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Evaluating Tallaga Cheese Chemically and Microbiologically with Focusing on its Fraud Depending on Chromatographic Analysis

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Abstract

The current study was deliberated to evaluate safety and quality of small scale Tallaga cheese sold in Egyptian markets, as well as detecting its fraud. Fifty samples were examined chemically and microbiologically with special reference to fatty acid profile and presence of inhibitory substances. Results showed that the mean values of fat, T.S, moisture and fat/T.S % were 38.13, 47.17, 52.80 and 69.24%, respectively. Samples from twelve small scale plants were examined for fatty acid profile, the majority of examined samples lack butyric fatty acid in their profile that characterizes milk fat. A high content of palmitic acid reached to 48% found in some market samples, others had a high percent of unsaturated fatty acids as compared to control treatments prepared in lab which indicated the skimming of milk fat with addition of vegetable oils. The ratio of n-6/n-3 in most examined samples (83.33%) exceeds the permitted limits. On detecting addition of inhibitory substance one sample was found to contain benzoate and other contains carbonate. Microbiological examination of samples revealed that mean values of total bacterial, yeast and mold counts were 70×10^7, 20×10^7 and 10×10^2 CFU/g, respectively. This study recommends application of restricted regulations on small factories, labeling of its products must be mandatory in order not to fraud consumers, as well as great attention must be paid for using fatty acid profile for detecting adulteration without depending only on determining fat percentage.

KEYWORDS
Benzoate, Fatty acid, Fraud, Gas chromatography, HPLC, Tallaga cheese

INTRODUCTION

The superior nutritious quality of milk and dairy products is mainly linked to the presence of versatile and essential constituents such as protein, fat, omega 3, 6 fatty acids, conjugated linoleic acid (CLA), as well as many vitamins and minerals (Markiewicz-Kęszycka *et al*., 2013). All these elements play an important role in human health through lowering the incidence and complexity of many diseases such as cancer, obesity, diabetes and cardiovascular diseases (Saad *et al*., 2023). Cheese is considered an important item in Egyptians diet; Tallaga cheese is the most widely consumed soft cheese in Egypt owing to its excellent flavor, spreadable smooth body and low salt concentration (El-Kholy *et al*., 2016). According to Egyptian standards ES 1008-5 (ES, 2005) Tallaga cheese is a white soft cheese that obtained after curdling of heat treated milk and preserved in brine solution inside refrigerator.

Milk fat is the most precious component in milk that significantly contributes to its price; it acts as a carrier of taste, aroma and excellent energy source. It is composed of saturated fatty acids (about 60-70% by weight), mono and polyunsaturated acids (25-30%). Amongst all natural fats, milk fat is the most complex as it comprises about 400-500 fatty acids (Hanuš *et al*., 2018). Since the year 2000, many research have opposed the theory that consumption of milk and dairy products would increase the synthesis of low density lipoproteins (LDL) and the risk of coronary diseases. Recently, many studies believed that lauric C12:0, myristic C14:0 and palmitic C16:0 saturated fatty acids only increase LDL blood concentration, but other saturated and unsaturated fatty acids present in milk counteract their effect by increasing high density lipoproteins (HDL) level (Markiewicz-Kęszycka *et al*., 2013). Consequently, milk fat has no adverse effect on health; moreover, many fatty acids in milk as conjugated linoleic acids (CLA), phytanic acid, and alpha-linolenic acid (ALA) have major health profits. Butyric acid helps in regeneration of intestinal cells and inhibition of the colon cancer (Kratz *et al*., 2013).

Egyptian organization for standardization stated that the only source of fat allowed to be found in Tallaga cheese is milk fat ES: 1008-5 (ES, 2005). Owing to its high economic value, milk fat has been a target of adulteration, some manufacturers replace dairy fat with lower cost oils or fats that may adversely affect human health without labeling the product. Soy oil, rapeseed oil, palm oil, or their hydrogenated forms may be used, palm oil is the most predominant vegetable oil used due to its low price. Owing to high amount of saturated fatty acids found in palm oil, it enhances the development of heart disease, besides it contains potentially harmful compounds as glycidols and chloropropanediols that generated during refining operations (Sharma *et al*., 2020). Fatty acid composition of milk fat has long been used to report adulteration with vegetable oils, owing to short-chain fatty acids found only in milk fat, GC and HPLC methods are the superior in detecting non-milk fats or oils in milk and dairy prod-

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ucts (El-Nabawy *et al*., 2023).

Removal of fat is not the only form of adulteration; other forms may be found as prolongation of shelf life by application of inhibitory substances. They mask the inferior hygienic quality under which the product had been manufactured. Sodium benzoate, benzoic acid, hydrogen peroxide, sorbic acid and others are an example of preservatives applied in cheese industry. Addition of preservatives must be controlled to limit their risk; excessive application has harmful effects like allergy, urticaria, asthma and dermatitis (Abusaloua *et al*., 2019).

Furthermore, it is important to evaluate the microbiological quality to ensure product safety and its compliance with standards and regulations. Presence of pathogens or their toxins has a great health hazard as well as spoilage microorganisms as yeast, mold and coliforms can cause undesirable flavors, discoloration and sliming that affect product quality (Hegab *et al*., 2021).

The objective of current study was to evaluate Tallaga cheese based on chemical and microbial analysis and reveal the fraud that may occur based on fatty acid profile and preservatives content. It is one of the fewest studies that detect Tallaga cheese adulteration.

MATERIALS AND METHODS

Sample collection

Fifty samples of small scale Tallaga cheese were collected randomly from different markets in Cairo and Giza Governorates, Egypt.

Laboratory manufacturing of Tallaga cheese

Tallaga cheese was prepared in lab following the rules of Egyptian Standards to compare its fatty acid profile (as control) with that of collected samples. Fresh buffalo's and cow's milk were obtained from Faculty of Agriculture, Cairo University, Egypt; fat % was measured before manufacturing using Gerber method (9% for buffalo's milk and 3.5% for cow's milk). Four treatments were made: buffalo's milk cheese with and without heat treatment (T1, T2) and cow's milk cheese with and without heat treatment (T3, T4), (unheated treatments were done as small scale samples were not labeled so some samples could be made from raw milk, as heat treatment may has an effect on fatty acid profile). Salt was added at a rate of 3% and then lab pasteurization was done in T1, T3 as stated by ES 1008-5 (ES, 2005), milk was heated at 80°C then suddenly cooled followed by raising temperature to 55ºC (temperature suitable for microbial rennet). Microbial rennet powder (Reniplus 2000 IMCU) was added according to the manufacturer instructions, calcium chloride (Sigma Chemical Company) was added at 0.02%. Coagulation takes about 3 hours, then drainage of whey takes about 12 hours. Finally, the cheese was cut into cubes and kept at refrigerator soaked in its whey.

Chemical analysis

Determination of fat, moisture and total solids according to AOAC (2019). Fat content was determined using Gerber method, while moisture and total solids by oven drying method.

Fatty acids profile analysis

Fatty acid profile was performed for 12 small scale processing plants (3 samples from each plant) and the 4 control treatments of prepared in lab.

F3 at extraction: was performed using chloroform and methanol mixture (in duplicate) (Nielsen, 2017).

Trans methylation process was done to form fatty acid methyl esters (FAMEs) by adding 1 ml of n-hexane to 15 mg of extracted fat followed by 1 mL of sodium methoxide (0.4 mol) (Zahran and Tawfeuk, 2019).

Separation and identification of fatty acids methyl esters

The FAMEs were separated and analyzed according to IUPAC (1992) using HP 6890 plus gas chromatography (Hewlett Packard, USA), a capillary column Supelco™ SP-2380 (60 m×0.25 mm×0.20 µm), (Sigma-Aldrich, USA) and Detector (FID). Carrier gas was helium at flow rate 1.2 mL min-1. FAMEs were identified by matching their relative and absolute retention times to those authentic standards of FAMEs (SupelcoTM 37component FAME mix). The fatty acid composition was reported as a relative percentage of the total peak area (Slover and Lanza, 1979).

Detection of inhibitory substances

Determination of benzoate and sorbate: using High-Performance Liquid Chromatography (HPLC) according to Pylypiw and Grether (2000). HPLC apparatus used with column (Supelcosil LC-18, 25 cm 34.6 mm, 5 mm, Supelco, Bellefonte, PA, USA). The temperature of the column was 20ºC.
Determination of nitrate and nitrite: applied by using methodology of Zhong *et al*. (2002) using high-performance ion chromatography (HPIC).
Detection of carbonate: by resolic acid test according to Gondim *et al*. (2021).

Microbiological examination: Preparation of samples, Total colony,

Total *Staphylococci*, Yeast, mold and Coliforms counts according to APHA (2004).

Statistical analysis

All results obtained were stated as mean ± standard error (SE). Data investigated by one-way ANOVA using SPSS 23 for windows where multiple comparisons of means were performed using least significant difference (LSD) at significance level ($P<0.05$).

RESULTS

Data represented in Table 1 illustrated that fat% in examined Tallaga cheese samples ranged between 22.40-55.0%. The total solid was ranged between 33.6-78.8%. Mean value of moisture % was 52.80. Fat/T.S % ranged between 46.66-75.82%.

Table 1. Compositional chemical analysis of collected Tallaga cheese samples (n=50).

Parameter	Min.	Max.	Mean±SE
Fat%	22.4	55	38.13±1.33
T.S%	33.6	78.8	47.17±1.10
Moisture%	21.1	66.4	52.80±1.10
Fat / T.S%	46.66	75.82	69.24±1.54

Results in Table 2 reported that laboratory manufactured Tallaga cheeses (control) were significantly higher in C4, C6 and C8 fatty acids compared to the majority of examined plants. Six plant samples contain no butyric acid in their profile and the

Table 2. Saturated fatty acids profile of Tallaga cheese samples.

	Surveyed market samples											Control samples			
	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8	Plant 9	Plant 10	Plant 11	Plant 12	T1	T2	T3	T4
C4:0	0.00^{a**}±0.00	0.00^{a} ±0.00	0.00^{a}±0.00	0.00^{a} ±0.00	0.69^{a}±0.01	1.93^{bcd}±0.04	0.00^{a} ±0.00	5.77^{ef}±0.10	0.00^{a} ±00	0.96^{ab}±0.05	0.49^{ab}±0.01	0.14^{a}±0.01	5.58^{ef}±0.02	6.63^{fg}±0.11	2.87^{bcd}±0.03	4.17^{f}±0.13
C6:0	0.00^{a}±0.00	0.00^{a}±0.00	0.05^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	2.89^{bcd}±0.03	0.35^{ab}±0.01	2.74^{bcd}±0.02	0.00^{a} ±0.00	1.18^{abc}±0.02	1.18^{abc}±0.11	0.11^{a}±0.01	2.45^{bcd}±0.03	3.34^{def}±0.05	1.89^{abc}±0.06	2.89^{bcd} ± 0.02
C8:0	0.00^{a}±0.00	0.00^{a}±0.00	0.94^{ab}±0.01	0.00^{a}±0.00	0.00^{a}±0.00	1.83^{abc}±0.01	2.36^{bcd}±0.03	1.34^{abc}±0.04	0.00^{a}±0.00	9.04^{de}±0.13	1.08^{abc}±0.10	0.09^{a}±0.01	1.25^{ab}±0.02	1.74^{abcd}±0.08	1.30^{ab}±0.01	1.70^{abc}±0.02
C10:0	0.00^{a}±0.00	0.00^{a}±0.00	0.95^{ab}±0.05	0.27^{a}±0.01	0.62^{a}±0.02	3.91^{cde}±0.02	1.68^{abc}±0.02	2.25^{bcd}±0.01	0.00^{a}±0.00	6.5^{de}±0.81	2.64^{bcd}±0.02	0.19^{a}±0.01	2.17^{bcd}±0.05	3.29^{def}±0.11	2.65^{abcd}±0.08	3.02^{bcd}±0.06
C12:0	0.28^{a}±0.01	0.00^{a}±0.00	11.15^{bc}±0.13	0.24^{a}±0.01	0.00^{a}±0.00	4.05^{cde}±0.15	14.05^{cde}±0.06	2.49^{bcd}±0.01	0.54^{a}±0.01	47.28^{fg}±0.50	3.34^{cde}±0.05	0.41^{a}±0.02	2.75^{bcd}±0.06	3.76^{def}±0.02	2.76^{bcd}±0.03	3.10^{bcd}±0.08
C13:0	0.00^{a}±0.00	0.00^{a}±00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.86^{ab}±0.01	0.00^{a}±0.00	0.00^{a}±0.00	0.34^{a}±0.02	0.00^{a}±0.00	0.42^{a}±0.01	0.56^{ab}±0.01	0.61^{ab}±0.01	0.42^{a}±0.01
C14:0	1.07^{a}±0.02	1.79^{abc}±0.08	4.90^{ab}±0.06	1.47^{a}±0.09	6.98^{abc}±0.07	11.25^{cde}±0.08	4.22^{bcd}±0.05	8.87^{cde}±0.06	1.83^{a}±0.07	10.95^{cde}±0.04	11.70^{de}±0.02	1.99^{a}±0.10	11.72^{de}±0.09	12.54^{de}±0.05	9.79^{cde}±0.02	10.06^{cde}±0.02
C15:0	0.00^{a}±0.00	0.00^{a}±0.00	0.50^{ab}±0.01	0.31^{a}±0.01	2.97^{bc}±0.04	1.50^{abc}±0.04	0.00^{a}±0.00	2.00^{bcd}±0.05	0.00^{a}±0.00	0.00^{a}±0.00	0.87^{ab}±0.03	0.08^{a}±0.00	1.46^{abc}±0.06	2.19^{bcd}±0.01	1.54^{abc}±0.03	1.43^{abc}±0.03
C16:0	42.47^{abc}±0.09	48.00^{cde}±0.15	35.34^{de}±0.06	46.33^{cde}±0.05	41.65^{cde}±0.07	29.65^{bcd}±0.08	33.59^{cde}±0.02	27.02^{bcd}±0.06	42.42^{abcd}±0.13	9.25^{ab}±0.12	29.16^{bcd}±0.06	43.33^{cde}±0.08	33.27^{cde}±0.05	29.38^{bcd}±0.07	24.18^{bcd}±0.10	23.92^{bcd}±0.03
C17:0	0.10^{a}±0.01	0.00^{a}±0.00	0.00^{a}±0.00	0.27^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	1.36^{ab}±0.06	0.00^{a}±0.00	0.00^{a}±0.00	0.83^{ab}±0.03	0.18^{a}±0.01	0.42^{a}±0.07	0.24^{a}±0.01	0.75^{ab}±0.01	0.83^{ab}±0.06
C18:0	5.29^{abcd}±0.06	6.56^{bcde}±0.80	11.02^{cde}±0.50	4.68^{abc}±0.20	7.21^{bcde}±0.26	8.40^{bcde}±0.55	6.84^{abcde}±0.34	10.02^{cde}±0.06	5.1^{abc}±0.33	7.73^{bcde}±0.05	11.98^{cde}±0.04	5.33^{abcde}±0.07	9.39^{cde}±0.63	7.93^{abcde}±0.20	12.99^{cde}±0.31	11.52^{cde}±0.71
C20:0	0.37^{a}±0.05	0.00^{a}±0.00	0.37^{a}±0.02	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.97^{ab}±0.08	0.44^{a}±0.03	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00

T1: heat treated buffalo's milk cheese, T2: raw milk buffalo's cheese, T3: heat treated cow's milk cheese, T4: raw milk cow's cheese. *a-j Values with different superscripts within the same raw are significantly ($P < 0.05$) different: Data represent Mean±Standard error.

other 6 plants had butyric acid in range of 0.14-1.93, only one plant (No. 8) had butyric acid (5.77) not significantly different with control cheese. The most plentiful saturated fatty acids in all cheeses were palmitic C16:0, stearic C18:0 and myristic C14:0 acids. Palmitic acid was found to be significantly higher in plants no. 1, 2, 4, 5, 9 and 12 (42.47, 48, 46.33, 41.65, 42.42 and 43.33%, respectively) when matched with control cheese as declared in Table 2. It was noted that plant no. 10 had highest incidence value of lauric acid C12:0 (47.28%) and the lowest value of palmitic acid (9.25%). Data presented in Table 3, showed that oleic acid C18:1n 9c was detected in very high ratio reached to 40% (in plant no. 1) and had a significant difference with normal value in control cheeses. Linoleic acid C18:2 was found to be higher in the majority of examined plants when matched with control cheese. Results in Table 4, reported that SFAs were variable, fluctuated from 49.58 (plant 1) to 92.89 (plant 10), while in control samples from 61.33- 71.60. MUFAs extend to 41.25 and the lowest value was 7.13 in collected samples. Moreover, PUFAs extended to 9.71. Control cheeses had n6/n3 ratio lower than recommended limit but significantly different from other collected samples that were ranged from 19.42 to 87.27, only 2 collected samples from different plants were within normal ranges that were 1.14 and 0.10, respectively. Trans fatty acids (TFA) was detected in 4 plant samples with ratio of 0.19, 0.32, 0.9 and 2.12, respectively.

Concerning presence of inhibitory substances one sample contained benzoate with a level of 87mg/kg. Sorbate was not detected above permissible limit (1000 mg/kg) in examined samples, the obtained results ranged from 357-869 mg/kg. Nitrate and nitrite were not detected in all examined samples. Carbonate was found in 2% of examined samples.

As shown in Table 5 total bacterial count ranged between 50×10-50×10^8 with average of 70×10^7 CFU/g. The minimum total *Staphylococcus* count was 10×10^2, while the maximum was 70×10^7 CFU/g with mean value of 60×10^6 CFU/g. Total yeast was detected in 44% of samples with count ranged from 30×10^2-20×10^8 with mean value 20×10^7CFU/g. Mold count ranged from 10×10-40×10^2 with mean value 10×10^2CFU/g. The mean value of coliforms was 10×104 and ranged from 20-10×10^5 CFU/g.

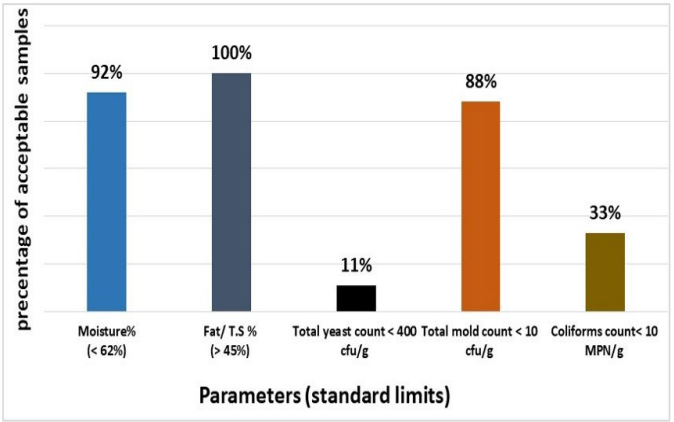

Fig. 1. Degree of acceptability of Tallaga cheese samples according to Egyptian standards (ES 1008-5, 2005).

DISCUSSION

The mean value of fat% in collected samples was 38.13 (Table 1), lower values obtained by Mohamed and El-Zubeir (2018). The average of total solid was 47.17 % that result was lower than that reported by Salih *et al.* (2012) and higher than those results obtained by Ibrahim *et al.* (2008). However nearly similar results reported by Mohamed and El-Zubeir (2018). Concerning moisture

Table 3. Unsaturated fatty acids profile of Tallaga cheese samples.

	Surveyed market samples												Control samples			
	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8	Plant 9	Plant 10	Plant 11	Plant 12	T1	T2	T3	T4
C10:1	0.00^{a}*±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.45^{b}±0.01	0.00^{a}±0.00	0.25^{c}±0.00	0.00^{a}±0.00	00^{a}±00	0.00^{a}±0.00	0.00^{a}±0.00	0.25^{c}±0.01	0.26^{c}±0.01	0.43^{b}±0.01	0.45^{b}±0.01
C14.1	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	1.00^{b}±0.01	0.00^{a}±0.00	1.06^{b}±0.02	0.00^{a}±0.00	0.00^{a}±0.00	1.00^{b}±0.06	0.09^{a}±0.01	0.95^{b}±0.02	1.71^{c}±0.08	0.92^{b}±0.01	0.97^{b}±0.06
C14.2	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	1.00^{b}±0.01	0.00^{a}±0.00	1.05^{b}±0.01	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	1.05^{b}±0.01	1.47^{b}±0.05	1.13^{b}±0.05	0.95^{b}±0.02
C15:1	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.37^{a,b}±0.01	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.81^{b}±0.01	0.00^{a}±0.00	0.00^{a}±0.00	1.37^{c}±0.01	0.18^{a,d}±0.01	0.69^{b}±0.02	1.46^{c}±0.09	0.61^{b,d}±0.07	0.61^{b,d}±0.01
C16:1, n9	0.16^{a,b}±0.01	0.00^{a}±0.00	0.18^{a,b}±0.01	0.00^{a}±0.00	0.00^{a}±0.00	1.07^{bc}±0.05	0.00^{a}±0.00	2.96^{d}±0.03	0.71^{a,b,e}±0.01	0.00^{a}±0.00	0.43$^{a,\ b}$±0.05	0.05^{a}±0.01	2.84^{d}±0.03	3.54^{d}±0.07	1.44^{c,e}±0.06	1.70^{c}±0.06
C16:1, n7	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	1.21^{b,e}±0.01	0.00^{a}±0.00	0.00^{a}±0.00	1.65^{b}±0.08	0.37^{a,d}±0.02	0.77^{c,d}±0.01	0.00^{a}±0.00	0.71^{d}±0.01	0.68^{d}±0.01
C17:1	0.41^{a}±0.03	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.67^{c}±0.01	0.07^{b}±0.00	0.45^{a}±0.04	0.00^{b}±0.00	0.94^{d}±0.02	0.33^{a}±0.01
C18:1n9c	40.33^{a}±0.12	38.72^{a,b}±0.08	29.13^{c,d}±0.06	37.86^{b,e}±0.08	33.03^{f}±0.07	27.38^{g,h}±0.04	30.30^{c}±0.09	23.63^{i}±0.07	39.00^{a,e,b} ±0.14	7.13^{j}±0.03	26.18^{g}±0.04	37.86^{b}±0.06	20.72^{k}±0.05	16.81^{L}±0.03	29.66^{c,m}±0.02	28.27^{d,h,m}±0.05
C18:1n9t	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.90^{b}±0.01	0.00^{a}±0.00	0.56^{c}±0.02	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00
C18:2n6c	8.86^{a}±0.07	4.93^{b,c}±0.02	5.46^{b,d}±0.01	8.19^{a,e}±0.05	6.84^{d,e}±0.07	3.69^{c,f}±0.01	6.57^{d}±0.05	2.31^{f,g}±0.02	9.60^{a}±0.07	0.01^{h}±0.00	1.71^{g}±0.11	8.53^{a}±0.06	0.89^{g,h}±0.02	2.44^{f,g}±0.09	1.98^{g}±0.05	1.43^{g,h}±0.03
C18:2n6t	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.47^{b}±0.01	0.17^{c}±0.01	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00
C18:3n3	0.13^{a}±0.01	0.14^{a}±0.01	0.15^{a}±0.01	0.16^{a}±0.00	0.14^{a}±0.01	0.19^{a}±0.00	0.12^{a}±0.01	2.02^{b}±0.03	0.11^{a}±0.01	0.10^{a}±0.00	0.31^{a}±0.01	0.15^{a}±0.01	0.55^{c}±0.04	0.75^{c}±0.01	0.85^{c}±0.02	1.55^{b}±0.01
C18:3n6t	0.19^{a}±0.02	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	1.09^{c}±0.05	0.15$^{a±}$0.01	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00
C20:1	0.35^{a}±0.02	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00

T1: heat treated buffalo's milk cheese, T2: raw milk buffalo's cheese, T3: heat treated cow's milk cheese, T4: raw milk cow's cheese. *a-m Values with different superscripts within the same raw are significantly (P < 0.05) different: Data represent Mean±Standard error.

Table 4. Fatty acids indices and nutritionally essential ratios of Tallaga cheese samples.

	Surveyed market samples												Control samples			
	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8	Plant 9	Plant 10	Plant 11	Plant 12	T1	T2	T3	T4
SFAs**	49.58$^{a*±}$0.02	56.35$^{b±}$0.03	65.22$^{c±}$0.10	53.57$^{d±}$0.02	60.12$^{e±}$0.06	65.41$^{c±}$0.05	63.09$^{f±}$0.13	64.72$^{c,f±}$0.03	49.89$^{a±}$0.12	92.89$^{g±}$0.10	64.58$^{c,f±}$0.04	52.29$^{d±}$0.09	70.88$^{h±}$0.08	71.60$^{h±}$0.06	61.33$^{e±}$0.05	63.06$^{f±}$0.02
MUFAs	41.25$^{a±}$0.02	38.72$^{b±}$0.01	29.31$^{c±}$0.04	38.23$^{b±}$0.05	33.03$^{d±}$0.02	29.90$^{c,e±}$0.03	30.30$^{c,e±}$0.02	29.92$^{c,e±}$0.02	39.7$^{a,b±}$0.04	7.13$^{f±}$0.16	31.30$^{e±}$0.10	38.62$^{b±}$0.05	26.67$^{g±}$0.02	23.78$^{h±}$0.03	34.71$^{i±}$0.08	33.01$^{d±}$0.06
PUFAs	8.99$^{a±}$0.03	5.07$^{b±}$0.07	5.61$^{b,c±}$0.12	8.35$^{a,d±}$0.05	6.98$^{c,d±}$0.13	4.88$^{b±}$0.05	6.69$^{c±}$0.04	5.38$^{b,c±}$0.06	9.71$^{a±}$0.08	0.11$^{e±}$0.02	2.02$^{f±}$0.05	8.68$^{a±}$0.06	2.49$^{f,g±}$0.14	4.49$^{b±}$0.05	3.96$^{b,g±}$0.02	3.93$^{b,g±}$0.04
UFA	50.24$^{a±}$0.06	43.79$^{b±}$0.04	34.92$^{c,d±}$0.03	46.58$^{e±}$0.03	40.01$^{f±}$0.05	34.78$^{c,d±}$0.08	36.99$^{g±}$0.06	35.30$^{c,g±}$0.13	49.42$^{a±}$0.11	7.24$^{h±}$0.06	33.32$^{d±}$0.09	47.30$^{e±}$0.14	29.16$^{i±}$0.09	28.27$^{i±}$0.19	38.67$^{f±}$0.06	36.94$^{g±}$0.03
TFA	0.19$^{a±}$0.01	0.00$^{a±}$0.00	0.00$^{a±}$0.00	0.00$^{a±}$0.00	0.00$^{a±}$0.00	0.00$^{a±}$0.00	0.00$^{a±}$0.00	0.00$^{a±}$0.00	0.90$^{b±}$0.03	0.00$^{a±}$0.00	2.12$^{c±}$0.01	0.32$^{a±}$0.01	0.00$^{a±}$0.00	0.00$^{a±}$0.00	0.00$^{a±}$0.00	0.00$^{a±}$0.00
PUFAs/SFAs	0.18$^{a±}$0.02	0.09$^{c±}$0.01	0.09$^{c±}$0.00	0.16$^{b±}$0.01	0.12$^{c±}$0.01	0.07$^{d±}$0.01	0.11$^{c±}$0.01	0.08$^{c±}$0.01	0.19$^{a±}$0.02	0.00$^{f±}$0.00	0.03$^{e±}$0.00	0.17$^{a±}$0.01	0.04$^{e±}$0.01	0.06$^{d,e±}$0.01	0.06$^{d,e±}$0.01	0.06$^{d,e±}$0.01
n-6/n-3	68.15 ^{a}±0.09	35.21 ^{b}±0.04	36.40 $^{b±}$0.01	51.19 $^{e±}$0.06	48.86 ^{c}±0.05	19.42 ^{d}±0.09	54.75 $^{e±}$0.06	1.14 ^{e}±0.01	87.27$^{f±}$0.08	0.10 $^{g±}$0.01	5.52$^{h±}$0.01	56.87$^{e±}$0.08	1.62$^{e±}$0.03	3.25$^{i±}$0.13	2.33$^{i±}$0.12	0.92$^{e±}$0.01

T1: heat treated buffalo's milk cheese, T2: raw milk buffalo's cheese, T3: heat treated cow's milk cheese, T4: raw milk cow's cheese. ** Saturated fatty acid (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), Total unsaturated fatty acids (UFA) = MUFA+PUFA, trans fatty acids(TFA), polyunsaturated fatty acids / saturated fatty acids (P/S) ratio, Omega 6 (n6) / Omega 3 (n3).*a-i Values with different superscripts within the same raw are significantly (P < 0.05) different: Data represent Mean±Standard error.

Table 5. Microbiological evaluation of collected Tallaga cheese samples (n=50).

Parameter	No. (%) of +ve samples	Minimum	Maximum	Mean±SE
Total bacterial count (CFU/g)	44 (88%)	50×10	50×10^8	$70\times10^7\pm20\times10^7$
Total *Staphylococci* count (CFU/g)	42 (84%)	10×10^2	70×10^7	$60\times10^6\pm20\times10^6$
Yeast count (CFU/g)	44 (88%)	30×10^2	20×10^8	$20\times10^7\pm6.4\times10^7$
Mold count (CFU/g)	6 (12%)	10×10	40×10^2	$10\times10^{2\pm}3\times10^2$
Coliforms count (MPN/g)	32 (64%)	20	10×10^5	$10\times10^4\pm3.8\times10^4$

percent reached up to 66.40 as maximum and 21.10 as minimum. Fat/T.S mean value was 69.24± 1.54%, lower findings reported by Ibrahim *et al.* (2008). Comparing the obtained results with Egyptian Standard (ES 1008-5, 2005), 92% of examined samples were accepted as their moisture did not exceed 62 % and 100% of samples comply with E.S as their Fat /DM % not less than 45% (Fig. 1).

According to carbon chain length saturated fatty acids of milk are classified into short, medium and long chain fatty acids. Short chain fatty acids constitute around 11% of total saturated fatty acids. They are the most characteristic fatty acids that found only in milk especially butyric acid that act as a marker of milk adulteration (Sharma *et al.*, 2020). Absence of butyric acid in most of plants profile and lower percent in the rest of plants as declared in Table 2 indicates that the majority of examined plant samples replace milk fat with other cheaper fat sources. Replacing the valuable components with cheaper ones to achieve financial gains is unethical action especially if this action adversely affects human health (Nicolaou *et al.*, 2011). Comparing cheese treat ments made in lab revealed that cheese made from buffalo's milk had a different fatty acid profile and significant higher butyric acid than cow's milk cheese; similar results were obtained by Hamad and Baiomy (2010). On the other hand, heat treatment reduces short chain volatile fatty acids, as demonstrated by Pestana *et al.* (2015) that pasteurization and commercial sterilization processes can reduce volatile fatty acid as butyric, caproic and caprylic acids.

High lauric acid in plant no. 10 may indicate the use of coconut oil as it contains 82% saturated fatty acid about half of them is lauric acid (DebMandal & Mandal, 2011). Palmitic acid was found to be significantly higher. These results found to be exceeded the results obtained by Mustafa *et al.* (2013) and lower than result reported by Abo-Elwafa *et al.* (2015) for small producers. The highest ratio of palmitic fatty acid may indicate the presence of palm oil; many researchers mentioned its harmful effect on human heath, Pascual *et al.* (2021) declared that palmitic fatty acids had a motivated effect and cause progression of the cancer metastasis. Palm oil is produced in large quantities and applied in dairy industry due to its low price (Gunstone *et al.*, 2007). Small scale cheese producers must mention the using of vegetable oils on the label as in case of analogue cheese or it will be considered fraud to the consumers.

Myristic acid C14:0 and stearic acid C18:0 in collected plant samples were lower than that reported by Mustafa *et al.* (2013); Abo-Elwafa *et al.* (2015). Oleic acid is the highest monounsaturated fatty acid in milk; it helps in prevention of cardiovascular insulin resistance and prevents atherosclerosis (Perdomo *et al.*, 2015). oleic acid was detected in very high ratio (Table 3) which was over than data detected by Mustafa *et al.* (2013); Barać *et al.* (2018) and had a significant difference with normal value in control cheeses. Linoleic acid C18:2 was found to be higher in the majority of examined plants when matched with control cheese, this result may reflect adulteration with vegetable oil(s) as they are rich with C18:2 than milk (Sharma *et al.*, 2020). PUFAs illustrated in table 4 exceed the ratio reported by Soyeurt *et al.* (2006) and lower than result of Abo-Elwafa *et al.* (2015). Nutritional guidelines recommendation that PUFA/SFA ratio preferable to be between 0.4-1 to conserve a good health of cardiovascular system and diminishes the oxidative stress (WHO, 2003). All market samples within the recommended range as declared in table (4). It's worth to mention that n6/n3 ratio more than 5 may cause health risk (Naydenova *et al.*, 2010). Collected samples were found to have n6/n3 ratio higher than recommended limit which considered unsafe. Trans fatty acids (TFA) was detected in 4 plant samples. Consuming trans fats has been linked to high incidence of cancer, stroke, several food allergies, infertility, blindness, liver diseases and increase the risk of cardiovascular disease and type 2 diabetes (Zaki *et al.*, 2021).

Small scale plants may use preservatives to cover unhygienic conditions, owing to the absence of control on their processing. Many methods can be used in order to control spoilage among them addition of preservatives, but they must be added within permissible limits. Benzoate is not allowed to be added in fresh cheeses according to Egyptian standards. It was reported by Amirpour *et al.* (2015) that benzoate level in different types of cheese ranged from 17-50 mg kg−1 that was lower than the obtained result, additionally Gul and Dervisoglu (2013) also determined lower ratio of benzoate. However, Salehi *et al.* (2017) detected higher benzoate amount in cheese samples. Benzoate may cause hypersensitivity, hyperactivity, metabolic acidosis and convulsions in sensitive persons. Carcinogen benzene may be produced when benzoate interact with ascorbic acid. Benzoate and sorbate can affect human lymphocytes causing chromosomal aberrations (Piper and piper, 2017). Sorbate not found in collected samples above permissible limit, higher findings obtained by Salehi *et al.* (2017), but lower results recorded by Özdemir *et al.* (2020). Nitrate and nitrite were not detected in all examined samples. Carbonate was found; it used to neutralize acidity and masking the unsanitary condition under which the product manufactured (Rahman *et al.*, 2018).

One of the most common methods to evaluate the overall quality of the product is the aerobic plate count. Result was higher than result reported by Eid *et al.* (2022) and slightly similar to Aiad (2013). *Staphylococci* can be transmitted through contact surfaces, surrounding environment, and food handlers (Hegab *et al.*, 2020). Lower total *Staphylococcus* count results obtained by EL Sayed *et al.* (2011). Egyptian organization for standardization clarified that yeast, mold and coliforms count must not exceed 400, 10 CFU/g and 10 MPN/g, respectively. It was found that 11, 88 and 33% of samples meet the limits of E.S (fig. 1). Yeasts and molds not only affect food spoilage but also affect consumer's health. Poisoning and even cancers may be induced by the mycotoxins (Eid *et al.*, 2022). Yeast result was in line with that obtained by ELbagory *et al.* (2014). Other authors reported lower results as Hassan *et al.* (2019); Eid *et al.* (2022). Mold count approximately similar to ELbagory *et al.* (2014). Coliforms act as indication of the fecal contamination; however, they simply destroyed by heat treatment so it is used as indicator of efficient heat treatment. Increase population of coliform has a great impact on the safety and quality of the product as it one of gas forming organisms that produce holes that render the final product unacceptable to consumers (Gran *et al.*, 2002). Our result agreed with that of Mohamed *et al.* (2022). Lower results reported by Hassan *et al.* (2019); Eid *et al.* (2022) while higher results stated by Ortolani *et al.* (2010).

CONCLUSION

This study concluded that small scale Tallaga cheese subjected to many types of fraud. Replacing milk fat with vegetable oils was detected extensively in our present study, the product

introduced to consumers without label as natural cheese without reduction of its price which leads to consumer exploitation and misinformation. Moreover, the oil used found to be mostly the palm oil which known to have bad effect on human health. Another form of falsification was detected through addition of some preservatives that are forbidden to be used as benzoate. On the other hand, microbial analysis of the examined samples showed a high bacterial, yeast and coliforms counts which express the unsanitary environment in small plants. So, this study recommended that small scale plants must label their products and follow the Egyptian standards in their manufacture, also authorities must care about fatty acid profile as one of the method detecting milk fat fraud. Additionally, more follow up inspections by local authorities must be adopted to control the commercial fraud in Tallaga cheese processing steps.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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