



## Assessment of Selected Varieties of Cheese Analogues sold in Egyptian Markets

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### Abstract

The higher price of full fat dairy cheese is the major cause that encourages consumers to purchase analogue cheese that depends on vegetable oils. Consequently, this study was designed to assess the various cheese analogues sold in Egypt. Ninety-five cheese analogue samples (35 each of Mozzarella analogues and processed spread, as well as 25 processed Cheddar) were examined microbiologically and chemically with an evaluation of their fatty acids profile. The moisture and fat/dry matter % of Mozzarella, processed cheese spread, and processed Cheddar were declared with the mean values of 49.53, 58.98, 51.88% and 62.53, 73.14, 60.16%, respectively. The total colony count mean was  $6 \times 10^7$ ,  $19 \times 10^2$ , and  $1 \times 10^6$  CFU/g in Mozzarella, processed spread and Cheddar cheese, respectively. The highest yeast count was noticed in Mozzarella and Cheddar in a percentage of 94.29 and 92.00, respectively, while the maximum unacceptable ratio of molds was counted in processed cheese spread (22.9%) based on Egyptian Standards (ES: 1132/2005). A high incidence of Staphylococcus and Coliform counts were detected in the examined Mozzarella cheese samples. The results of potassium sorbate revealed that two Mozzarellas, two Cheddar, and seven processed samples exceeded 1000 ppm in disagreement with the label. The saturated fatty acids % in examined products ranged from 46.52 to 57.32. No samples were acceptable for ratios of poly unsaturated fatty acids/saturated fatty acids (PUFA/SFA) and Omega-6 /Omega-3 (n6/n3) that were found uncompatible with the nutritional guideline for healthy food announced by WHO (2003). This is one of few studies in Egypt that is designed to evaluate the comparison between the selected analogue cheeses. Therefore, we recommend more studies on assessment of analogue cheeses as well as types of fat that are used in their processing should be identified in regulations and included on the label by law.

**Keywords:** cheese analogue; fat/dry matter %; fatty acids profile; microbiological assessment; potassium sorbate.

### 1. Introduction

Cheese is found to be one of the primary favorable and public dairy products consumed in Egypt and over the world as well. But dairy cheese is characterized mainly by expensive ingredients and elevated manufacturing and storage costs. Therefore, producers start tending to replace the entire natural product with another low-cost product represented in cheese analogues [1, 2].

The concept of cheese analogue expanded due to the economic causes mostly as the reduction of the cost of the manufacturing and the cheaper initial ingredients with the simpler and rapid manufacturing process [3, 4]. This type of cheese also known as imitation cheese is designed to be prepared by either partially or totally replacing the protein and/or fat specific to milk with other sources of plant origin and addition of other

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ingredients as stabilizers and emulsifiers to produce the final product that similar to natural cheese in appearance [4-6]. The other ingredients that may be used are rice, soya bean, almond, and other products free from milk fat [7].

The commonly developed examples of analogue cheese are Mozzarella (due to highly involved in pizza manufacturing), processed Cheddar cheese with increased demand for a cheeseburger as well as processed cheese spread packed in triangles form referred to high popularity, especially to young children [8]. The high meltability and ability to stretch are the most attractive properties of low-moisture unripened Mozzarella cheese [9, 10]. While processed cheese spread with vegetable oils and fats is a cheese that can be manufactured by heating a mixture of one or more types of cheese with various degrees of ripening plus the addition of emulsifying salts and other ingredients as flavoring agents as well as may use a dairy portion [11, 12]. The determination of cheese composition as moisture and fat content are considered important factors to define its acceptability accompanied by clarifying and evaluating their microbiological state [9, 13].

As well the addition of food preservatives is permitted in cheese analogues to keep nutritional value unaffected by enzymatic or chemical alteration and the growth of spoilage microorganisms. Generally, potassium sorbate is the predominant common preservative used in cheese manufacture all over the world [14]. The main aim of using sorbates is to inhibit fungal growth, avoid spoilage and keep products fresh as long as possible. Increase the level of consumption of this preservative above the allowable limit permitted by low as 0.1% in unripened and prepacked or sliced cheese, as elevated levels of consumption perhaps cause a hypersensitivity and allergenicity reaction in some cases as well as genotoxicity [15-18]. Hydrogenated or nonhydrogenated palm oil is one of the main popular vegetable oils that are highly used in the formulation and production of cheese analogues. Although based on an announcement by World Health Organization (WHO, 2003), the consumption of a diet high in palm oil content daily may lead to a high probability of cardiovascular disease [19]. Palm oil comprises a high percentage reaching up to 50% saturated fatty acid that it is consisting of about 44% palmitic acid with about 5% stearic acid. The high levels of saturated fatty acid may probably be linked with increased cholesterol in the blood with a great incidence of cardiovascular diseases [20]. A recent study by Pascual et al. [21]; showed that dietary consumption of a palm oil-rich diet enhanced the development of metastasis. High consumption and widespread use of Mozzarella and the palatability of processed cheese spread help us to

think about their quality and safety states evaluation. So, this study is designed to investigate the microbiological and chemical of these common cheese analogues sold in Egypt markets as well as presentation of their fatty acids profile, and some chemical preservatives. In addition to determining to what extent these products comply with Egyptian standards.

## 2. Materials and Methods

### Collection of samples

A total of 95 random samples (35 analog Mozzarella cheese, 35 processed cheese spread, and 25 processed Cheddar cheese samples) were collected from different markets in Giza and Cairo Governates, Egypt to be examined for some chemical and microbiological parameters. In a complete aseptic situation, all samples were transported in a cool isolated icebox as soon as possible to the laboratory.

### Chemical examination, determination of preservatives, and fatty acids profile analysis of cheese analogue samples

All samples examined by determination of total solids and moisture content using oven drying method, while fat content by Gerber method as chemical examination is performed based on AOAC [22]. The determination of chemical preservatives benzoate and sorbate was done using High-Performance Liquid Chromatography (HPLC, model HP Agilent 1200 series from Germany). according to Pylypiw and Grether [15]; that done by homogenization and blending of 10 g. of sample by 50 ml mobile phase then allowed to separate for 5 minutes with transfer of 1 ml supernatant and diluted using 10 ml mobile phase. As well as filtering via Acrodisk filter (25 mm×0.45mm) for samples and finally 2 ml was taken for examination. While based on the method described by Zhong et al. [23]; for nitrate and nitrite, the samples were prepared by adding ten grams of samples into 50 ml deionized water then heating up to 70°C for approximately 30 minutes. Finally, all prepared samples were filtrated with added deionized water up to 100 ml. and transferred to HPLC for analysis. The fat extraction was applied using the Soxhlet method [24, 25] and Folch method [26] for the evaluation and identification of fatty acids profile for 30 samples for different brands (10 for each) using Gas Chromatography (GC, Perkin Elmer Auto System XL) based on Zahran and Tawfeuk [27]. Firstly, the analysis was done by transmethylation process by forming Fatty Acid Methyl Esters (FAMES). Following by separation of FAMES using injector and detector temperature was 250°C with using a helium as the carrier gas at flow rate of 1.2 ml/min. While the column temperature was 140°C hold/5 min. and reach 240°C at a rate of 4°C every one minute with holding

at final temperature (240°C) for 10 minutes. Each 0.001 ml of prepared sample (in n-hexane) was injected in splitting ratio of 100:20. The identification of FAMES were performed by contrasting their times of relative and absolute retention to 37 component standards FAMES. The composition of fatty acids was recorded as the total peak area that converted into percentage using GC.

### Microbiological examination

The samples were prepared by the addition of 10 g of sample and 90 ml of sodium citrate 2% solution (HiMedia, R014) as a diluent according to ISO 6887-5: 2010 [28]. For additional dilutions, 9 ml of diluent to was added to 1 ml of the preceding dilution. Utilizing a vortex, agitated the tube carefully. For enumeration of aerobic mesophilic count, an inoculum of 1 ml from the prepared dilutions was transferred and the plate count agar was poured on it (HiMedia, M091) and then plates were incubated at 32 ±1°C for 48 ±2 hours with counting the plates 25-250 colonies referred to Ryser and Schuman [29]. The total Staphylococci count was enumerated by inoculation with 0.1 ml of previously prepared serial dilutions on Barid Parker agar media (HiMedia, MU043) at 35°C ±2°C for 48 hours based on ISO 6888-1:2021 [32]. The yeast and mold count were determined using Dichloran Rose Bengal chloramphenicol (DRBC) agar

(HiMedia, M1881) plates (for foods with water activity >0.95). All the inoculated media (using the spreading method) was incubated at 25°C for five days. The plates with 10 to 150 colonies were counted and calculated as described by Ryu and Wolf-Hall [30]. Coliforms count MPN/g was assessed referenced to Kornacki et al. [31]; using three tubes method of Lauryl Sulphate Tryptose (LST) broth (HiMedia, M080).

### Statistical analysis

All results obtained were analyzed in SPSS program V25 (SPSS, 2017) [33] and were described as mean ±SEM. In addition to the estimation of the Least Significant Difference (LSD) by using one-way ANOVA was done, as a p-value ( $P < 0.05$ ) for the recorded mean values.

## 3. Results

**Table 1.** Statistical analytical results of the tested chemical parameters of the examined samples and their degree of acceptability based on the Egyptian standards

Type of samples	Parameters	Values			Legal values*	No. of acceptable samples (%)
		Minimum	Maximum	Mean ±SEM		
Mozzarella cheese (n= 35)	Total solid %	40.00	56.80	50.47±0.66	NA	-----
	Fat %	20.00	46.00	31.53±0.87	NA	-----
	Moisture %	43.20	60.00	49.53±0.66	< 60	35 (100.00%)
	Fat /DM%	50.00	85.59	62.53±1.58	> 60	18 (51.43%)
Processed cheese spread (n= 35)	Total solid %	36.00	47.20	41.02±0.50	NA	-----
	Fat %	20.00	38.00	29.97±0.59	NA	-----
	Moisture %	52.80	64.00	58.98±0.50	< 55	20 (57.14%)
	Fat /DM%	49.02	86.73	73.14±1.24	>65	31 (88.57%)
Processed Cheddar cheese (n= 25)	Total solid %	35.60	65.60	48.11±1.63	NA	-----
	Fat %	18.00	49.00	29.12±1.74	NA	-----
	Moisture %	34.40	64.40	51.88±1.63	< 48	14 (56.00%)
	Fat /DM%	39.13	85.66	60.16±2.49	>65-60	14 (56.00%)

n: number of samples examined; SEM: standard error of mean

n: number of samples examined; SEM: standard error of mean; DM: Dry Matter

\* Egyptian Standards (ES: 1132/2005 and ES: 1867/2005)

Data represented in (Table 1); revealed that the fat% in all examined samples was found in a range of 20-46%, 20-38%, and 18-49% in Mozzarella, processed cheese spread, and processed Cheddar cheese, respectively. While minimum to maximum

percentages of fat/ DM were 50-85.59, 49.02-86.73, and 39.13-85.66%, respectively. The mean values of moisture % and total solid % were 49.53±0.66 and 50.47±0.66 in examined Mozzarella cheese samples, respectively, followed by high moisture percent of

processed Cheddar cheese with a mean value of  $51.88 \pm 1.63\%$  and  $58.98 \pm 0.50\%$  in processed cheese spread samples.

Referenced to the results obtained in (Table 1); for evaluation of the compatibility of examined samples with limits set by Egyptian Standard according to the chemical parameters, the highest percentage of samples acceptability referred to the

moisture content is Mozzarella cheese samples in a percentage of 100.00. On the other side, the highest ratio of accepted samples according to the Fat/ DM % was the processed cheese spread (88.75%) followed by the processed Cheddar cheese (56.00%) compared to the lowest acceptable percentage in Mozzarella cheese by 51.00%.

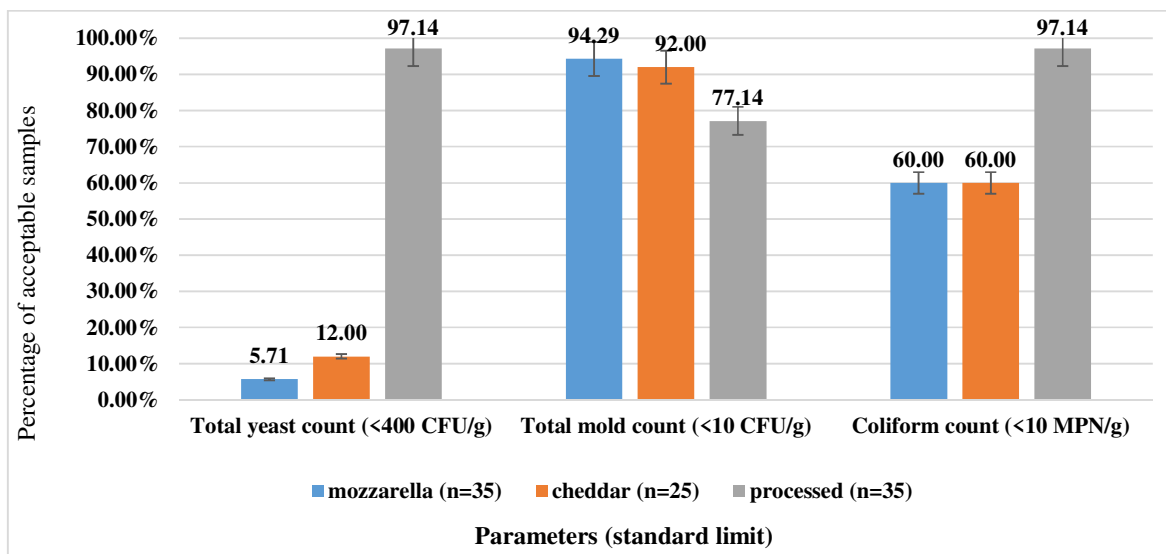
**Table 2.** Statistical analytical results of the tested microbiological parameters of the examined samples

Parameters	Mozzarella cheese (n= 35)				Processed cheese spread (n= 35)				Processed Cheddar cheese (n= 25)			
	No. (%)	Min.	Max.	Mean $\pm$ SEM	No. (%)	Min.	Max.	Mean $\pm$ SEM	No. (%)	Min.	Max.	Mean $\pm$ SEM
Total aerobic mesophilic count (CFU/g)	33 (94.3%)	$9 \times 10^2$	$37 \times 10^7$	$6 \times 10^7 \pm 0.17 \times 10^7$	5 (14.3%)	80	$4 \times 10^3$	$19 \times 10^2 \pm 7 \times 10^2$	23 (92.0%)	$3 \times 10^2$	$19 \times 10^6$	$17 \times 10^5 \pm 8 \times 10^5$
Total Staphylococci (CFU/g)	32 (91.4%)	$1 \times 10^3$	$1 \times 10^8$	$7 \times 10^6 \pm 3.90 \times 10^6$	1 (2.9%)	$4 \times 10^2$		$4 \times 10^2 \pm 0.00$	21 (84.0%)	$5 \times 10^2$	$15 \times 10^5$	$17 \times 10^4 \pm 8 \times 10^4$
Total yeast count (CFU/g)	33 (94.3%)	$1 \times 10^3$	$55 \times 10^7$	$5 \times 10^7 \pm 2 \times 10^7$	1 (2.9%)	$7 \times 10^2$		$7 \times 10^2 \pm 0.00$	23 (92.0%)	$3 \times 10^2$	$2 \times 10^6$	$4 \times 10^5 \pm 1 \times 10^5$
Total mold count (CFU/g)	2 (5.7%)	$1 \times 10^2$	$2 \times 10^2$	$15 \times 10 \pm 5 \times 10$	8 (22.9%)	$1 \times 10^2$	$3 \times 10^2$	$1 \times 10^2 \pm 0.28 \times 10^2$	2 (93.0%)	$1 \times 10^2$	$1 \times 10^2$	$1 \times 10^2 \pm 0.00$
Coliform count (MPN/g)	19 (54.3%)	3	$2 \times 10^5$	$24 \times 10^3 \pm 14 \times 10^3$	1 (2.9%)	$4 \times 10^3$		$4 \times 10^3 \pm 0.00$	13 (52.0%)	4	$1 \times 10^3$	$16 \times 10 \pm 5 \times 10$

n= number of examined samples; No.= number of positive samples; Min.= minimum; Max.= maximum; S.E.M.= Standard Error of Mean; CFU= Colony Forming Units

Microbiological data analysis in (Table 2); showed that the incidence of total aerobic mesophilic count in Mozzarella, processed spread and processed Cheddar cheese were 94.3, 14.3, and 92.0% with the range of  $9 \times 10^2$ - $37 \times 10^7$ ,  $80$ - $4 \times 10^3$ , and  $3 \times 10^2$ - $19 \times 10^6$  CFU/g, respectively. However, Staphylococci were counted in 91.4% and 84.0% of the tested Mozzarella, and processed Cheddar cheese samples, respectively. The Mozzarella cheese samples were the maximum contaminated analogue with yeast (94.3%) compared to processed Cheddar cheese (92%) with mean values of  $5 \times 10^7 \pm 2 \times 10^7$  and  $4 \times 10^5 \pm 1 \times 10^5$  CFU/g, respectively.

Furthermore, Coliforms prevalence in Mozzarella and Cheddar cheese was 54.3 and 52.0% with MPN/g range of  $3$ - $24 \times 10^3$  and  $4$ - $1 \times 10^3$ , respectively. The lowest prevalence and contamination were observed in processed cheese spread (2.85%) for total staphylococci, yeast, and coliform count with the following mean values  $4 \times 10^2$ ,  $7 \times 10^2$  CFU/g, and  $4 \times 10^3$  MPN/g, respectively, while it was the highest contaminated by mold in eight processed spread samples only with a mean value of  $1 \times 10^2 \pm 0.28 \times 10^2$  CFU/g.



**Figure 1:** Degree of microbiological acceptability of Mozzarella, Cheddar and processed cheese spread samples according to Egyptian standard ES: 1867/2005 and ES:1132/2005; n= number of examined samples

As declared in (Figure 1); comparing to the Egyptian Standards in microbiological data analysis for the tested cheese analogue samples, processed cheese spread samples were found to be the most compatible product to the ES in a percentage of 97.14 based on total yeast and coliforms count. However, the processed spread samples revealed the lowest accepted

category in the total mold count parameter. While based on the Egyptian Standards ES: 1867/2005 [34] and ES: 1132/2005 [11], only 5.71 and 12.00% of the examined Mozzarella and processed Cheddar cheese samples, respectively, agreed with the Egyptian standards for total yeast count and 60% for coliforms count.

**Table 3:** Statistical analytical results of sorbate in the examined cheese samples (Permissible  $\leq 1000$  ppm)\*

Type of samples	No. of samples (%)	Mean $\pm$ SEM
Mozzarella	2 (5.71)	1370 $\pm$ 24.75
Processed Cheddar	2 (8.00)	1471 $\pm$ 47.73
Processed spread	7 (20.00)	1261 $\pm$ 35.35

\* = referenced to EU: 1129/2011

No.= numbers of positive samples exceed permitted limit and data on label, SEM: standard error of mean

All samples showed a total aerobic mesophilic colony count of  $< 10$  CFU/g. were examined using HPLC for their sorbate concentration in two of Mozzarella, two of processed Cheddar, and thirty processed cheese spreads. Some samples were found to have sorbate salts above the permissible limit (1000 ppm) set by EU: 1129/2011 [16] regulations for unripened and sliced ripened cheeses. It was detected

in mean values of 1370 $\pm$ 24.75, 1471 $\pm$ 47.73 and 1261 $\pm$ 35.35 ppm in Mozzarella (5.71%), processed Cheddar (8.00%), and processed spread cheese (20.00%), respectively (Table 3). In addition, we applied nitrate, nitrite, and benzoate on the same low-count cheese samples which could not be determined in examined samples by HPLC.

**Table 4.** Individual fatty acid contents (fatty acids %) of examined cheese samples

Fatty acids	Type of samples (Mean $\pm$ SEM)		
	Mozzarella cheese	Processed cheese spread	Processed Cheddar cheese
<b>Saturated fatty acids (SFA)</b>			
Butyric acid (C4:0)	0.01 $\pm$ 0.00 <sup>a</sup>	ND	0.14 $\pm$ 0.089 <sup>b</sup>
Caproic acid (C6:0)	0.01 $\pm$ 0.00 <sup>a</sup>	ND	0.31 $\pm$ 0.203 <sup>b</sup>
Caprylic acid (C8:0)	0.03 $\pm$ 0.017 <sup>a</sup>	ND	0.42 $\pm$ 0.269 <sup>b</sup>
Capric acid (C10:0)	0.06 $\pm$ 0.026 <sup>a</sup>	ND	0.97 $\pm$ 0.625 <sup>b</sup>
Lauric acid (C12:0)	0.36 $\pm$ 0.054 <sup>a</sup>	ND	1.51 $\pm$ 0.974 <sup>b</sup>
Myristic acid (C14:0)	1.72 $\pm$ 0.035 <sup>a</sup>	0.50 $\pm$ 0.082 <sup>b</sup>	4.77 $\pm$ 2.331 <sup>c</sup>
Pentadecanoic acid (C15:0)	0.08 $\pm$ 0.038 <sup>a</sup>	0.14 $\pm$ 0.00 <sup>a</sup>	0.17 $\pm$ 0.111 <sup>a</sup>
Palmitic acid (C16:0)	44.52 $\pm$ 0.441 <sup>a</sup>	29.17 $\pm$ 2.524 <sup>b</sup>	42.21 $\pm$ 2.108 <sup>a</sup>
Heptadecanoic acid (C17:0)	0.08 $\pm$ 0.028 <sup>a</sup>	ND	0.11 $\pm$ 0.073 <sup>b</sup>
Stearic acid (C18:0)	5.23 $\pm$ 0.066 <sup>a</sup>	16.71 $\pm$ 1.988 <sup>b</sup>	6.72 $\pm$ 1.223 <sup>a</sup>
Arachidic acid (C20:0)	0.51 $\pm$ 0.077	ND	ND
<b>Monounsaturated fatty acids (MUFA)</b>			
Myristoleic acid (14:1)	0.10 $\pm$ 0.023 <sup>a</sup>	ND	0.30 $\pm$ 0.192 <sup>b</sup>
Cis-10-Pentadecenoic (C15:1)	0.09 $\pm$ 0.045 <sup>a</sup>	ND	0.37 $\pm$ 0.242 <sup>b</sup>
Palmitoleic acid (C16:1), n9	0.26 $\pm$ 0.042 <sup>a</sup>	0.57 $\pm$ 0.096 <sup>a</sup>	0.49 $\pm$ 0.317 <sup>a</sup>
Oleic acid (C18:1n9c)	37.83 $\pm$ 0.391 <sup>a</sup>	44.64 $\pm$ 0.676 <sup>b</sup>	33.64 $\pm$ 3.289 <sup>a</sup>
Gadoleic acid (C20:1)	0.03 $\pm$ 0.018	ND	ND
<b>Poly unsaturated fatty acids (PUFA)</b>			
Linoleic acid (C18:2n6c)	8.42 $\pm$ 0.105 <sup>a</sup>	7.30 $\pm$ 0.340 <sup>a</sup>	7.28 $\pm$ 1.538 <sup>a</sup>
Linoleic acid (C18:2n6t)	0.03 $\pm$ 0.022	ND	ND
$\alpha$ - Linolenic acid (C18:3n3)	0.13 $\pm$ 0.020 <sup>a</sup>	0.69 $\pm$ 0.116 <sup>b</sup>	0.59 $\pm$ 0.378 <sup>b</sup>
$\gamma$ - Linolenic acid (C18:3n6t)	0.45 $\pm$ 0.187 <sup>a</sup>	0.27 $\pm$ 0.044 <sup>a</sup>	ND

n: no. of examined samples; ND: Not detectable; Means with different superscript within the same row indicates significant difference ( $P < 0.05$ )

The fatty acids fractionation presented in (Table 4); showed that palmitic fatty acid was the most predominant fatty acid in Mozzarella and processed Cheddar cheese with means of 44.52 and 42.21%, followed by oleic acid with means of 37.83 and 33.64%, respectively. But in processed cheese spread, the oleic fatty acid was the major fatty acid in the percentage of 44.64 with the lowest palmitic FA (29.17%) and the highest Stearic acid (16.71%) compared to other products. Consequently, there was a significant difference between processed cheese and

other examined cheese analogues based on their palmitic, oleic, and stearic fatty acids content.

Based on the main constituent of PUFA was linoleic acid in percentages of 7.28, 7.30, and 8.42 for processed Cheddar, processed cheese spread, and Mozzarella cheese, respectively (Table 4). Also, there was a significant difference in  $\alpha$ - Linolenic acid (C18:3n3) between Mozzarella and other analogue types that were lower than the processed spread and processed Cheddar.

**Table 5.** Fatty acids indices and nutritionally important ratios of examined cheese samples

Parameter	Type of samples (Mean $\pm$ SEM)		
	Mozzarella cheese	Processed cheese spread	Processed Cheddar cheese
SFA	52.62 $\pm$ 0.392 <sup>a</sup>	46.52 $\pm$ 0.593 <sup>b</sup>	57.32 $\pm$ 3.700 <sup>a</sup>
MUFA	38.31 $\pm$ 0.467 <sup>a</sup>	45.22 $\pm$ 0.772 <sup>b</sup>	34.81 $\pm$ 2.538 <sup>a</sup>
PUFA	8.55 $\pm$ 0.152 <sup>a</sup>	7.99 $\pm$ 0.224 <sup>a</sup>	7.86 $\pm$ 1.160 <sup>a</sup>
Trans fatty acids (TFAs)	0.48 $\pm$ 0.182 <sup>a</sup>	0.27 $\pm$ 0.044 <sup>a, b</sup>	ND
UFA	46.86 $\pm$ 0.536 <sup>a</sup>	53.21 $\pm$ 0.547 <sup>b</sup>	42.67 $\pm$ 3.697 <sup>a</sup>
n3	0.13 $\pm$ 0.020 <sup>a</sup>	0.69 $\pm$ 0.116 <sup>b</sup>	0.58 $\pm$ 0.374 <sup>a, b</sup>
n6	8.42 $\pm$ 0.105 <sup>a</sup>	7.30 $\pm$ 0.340 <sup>a</sup>	7.23 $\pm$ 1.530 <sup>a</sup>
PUFA/ SFA	0.16 $\pm$ 0.0025 <sup>a</sup>	0.17 $\pm$ 0.0024 <sup>a</sup>	0.14 $\pm$ 0.025 <sup>a</sup>
n6/n3	64.77 $\pm$ 7.039 <sup>a</sup>	10.58 $\pm$ 0.269 <sup>b</sup>	12.47 $\pm$ 0.174 <sup>b</sup>

ND: Not detectable; SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= poly unsaturated fatty acids; UFA= unsaturated fatty acids; n3= omega 3; n6= omega 6; Means with different superscript within the same row indicates significant difference ( $P < 0.05$ )

Data presented in (Table 5) showed that the highest concentration of saturated fatty acid was in processed Cheddar cheese followed by Mozzarella and the lowest one in processed cheese spread with values of 57.32, 52.62, and 46.52%, respectively. Consequently, the maximum levels of monounsaturated fatty acids were present in processed cheese spread samples (45.22) and the minimum in processed Cheddar cheese (34.81). Concerning PUFA, the processed Cheddar cheese had a low value (7.86)

and 7.99% in processed cheese spread samples with the peak concentration of Mozzarella cheese (8.55).

On the calculation of the nutritional significance of cheese analogue samples; the PUFA/SFA were estimated in a range of 0.14-0.17. While n6/n3 ratio was observed to be higher in all examined cheese samples with the highest ratio of 64.77 for Mozzarella cheese samples that had a significant difference compared to processed spread and processed Cheddar cheese (Table 5).

#### 4. Discussion

In general, variations in manufacturing circumstances between producers, utilizing different milk sources, manufacturing techniques that involve temperature variations and the utilization of starter cultures may be the main cause for variations in the chemical structure of cheese samples [35]. The moisture and fat to dry matter (%) were important parameters mentioned by Egyptian Standards to be followed, so it was necessary to be determined and evaluated. In this study, referring to analytical data in (Table 1), the fat (%) of Mozzarella cheese samples was high among other types of examined analogues and the results were higher than that reported by Helal [36], while nearly similar to Mehta [37]. Fat to DM (%) for Mozzarella cheese was found to comply with the regulations set by Egyptian Standards (ES:1132/2005) [11] as > 60.00% in high-fat soft cheese with vegetable oil on the opposing lower result obtained by Zedan et al. [13]. Total solids (%) results in Mozzarella cheese were similar to the results of Zedan et al. [13] and Mailam [38], but below than data of Abdalla and Ibrahim [35]. While the moisture (%) was similar to findings reported by Helal [36] and Mailam [38]. The fat% in processed spread cheese samples was approximately similar to data recorded by Suleiman et al. [39] and higher than the results obtained by El-Shibiny et al. [40], Cunha et al. [41], and Ghita et al. [42]. In addition, the fat/DM (%) in our samples exceeded the findings of El-Shibiny et al. [40] and Ghita et al. [42]. However, moisture (%) was nearly similar to Cunha *et al.* [41] and higher than Suleiman et al. [39]. On the other side, the T.S% is lower than the result reported by El-Shibiny et al. [40] but almost identical to Ghita et al. [42]. The results of fat content in this study on cheddar cheese were in agreement with the findings of Kwak et al. [43] and Suleiman et

al. [39]. However, Piska & Štětina [44] and Abdel Razig & Yousif [45] described moisture content in processed cheese spread in the range of 51.0-64.21% which was similar to our results. About 42.86 and 44.00% of examined processed spread and processed Cheddar cheese samples were unacceptable to legal moisture (%) announced by ES:1867/2005 [34], respectively. de Medeiros Carvalho et al. [46]; was declared that the difference in the moisture percentages among cheese products is mainly attributed to changes in the modes of its manufacture. As well as some producers may choose to increase the levels of moisture with the reduction in other ingredients to improve their earnings [47]. The relative variation and elevation in moisture content in cheese products are considered as the favorable growth of several microbes, especially the flourishing of fungi that may be get via raw milk or throughout any step of the production [48, 49]. APHA, 2004 [50]; stated that total colony count, coliform as well as yeast and mold count were regarded as significant indicators for the products' microbiological and hygienic quality. The aerobic bacterial count provides an overall safety assessment of the product as a standard criterion for inferior sanitation, faults during processing, handling, and storage [51]. In (Table 2); the obtained result of total colony count in Mozzarella was similar to Tanweer [52] and Ali & Elsherif [9], but Francesca et al. [53] reported lower results. The highest count of staphylococci in our study with an incidence of 91.4% which was nearly identical to the result obtained by Garbaj et al. [54]. The findings of TCC for cheddar cheese were similar to El-Diam and El-Zubeir [55], while the result of total Staphylococcus count in cheddar cheese was similar to that found by Halim et al. [56]. Staphylococci serve as indicators of poor cleanliness, and their significant prevalence suggests

that proper hygiene standards were not followed during the production, processing, and distribution of dairy products [57]. Exposure of Mozzarella cheese to the cutting process allowed them to come into contact with surfaces, tools, and manipulators. Furthermore, fractionating any solid product increases its exposed surface, which raises the possibility of contamination [58]. Putting into consideration the shredding and slicing processes applied to Mozzarella and processed Cheddar cheese, also a lack of process monitoring explains the high count of bacteria determined in our survey. Although, the processed cheese samples results showed the lowest incidence in a percentage of 14.3 and 2.9 for aerobic plate count and Staphylococcus counts, respectively, in agreement with results declared by Nazem et al. [59]. That lowest count mainly occurs due to the product undergoing heat treatment and then packaging that there is no further processing that encourages product contamination. Due to the coliform organism's extreme sensitivity to heat, the high occurrence of coliform is a result of product pollution during or after heat treatment or inefficient heat treatment [60]. The result of coliform count in Mozzarella cheese was in agreement with Garbaj et al. [54], while higher counts were obtained by Soliman [61]. In contrast, it was higher than the results recorded by Marinheiro et al. [58] and Mohamed et al. [62]. However, in cheddar cheese, the coliform count is lower than that recorded by Kamel and Halim et al. [63, 56], on the contrary, the coliform count detected in processed spread was nearly similar to the result clarified by Sallam et al. [64]. The counts for positive samples were unacceptable and much higher than the limits suggested by the Egyptian Standards ES: 1867/2005 [34] and ES:1132/2005 [11] that mentioned not exceeding 10 coliforms/g for Mozzarella and processed Cheddar in numbers of 19 and 13 samples, respectively and only one unacceptable sample of processed cheese spread that may be attributed to post-pasteurization contamination. The yeasts and molds count obtained in Mozzarella cheese samples were nearly comparable to Ali and Elsherif [9] and Serna et al. [65], although our results were slightly higher than those reported by Tanweer [52] and Mohamed et al. [62]. On the other hand, the result obtained in our study of processed Cheddar cheese was nearly in the same line same line as Halim et al. [56] and Kamel [63]. Conversely, the prevalence of mold was the most reported in processed cheese spread that occur in eight samples followed by Mozzarella and processed Cheddar which was counted in two samples each. The yeast and mold counts of processed spread were similar to Nazem et al. [59]. Mold and yeast analyzed in cheese may be associated with poor sanitary measures during manufacturing, also raw milk was measured as the chief reason, as cross-contamination

and open-air or improper storage [63]. The way into using chemical preservatives has increased currently, the main one used in cheese manufacture is sorbic acid or using of its potassium, calcium, and sodium salts. Sorbic acid was identified as nontoxic and secure unless some studies proved using it in large quantities in a diet daily may cause allergies. As well as it may assist in the elevation of potassium levels that may be the cause of hyperkalemia [18, 66]. Sorbic acid has been used in a variety of foods as a preservative because of its anti-mold, anti-yeast, and antibacterial activities, The Code of Federal Regulations [67]; expresses sorbic acid as generally recognized as safe (GRAS) with good manufacturing practice limitations, however European regulatory set a level for using sorbic acid in sliced, pre-packed and layered cheese lower at 1000 mg/kg [16, 68]. On the other hand, some studies reported that an increase in the dose above 1000 ppm was found to be the reason for chronic urticarial and atopic dermatitis [69]. Additionally, it may cause off-flavor as kerosene flavor that occurs from spoilage microorganisms in cheese contain sorbic acid by decarboxylation of it [70]. Referenced to our Egyptian labels on all examined samples, there 5.71, 8.00 and 20.00% of tested Mozzarella, processed Cheddar, and processed cheese spread samples, respectively, exceeded 1000 mg/kg of sorbates in a range of 1084-1795 mg/kg. While Guarino et al. [71]; Zamani Mazdeh et al. [72] and Özdemir et al. [66]; reported a sorbate level less than our examined samples and lower than the limit allowed by law. The limit of using potassium sorbate must be more restricted and defined in the standard as well as the actual concentration should be declared on the label as the studies are still progressing in determining the safety of this preservative, especially for daily dietary usage. Egypt has insufficient adequate evaluation and observation of saturated fatty acids percentages in dietary foods with a lack of frequent upgrades. The main issue is that SFAs are known to cause low-density lipoprotein levels elevation, associated with cardiovascular diseases and cancer progression [73-75]. The fatty acids profile of processed Cheddar and Mozzarella analogue cheese samples were characterized by a high content of saturated fatty acids expressed mostly in palmitic fatty acids as presented in (Table 4 & 5). The processed cheese spread was high in unsaturated fatty acids demonstrated in oleic acid (44.64%) and SFAs (46.52%) as palmitic (29.17%) and stearic acids (16.71%). Generally, these results are nearly similar to Abdel-Ghany et al. [76] but below in SFAs than data recorded by Al-Amiri et al. [77]. However, Ismail et al. [75] reported that triangles processed cheese showed a high ratio of saturated compared to MUSFs and Mozzarella cheese samples with lower saturated than unsaturated fatty acids. Although, all examined samples have PUFAs/SFAs



ratio (0.14-0.17) minimum than specified by nutritional health guidelines (< 0.45) set by WHO, 2003 [78]. While the n6/n3 ratio that was approved to be less than 10 referenced to health guidelines was found to be unacceptable in a range of 10.58-64.77% in all examined cheese samples [79, 80]. Our reported results of processed cheese were lower than that recorded by Abbas et al. [81] for nutritional ratios (n6/n3 and PUFAs/SFAs). The maximum percentage of omega 3 was detected in processed cheese spread samples 0.69% but omega 6 in Mozzarella cheese with mean of 8.42% with the highest PUFAs %. In agreement with our result, Al-Amiri et al. [77]; described a low P/S ratio (0.01) for triangle spreadable processed cheese which was assessed as unhealthy. From the nutritional point of view, the consumption of dietary PUFAs would help in reducing the cholesterol serum level resulting in less probability of CVD (cardiovascular diseases) as well as may be associated with prohibition in diabetes, asthma, hypertension, and some cancers [77, 82].

### 5. Conclusion

The processed cheese spread was the most acceptable product for Egyptian regulations, while processed Cheddar and Mozzarella were low agreement and recommended to be developed and monitor during processing. In addition, as recognized in the fatty acid profile that the ratio of saturated fatty acids was very high, which counteracts the lead of initiation of cheese analogue concept that replacing saturated fatty acid with unsaturated one to ameliorate the consumer's health. So, we suggested that types of fat and its fractions that are used in cheese analogue must be set in regulations and the moisture % must be included on the label. Also as reported that the potassium sorbates did not comply with the label even if it is safe for humans, it is considered fraud. The HACCP system is commended to be established for developing products' hygiene and application of sanitary standards during product industrial procedures. Further studies are needed to declare the overall acceptability of cheese analogues sold in Egypt as well as determine the safety of replacing milk fat with palm oil.

### 6. Conflict of Interest

The authors declare no conflict of interest.

### 7. References

- [1] Tamime, A.Y., 2011. Processed cheese and analogues: An overview. In Tamime (Ed.). Processed cheese and analogues. Wiley Blackwell.
- [2] Abd El-Gawad, I.A., Hamed, E.M., Zidan, M.A., Shain, A.A., 2015. Fatty acid composition and quality characteristic of some vegetable oils used in making commercial imitation cheese in Egypt. *Nutr. Food Sci.j.* 5, 380-384.
- [3] Verma, S.K., Upadhyay, S., Chandra, R., Paul, A., 2013. Preparation of processed cheese spread using tofu, Mozzarella and Cheddar cheese. *Int J Food Sci Nutr.* 2, pp. 19-23.
- [4] Hussein, G., Shalaby, S., 2018. Properties of imitation cheese products prepared with non-dairy ingredients. 3, pp. 578-587.
- [5] Kadbhane, V. S., Shelke, G. N., Thorat, S. L., 2019. Preparation of non-dairy cheese analogue enriched with coconut milk. *Pharma Innov.* 8, pp. 56-60.
- [6] Kamath, R., Basak, S., Gokhale, J., 2021. Recent trends in the development of healthy and functional cheese analogues-a review. *LWT*, 112991.
- [7] Shurtleff, W., Aoyagi, A., 2013. History of Cheese, Cream Cheese and Sour Cream Alternatives (with or without Soy) (1896-2013), Lafayette, California.
- [8] Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney, P.L., 2017. Processed cheese and substitute/imitation cheese products. In *Fundamentals of cheese science*. Springer, Boston, MA. pp. 589-627.
- [9] Ali, D. N., Elsherif, W.M.A., 2015. Microbiological evaluation of Mozzarella cheese. *Assiut Vet. Med. J.* 61, 151-158.
- [10] Berta, M., Muskens, E., Schuster, E., Stading, M., 2016. Rheology of natural and imitation Mozzarella cheese at conditions relevant to pizza baking. *Int. Dairy J.* 57, 34-38.
- [11] ES: 1132/2005 (Egyptian Standards), 2005. Processed cheese with vegetable oils and fats. Egyptian Organization for Standardization.
- [12] Ehsannia, S., Sanjabi, M.R., 2016. Physicochemical, microbiological and spoilage analysis of probiotic processed cheese analogues with reduced emulsifying salts during refrigerated storage. *JFST.* 53, 996-1003.
- [13] Zedan, I.A., Abou-Shaloue, Z., Zaky, S.M., 2014. Quality evaluation of Mozzarella cheese from different milk types. *Alexandria Sci Exc J.* 35. pp. 162-177.
- [14] El-Leboudy, A. A., Amer, A. A., Nasief, M. E., Eltony, S. M., 2015. Occurrence and Behavior of Pseudomonas Organisms in White Soft Cheese. *AJVS.* 44, pp. 74-49.
- [15] Pylypiw, H. M., Grether, M.T., 2000. Rapid high-performance liquid chromatography method for the analysis of sodium benzoate and potassium sorbate in foods. *Journal of chromatography A.* 883, pp. 299-304.
- [16] EU Commission. Commission Regulation (EU) no 1129/2011 of 11 November 2011 Amending Annex II to Regulation (EC) no 1333/2008 of the European Parliament and of the Council by Establishing a Union List of Food Additives. *Off. J. Eur. Union L* 2011, 295, 1.

- [17] Mamur, S., Yüzbaşıoğlu, D., Ünal, F., Aksoy, H., 2012. Genotoxicity of food preservative sodium sorbate in human lymphocytes in vitro. *Cytotechnology*, 64, pp. 553-562.
- [18] EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2015. Scientific Opinion on the re-evaluation of sorbic acid (E 200), potassium sorbate (E 202) and calcium sorbate (E 203) as food additives. *EFSA Journal*, 13, 4144.
- [19] WHO, 2003. Diet, Nutrition and the Prevention of Chronic Diseases. WHO Technical Report Series 916. Geneva: WHO, P. 82, 88 & 88c.
- [20] Mukherjee, S., Mitra, A., 2009. Health effects of palm oil. *Hum. Ecol. J.* 26, pp. 197-203.
- [21] Pascual, G., Domínguez, D., Elosúa-Bayes, M., Beckedorff, F., Laudanna, C., Bigas, C., Benitah, S. A., 2021. Dietary palmitic acid promotes a prometastatic memory via Schwann cells. *Nature*, 599, pp. 485-490.
- [22] AOAC (Association of Official Analytical Chemists), 2019. Official Methods of Analysis Of AOAC International, 21st ed. Rockville, MD: AOAC Int.
- [23] Zhong, W., Hu C., Wang, M., 2002. Nitrate and nitrite in vegetables from north China: content and intake. *Food Addit Contam.* pp. 1125-9.
- [24] Nielsen, S.S., 2017. Food analysis laboratory manual. Springer International Publishing, Switzerland.
- [25] SenÖrāns, F.J., Luna, P., 2012. Sample preparation techniques for the determination of fats in food. *Comprehensive Sampl. Sample Preparat.* 4, 203–211.
- [26] Liu, Z., Ezernieks, V., Rochfort, S., Cocks, B., 2018. Comparison of methylation methods for fatty acid analysis of milk fat. *Food Chem.* 261, pp. 210–215.
- [27] Zahran, H.A., Tawfeuk, H.Z., 2019. Physicochemical properties of new peanut (*Arachis hypogaea* L.) varieties. *OCL* 26. 19, pp. 1-7.
- [28] ISO 6887-5:2010 (International Organization for Standardization), 2010. Microbiology of Food and Animal Feeding Stuffs: Preparation of Test Samples, Initial Suspension and Decimal Dilutions for Microbiological Examination, Part 5: Specific Rules for the Preparation of Milk and Milk Products. 1<sup>st</sup> edition. ISO, Geneva, Switzerland.
- [29] Ryser, E.T., Schuman, J.D., 2015. Mesophilic aerobic plate count. In: Salfinger, Y. & Tortorello, M.L. (eds) *Compendium of Methods for the Microbiological Examination of Foods*. 5th edition. American Public Health Association, Washington, DC, USA. Chapter 8, pp. 95–101.
- [30] Ryu, D., Wolf-Hall, C., 2015. Yeasts and molds. In: Salfinger, Y. & Tortorello, M.L. (eds) *Compendium of Methods for the Microbiological Examination of Foods*. 5th edition. American Public Health Association, Washington, DC, USA. Chapter 21, pp. 277–286.
- [31] Kornacki, J.L., Gurtler, J.B., Stawick, B.A., 2015. Enterobacteriaceae, coliforms, and *Escherichia coli* as quality and safety indicators. In: Salfinger, Y. & Tortorello, M.L. (eds) *Compendium of Methods for the Microbiological Examination of Foods*. 5th edition. American Public Health Association, Washington, DC, USA. Chapter 9, pp. 103–120.
- [32] ISO 6888-1:2021 (International Organization for Standardization), 2021. Microbiology of the food chain Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) — Part 1: Method using Baird-Parker agar medium. 2nd edition. ICS07.100.30 Food microbiology.
- [33] SPSS, 2017. Version 25. Armonk, NY: IBM Corp.
- [34] ES: 1867/2005 (Egyptian Standards), 2005. Soft cheese with vegetable fats. Egyptian Organization for Standardization.
- [35] Abdalla, M.O.M., Ibrahim, N.N.M., 2010. Chemical and Microbiological Evaluation of Mozzarella Cheese during Storage. *Australian Journal of Basic and Applied Sciences*. 4, 532-536
- [36] Helal, A.M.I., 2006. Manufacturing of Mozzarella Cheese from Cow's and Buffalo's Milk with Complete Substitution of Milk Fat with Vegetable Oils. Ph. D. Thesis, Fac. of Agric. (Damanhour), Alex. Univ., Egypt.
- [37] Mehta, M., 2014. Modelling the Grade Value of Cheese. *J. Food Technol.* pp. 2347-2359.
- [38] Mailam, M.A., 2015. Studies on the use of milk concentrates in Mozzarella cheese manufacture (Doctoral dissertation, Ph. D. Thesis, Dairy Sci. and Tech., Fac. Agric., Ain Shams Uni., Egypt.
8. [39] Suleiman, T.A.E., Abdalla, M.O.M., El Haj, N.H.M., Elsiddig, H.M.O., 2011. Chemical and microbiological evaluation of processed cheese available in Khartoum market, Sudan. *Am. J. Food. Nutr.* 1, pp. 28-33.
- [40] El-Shibiny, S., Shenana, M.E., El-Nagar, G.F., Abdou, S.M., 2007. Preparation and properties of low fat processed cheese spreads. *Int. J. Dairy Sci.* 2, pp.13-22.
- [41] Cunha, C.R., Dias, A.I., Viotto, W.H., 2009. Microstructure, texture, colour and sensory evaluation of a spreadable processed cheese analogue made with vegetable fat. *Food Res. Int.* 43, 723–729.

- [42] Ghita, E. I., Hassan. M. N. A., Hamad. E. A., Elaaser. Y. M., 2017. Evaluation of White Soft and Processed Cheese Brands Available in the Egyptian Local Market and Manufactured According to the Egyptian Standard Specification. *J. Food and Dairy Sci.*, Mansoura Univ. 8, pp. 99-101.
- [43] Kwak, H.S., Choi, S.S., Ahn, J., and Lee, S.W., 2002. Casein hydrolysis are fractions act as emulsifiers in process cheese. *J. Food Sci.* 67, pp.821-825.
- [44] Piska, I., Štětina, J., 2004. Influence of cheese ripening and rate of cooling of the processed cheese mixture on rheological properties of processed cheese. *J. Food Eng.* 61, pp. 551-555.
- [45] Abdel Razig, K.A., Yousif, A.M., 2010. Utilization of groundnut milk in manufacturing spread cheese. *Pak. J. Nutr.* 9, 314-319.
- [46] de Medeiros Carvalho, M., de Fariña, L.O., Strongin, D., Ferreira, C.L.L., Lindner, J.D.D., 2019. Traditional colonial-type cheese from the south of Brazil: A case to support the new Brazilian laws for artisanal cheese production from raw milk. *JDS.* 102, 9711-9720.
- [47] Andrade, A.P.C.D., Quirino, M.F., Silva, T.L., Carvalho, J. D. G., 2020. Evaluation of the physical and chemical parameters of Minas Frescal and Ricotta cheese marketed in Fortaleza, Ceará. *Revista Ciência Agronômica*, 51, 1-6.
- [48] Aly, S.A., Morgan, S.D., Moawad, A.A., Metwally, B. N., 2007. Effect of moisture, salt content and pH on the microbiological quality of traditional Egyptian Domiati cheese. *Assiut Vet. Med. J.* 53, 68–81.
- [49] ELbagory, A.M., Amal, M.E., Hammad, A.M., Salwa, A.D., 2014. Prevalence of fungi in locally produced cheese and molecular characterization of isolated toxigenic molds. *BVMJ.* 27, pp.9-20.
- [50] APHA (American Public Health Association), 2004. *Standard Methods for Examination of Dairy Products*. 17th ed. Washington DC.
- [51] Silva, N.D., Taniwaki, M.H., Junqueira, V.C.A., Silveira, N.F.D., Okazaki, M.M., Gomes, R.A.R., 2019. *Microbiological examination methods of food and water a laboratory manual*, 2nd ed. London, UK: CRC Press, Taylor & Francis Group.
- [52] Tanweer, A.G., 2011. Effect of MAP on microbiological quality of Mozzarella cheese stored in different packages at 7±1 °C. *J Food Sci Technol.* 48, pp. 120–123.
- [53] Francesca, L., Alyxandra, A., Giorgia, B., Francesca, R.P., Alberto, M., and Giovanni, A., 2014. Microbiological safety and quality of Mozzarella cheese assessed by the microbiological survey method. *J. Dairy Sci.* 97, pp. 46–55.
- [54] Garbaj, A.M., Naas, H.T., Gammoudi, F.T., Moawad, A.A., 2007. Bacteriological quality of Mozzarella cheese sold in Tripoli governorate. *Beni-Suef Vet. Med. J.* 17, pp. 99-104.
- [55] El-Diam, M.S.A., El-Zubeir, I.E.M., 2006. Comparison of microbiological quality of processed and non-processed Sudanese white cheese. *Res. J. Microbiol.* 1, 273-279.
- [56] Halim, E.Y.A., El-Essawy, H., Awad, A.A.N., El-Kutry, M.S., Ahmed, L.I., 2022. Estimating the microbial safety and sensory characteristics of some imported dairy products retailed in the Egyptian markets. *Adv. Anim. Vet. Sci.* 10, pp. 488-499.
- [57] Ostyn, A., De Buyser, M.L., Guillier, F., Groult, J., Felix, B., Salah, S., Delmas, G., Hennekinne, J. A., 2010. First evidence of a food poisoning outbreak due to staphylococcal enterotoxin type E, France, 2009. *Euro surveillance.* 15, pp. 1-4.
- [58] Marinheiro, A. F., Lucas, G. G., Natacha D.C., Helenice, G.O., Cláudio, D.I., Semina, 2015. Microbiological quality of sliced and block Mozzarella cheese. *Ciências Agrárias, Londrina.* 36, pp. 1329-1334.
- [59] Nazem, A.M., El-Maghraby; M.M., Amer, A.A., Hassan, O.M.E., 2010. Quality assessment of different varieties of processed cheese at Alexandria markets. *ALEX. J. VET. Science.* 30, pp.13-19.
- [60] Gran, H.M., Mutukumira, A.N., Wetleson, A., Narvhus, J.A., 2002. Small holder dairy processing in Zimbabwe: the production of fermented milk products with particular emphasis on sanitation and microbiological quality. *Food Control.* 13, pp. 161–168.
- [61] Soliman, N.M.S., 2002. Hygienic quality of Mozzarella cheese in Egyptian markets. *MVSc. Thesis, Fac. Vet.Med., Cairo University. Egypt.*
- [62] Mohamed, S.M., Abdou, M.A., Elbarbary, A.H., Elbaba, H.A., 2019. Assessment of microbiological quality in some cheese varieties in Egypt. *BVMJ.* 36, pp. 164-174.
- [63] Kamel, M. K., 2002. Microbiological studies on Cheddar and Mozzarella cheeses. *Thesis, M.V.Sc. Benha University, Egypt.*
- [64] Sallam, S., Aly, S., Banna, Al. (2016). Occurrence of *Escherichia coli* and coliforms in processed cheese. *Int. J. Chemtech Res.* 9, pp. 131-139,
- [65] Serna, C.M.B., Jimenez Obando, E.M., García, A.V., Silva, W.J.M.D., Capodifoglio, E., Godoy, S.H.S.D., Sousa, R.L.M.D. and Fernandes, A.M., 2015. Microbiological quality of sliced Mozzarella type cheese from Pirassununga/SP. In *Abstracts. São Paulo: Brazilian Society of Microbiology.*

- [66] Özdemir, A., Şanlı, S., Sardoğan, B., Sardoğan, S., 2020. Determination of Sorbic Acid in Cheese Samples by Rapid HPLC-DAD Method. *Int. J. Anal. Chem.* pp. 1-4
- [67] CFR (Code of Federal Regulations), 2012. Title 21. Part 182. U.S. Government Printing Office. Washington, D.C.
- [68] Silva, M.M., Lidon, F.C., 2016. Food preservatives-An Overview on applications and side effects. *Emir. J. Food and Agric.* 28, pp. 336–373.
- [69] Bush, R., Taylor, S., 2014. Reactions to food and drug additives. In *Middleton's Allergy: Principles and Practice: Eighth Edition*, pp.1340-1356.
- [70] Pitt, J.I., 2006. *Penicillium and related genera*. In: *Food Spoilage Microorganisms*. Blackburn, C.W. (Ed), Published by, Woodhead Publishing Limited, England, pp.437-450.
- [71] Guarino, C., Fuselli, F., La Mantia, A. and Longo, L., 2011. Development of an RP-HPLC method for the simultaneous determination of benzoic acid, sorbic acid, natamycin and lysozyme in hard and pasta filata cheeses. *Food chemistry*. 127, pp. 1294-1299.
- [72] Zamani Mazdeh, F., Sasanfar, S., Chalipour, A., Pirhadi, E., Yahyapour, G., Mohammadi, A., Rostami, A., Amini, M., Hajimahmoodi, M., 2017. Simultaneous determination of preservatives in dairy products by HPLC and chemometric analysis. *Int. J. Anal. Chem.* pp 1-8.
- [73] National Nutrition Institute, 2006. *Food Composition Tables for Egypt*, 2nd ed. ARE. Cairo, Egypt.
- [74] Islam, M.A., Amin, M.N., Siddiqui, S.A., Hossain, M.P., Sultana, F., Kabir, M.R., 2019. Trans fatty acids and lipid profile: A serious risk factor to cardiovascular disease, cancer and diabetes. *Diabetes Metab. Syndr. Clin. Res. Rev.* 13, 1643–1647.
- [75] Ismail, G., Abo El Naga, R., El Sayed Zaki, M., Jabbour, J. and Al-Jawaldeh, A., 2021. Analysis of fat content with special emphasis on trans isomers in frequently consumed food products in Egypt: the first steps in the trans fatty acid elimination roadmap. *Nutrients*. 13, pp.3087.
- [76] Abdel-Ghany, I.H.I., Sakr, S.S., Sleem, M.M., Shaaban, H.A., 2020. The effect of milk fat replacement by some edible oils on chemical composition, antioxidant activity and oxidative stability of spreadable processed cheese analogues. *Int. Res. J. Food Nutr.* 2, pp.6-14.
- [77] Al-Amiri, H.A., Ahmed, N. and Al-Sharrah, T., 2020. Fatty acid profiles, cholesterol composition, and nutritional quality indices of 37 commonly consumed local foods in Kuwait in relation to cardiovascular health. *MedRxiv*, 1-28.
- [78] WORLD HEALTH ORGANIZATION, 2003. WHO Technical Report Series, no. 916 (TRS 916). *Diet, Nutrition and the Prevention of chronic diseases*, Geneva, pp. 87-88.
- [79] Lopes, A.P., Schneider, V.V.A., Montanher, P.F., Figueiredo, I.L., Santos, H.M.C., Maruyama, S.A., Angela, M.M., Visentainer, J.V., 2015. Levels of Soybean Oil and Time of Treatment for Nile Tilapia: a Factorial Design for Total n-3 Fatty Acids, n-6/n-3 and PUFA/SFA Ratios. *J. Brazil. Chem. Soc.* 26, pp. 572-579.
- [80] Simopoulos, A.P., 2016. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. *Nutrients*. 8, 128.
- [81] Abbas, K. A., Abdelmontaleb, H. S., Hamdy, S. M., and Aït-Kaddour, A., 2021. Physicochemical, Functional, Fatty Acids Profile, Health Lipid Indices, Microstructure and Sensory Characteristics of Walnut-Processed Cheeses. *Foods (Basel, Switzerland)*, 10(10), 2274.
- [82] Zahran, H.A., Mabrouk, A. M. M. and Salama, H.H., 2022. Evaluation of Yoghurt Fortified with Encapsulated Echium Oil Rich in Stearidonic Acid as a Low-Fat Dairy Food. *Egypt. J. Chem.* 65 (4), pp. 29 – 41.