9	4.6	4.4	4.5		3.7	4.0	4.5	4.0	4.2	4.3	
Oleic acid (C18:1n9c)	25.3	23.6	24.0	23.9	25.3	25.1		30.9	25.9	28.7	22.7	39.4	36.4		37.4	36.6	35.8	37.2	35.3	33.9	
Linoleic acid (C18:2n6c)	9.6	10.3	7.7	10.4	7.7	6.3		6.9	5.5	5.0	4.2	9.6	9.7		7.7	7.2	7.0	8.0	8.0	8.2	
α- Linolenic acid (C18:3n3)																					
Arachidic acid (C20:0)																					
Saturated fatty acids (SFA)	64.8	66.2	67.4	65.1	67.0	68.6		60.7	67.6	64.8	72.4	50.8	53.7		54.8	55.9	57.1	54.6	56.5	57.7	
Unsaturated fatty acids (UFA	35.2	33.8	32.2	34.9	33.0	31.4		39.1	32.1	34.2	27.6	49.2	46.3		45.2	44.0	42.9	45.4	43.5	42.3	
Peroxide value	1.3	1.4	1.4	1.4	0.9	1.1	1.2*	1.9	1.5	2.0	1.0	2.8	2.4	1.93*	2.1	2.0	1.9	2.1	2.2	2.1	2.1*

low concentration that was not enough to change the palmitic acid values but succeeded in altering the myristic, oleic and linoleic acid concentrations. Uzunov *et al*. (2018) reported that myristic, palmitic, oleic, stearic, linoleic acids are biomarkers for milk fat adulteration with vegetable oils.

All white and yellow feta cheese samples exposed increased oleic and linoleic acid values. The palmitic acid increased in all yellow feta samples but only in 2 white feta samples. All feta samples also confirmed decrease in the myristic and stearic acids. Therefore, all examined white and yellow feta cheese samples contained vegetable oils. These results agreed with the outcomes of studies done by Kesenkaş *et al*. (2009) and El-Nabawy *et al*. (2023)

Saturated and unsaturated FA content and Peroxide values of the examined cheese samples

The SFA and USFA contents of the control milk sample were 69.83 and 30.26%, respectively, and its peroxide value was 1.2 mEq/kg (Table 2). Nearly alike results were obtained with Kiri, Baramili spread and Baramili cheese samples.

Three samples out of 6 (50%) of the examined Talaga and white Feta cheese samples in addition to all yellow Feta samples contained lower SFA and higher USFA which indicate the presence of vegetable oils. The peroxide values of these samples were higher than the control milk sample suggesting the susceptibility for occurrence of rancidity due to oxidation of the USFA which results in hazardous effects on the consumer's health. The means of the peroxide values of the examined cheese samples showed that the highest peroxide value mean was referred to yellow Feta followed by white Feta, Talaga, Baramili spread, Kiri spread then Baramili, in descending order (Tables 2.1 and 2.2). Consequently, yellow Feta cheese was not only the most adulterated cheese with vegetable oils, but also the one acquired deterioration and rancidity. This finding agreed with Abd El-Gawad *et al*. (2015) who mentioned that cheese with vegetable oils specially palm oil had significant increase in peroxide values. Kesenkaş *et al*. (2009) stated that the SFA content considered as a good indicator of dairy products adulteration with vegetable oils, and Uzunov *et al*. (2018) revealed that the degree of change in the SFA and USFA depend on the type and amount of the added vegetable oil.

CONCLUSION

The cheese industry has recently become uncontrolled, and those in command of this industry have increasingly tended to adulterate milk fat with vegetable oils for economic and profitability reasons, which in turn would affect the consumer's health badly. There are many approaches for detection of such adulteration including chemical and chromatographic methods. Fatty acids profile estimation could be used as useful and efficient detection method for adulteration of dairy products with vegetable oils.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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