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Evaluation of the chemical and microbiological properties of several cheese kinds available in Menoufia Governorate

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ABSTRACT

In this study, a total of 75 random samples of some locally manufactured Egyptian, cheeses were collected from various dairy sources in Menoufia Governorate, Egypt. The samples were represented as kareish cheese, tallaga cheese, and ras cheese (25 samples each). To assess their quality, the samples were examined chemically. Fat%, titratable acidity, total solids, salt content and moisture content and microbiologically for incidence and count of coliforms, E coli, *Staphylococcus aureus* and yeasts and molds. Compared with the Egyptian standards, the examined tallaga cheese samples were found to be of better quality than the other types of cheeses examined. The incidence of Coliforms count in Kareish, tallaga and ras cheese with mean values $8.56 \times 10^3 \pm 3.20 \times 10^3$, $4.30 \times 10^2 \pm 2.62 \times 10^2$ and $4.86 \times 10^2 \pm 2.24 \times 10^2$, respectively. While, the incidence of *Staph. aureus* was (2%), (32%) in kareish cheese and tallaga cheese respectively. In Kareish, tallaga, and ras cheese, the mean yeast and mold count was $3.84 \times 10^3 \pm 2.42 \times 10^3$, $1.76 \times 10^3 \pm 1.04 \times 10^3$, and $3.18 \times 10^3 \pm 1.88 \times 10^3$CFU/g, respectively. While chemical result of fat/ dry matter % in all examined cheese samples were 47.8± 1.44, 44.9± 1.44 and 43.4±0.83 in kareish, tallaga and ras cheese, respectively. While the mean values of moisture % were 62.4±1.61, 46.7±0.56 and 39±1.08 and total solid % were 26.6 ±0.66, 36.3±0.58, 26.6±0.66 and 63.3±1.15 in examined Kareish, tallaga and ras cheese samples, respectively. Although all types of the present study soft cheeses need to be improved microbiologically. The design and facilities of food establishments producing Egyptian kareish cheeses should be in compliance with licensing requirements and conditions stated by the international standards. The data obtained revealed unsanitary circumstances throughout the manufacturing, handling, and distribution of various cheese kinds; thus, we advocate the implementation of stringent sanitary procedures throughout the phases of storage and production.

1. INTRODUCTION

Dairy products are the main regular diets which favorable consumed in Egypt and all over the world (Anema, 2020). Cheese has a long history as an important part of the Egyptian diet of both young and old people; it is an brilliant source of bioactive peptides, fat, minerals, vitamins and amino acids (Walther et al., 2008 and IDFA, 2010). Improvements in technology resulted in a wide range of cheeses available in the market, differing in texture and flavor. (Abd-Elhamid, 2012 and Brown, 2004)

Kareish cheese is a highly favored cheese variety in Egypt, particularly in rural areas, due to its high protein content, low fat, and affordability (Metwalli, 2011). The majority of kareish cheese is manufactured by dairy farmers, who often do not follow the proper hygiene precautions. (Moharram et al., 2018). Tallaga (white soft) cheese has a high moisture content and soft creamy consistency. This cheese must be stored in low temperature conditions. The shelf life of tallaga cheese affected by salt, enzyme activity and microbial proliferation during storage (Hamad, 2015).

Ras cheese (Romy) is Egypt's national hard cheese variety, and like Greek cheeses, it is manufactured in a large percentage from cow's or a blend of cow's and buffalo's milk, without employing starter cultures, and marketed when it has a strong harsh taste after 3 to 6 months. (Abou-Donia, 2008 and Ahlam *et al* 2014).

The microbiological quality and shelf-life of cheese are influenced by the initial flora of raw milk, equipment, storage temperature, transportation, and environmental hygiene during manufacturing, packaging, and handling (Barukčić et al., 2020)

Coliform bacteria, the most prevalent pollutants in raw milk and dairy products, are widely utilized as a quality indicator (Wolfe et al., 2014). However, coliforms are easily destroyed by heat treatments of milk, and their presence in dairy products indicates pasteurization failures or post-pasteurization contamination (Okura et al., 2010).

Escherichia coli is a common contaminant and a good predictor of faecal contamination in unclean dairy products (Thaker et al. 2012).

Staphylococcus aureus is a foodborne pathogen that can cause staphylococcal food poisoning, toxic shock syndrome, pneumonia, surgical wound infection, and nosocomial bacteremia (FDA, 2012).

Staph aureus contamination can be identified in raw milk obtained from mastitis-infected cows or food handlers as a result of unsanitary procedures (Bingol et al., 2012). Fungal growth is a typical issue in cheese manufacturing, and it spreads rapidly on the cheese surface during ripening and

storage. The majority of these fungus might cause human and animal disease or create poisons by eating contaminated food can lead to gastrointestinal infections and Some fungi can invade the skin or mucous membranes. (Ghibaudo and Peano 2010).

Given the preceding facts, the goal of this work was to assess the chemical test were moisture content, total solid percent, titratable acidity, fat and dry matter percent and microbial features are isolation and identification of *E. coli*, *Staph aureus*, yeast and mold, isolated bacteria by using PCR technique.

2. MATERIALS AND METHODS

All methods used in this study was approved by Scientific Research Ethics Committee, faculty of Veterinary Medicine, Benha University (Ethical Approval Number: BUFVTM05-06-24).

2.1. Collection of samples:

A total of 75 marketed random samples of kareish cheese, tallaga cheese, and Ras cheese of different brands (25 of each) were gathered from various stores and supermarkets in Egypt's Menoufia Governorate. All samples were collected aseptically, stored in an insulated icebox, and delivered to the lab. Each sample was separated into two portions, the first for chemical analysis and the second for microbiological analysis.

2.2. Chemical examination:

Fat% was determined using ISO 3433:2008 (IDF 222:2008), titratable acidity was determined using A.O.A.C. 2005, moisture content was determined using Bradley and Vanderwarn (2001), total solids were determined using ISO 5534:2004, and salt content in cheese was determined using IDFA standards (2010).

2.3 Microbiological examination:

2.3.1 Preparation of samples: According to Roberts and Greenwood (2003) 10 g from each sample was homogenized in 90 mL of a sterile 2% worm sodium citrate solution for 1 minute. Decimal dilutions were made in 0.1% sterile peptone water, and suitable Tenth fold dilutions were employed for count the following

2.3.2 Coliform count: on violet red bile agar (LABM) plates, as described by the FDA (2002).

2.3.3 *E. coli* count: on Eosin Methylene Blue (E.M.B.) agar at 35-37°C Incubation within 24 hours according to the FDA (2002).

2.3.4 Staphylococcus aureus: was counted after 24 to 48 hours of incubation at 37°C using Baird Parker media using Antibiotics, Potassium Tellurite, Lecithin, Buffering Agents as supplements (Oxoid 2006).

2.3.5 Mold and yeast count: on Potato Dextrose Agar Media (PDA) at 25°C for 3-7 days
based on (APHA 2004).

2.4 Molecular identification of several virulent genes in isolated strains

2.4.1. E. coli primer sequences utilized for the PCR identification technique. using primers as indicated in Table (1).

2.4.2 Primer sequences of Staph. aureus used for PCR system: using primers as shown in table (2).

Table (1): Genes and primers targeted for detecting *E. coli* isolates

Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
stx1 (F)	5′ ACACTGGATGATCTCAGTGG ′3	614	Dhanashree and Mallya (2008)
Stx1 (R)	5′ CTGAATCCCCCTCCATTATG ′3		
Stx2 (F)	5′ CCATGACAACGGACAGCAGTT ′3		Dhanashree and Mallya (2008)
Stx2 (R)	5′ CCTGTCAACTGAGCAGCACTTTG ′3	779	
eaeA(F)	5′ GTGGCGAATACTGGCGAGACT ′3	890	Mazaheri *et al*. (2014)
eaeA (R)	5′ CCCCATTCTTTTTCACCGTCG ′3		
hylA (F)	5′ ACGATGTGGTTTATTCTGGA ′3	165	Fratamico *et al.* (1995)
hylA (R)	5′ CTTCACGTGACCATACATAT ′3		

Table (2): Genes and primers targeted for detecting *Staph. aureus* isolates

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
sea (F)	5′ TTGGAAACGGTTAAAACGAA′3	120	Rall *et al.* (2008)
sea (R)	5′ GAACCTTCCCATCAAAAACA ′3		
seb (F)	5′ TCGCATCAAACTGACAAACG ′3	478	
seb (R)	5′ GCGGTACTCTATAAGTGCC ′3		
sec (F)	5′ GACATAAAAGCTAGGAATTT ′3	257	
sec (R)	5′ AAATCGGATTAACATTATCC ′3		
sed (F)	5′ CTAGTTTGGTAATATCTCCT ′3	317	
sed (R)	5′ TAATGCTATATCTTATAGGG ′3		

2.5 Statistical analysis

The findings were presented using one-way ANOVA analysis. Mean differences were assessed for significance ($P<0.05$) following Hill and Lewicki's (2007) methodology. The data was statistically analyzed using ANOVA.

3. RESULTS

Table (3) demonstrated that, the mean values of fat/dry matter% in all tested cheese samples were 47.8 ±1.44, 44.9 ±1.44 and 43.4 ±0.83 for kareish, tallaga and ras cheese, respectively. The mean moisture % were 62.4 ±1.61, 46.7±0.56 and 39±1.08 while total solid % were 26.6 ±0.66, 36.3±0.58, and 63.3 ±1.15 in examined Kareish, tallaga and ras cheese samples, respectively.

Table (3) Statistical analytical data of fat/dry matter, T.s%, and moisture content of several analyzed cheese samples.

Cheese samples	Fat/ dry matter%	T.S%	Moisture content
Kareish	47.8 ± 1.44	26.6 ± 0.66	62.4 ± 1.61
Tallaga	44.9 ± 1.44	36.3 ± 0.58	46.7 ± 0.56
Ras(romy)	43.4 ± 0.83	63.3 ± 1.15	39 ± 1.08

The results in Table (4) indicated that, the mean Titratable acidity% of the investigated kareish, tallaga, and ras cheese samples were 1.57± 0.03, 0.78± 0.04, and 1.08± 0.10, respectively. While the average salt percentage in the examined cheese samples was 1.03 ± 0.05, 2.5 ± 0.27, and 5.09 ± 0.40

Table (4) Statistical analytical data of titratable acidity and salt% of different cheese samples

Cheese samples	Titratable acidity	Salt%
Kareish	1.57 ± 0.03	1.03 ± 0.05
Tallaga	0.78 ± 0.04	2.5± 0.27
Ras(romy)	1.08± 0.10	5.09 ±0.40

Table 5 showed the findings of coliform counts in cheese samples. The mean values of Coliforms count in Kareish, tallaga and ras cheese were 8.56 ×103 ± 3.20×103, 4.30×102 ± 2.62 ×102 and 4.86×102 ± 2.24 ×102, respectively. Similarly, the results of *Staph. aureus* and *E. coli* counts revealed that, the % positive samples of *Staph. aureus* and *E. coli* counts in Kareish cheese samples were higher than those of tallaga cheese followed by ras (romy) samples (table 6 and 7). Table 8 showed the findings for yeast and mould counts in cheese samples. The lowest percentage of positive samples (32%) and low mean count ($1.76 \times 10^3 \pm 1.04 \times 10^3$) were reported in tallaga cheese samples, followed by Ras (romy) cheese (40%) and mean count value ($3.18 \times 10^3 \pm 1.88 \times 10^3$) and Kareish cheese samples (44%) with the highest mean count ($3.84 \times 10^3 \pm 2.42 \times 10^3$). Table 9 illustrated the results of comparison of coliforms, microbiological quality and safety, yeast, and moulds in different studied soft cheeses with the Egyptian standard of 2005. As demonstrated in table 9, 52% and 56% of Kareish cheese samples met Egyptian criteria for coliform and *Staphylococcus aureus* counts respectively. While 76% and 68% of tallaga cheese samples and 80% of ras (romy) cheese samples met Egyptian standard for coliform count and *Staphylococcus aureus*, respectively. In contrast, 40% of Kareish cheese, 32% of tallaga cheese, and 28% of Ras (romy) cheese samples met Egyptian standard for *E coli*. Moreover, the percentage of samples comply with Egyptian standards for yeast and moulds were 56, 68 and 60% for Kareish cheese, tallaga cheese and ras cheese samples respectively.

Table (5) shows the statistical analysis findings of Coliforms count/g in several varieties of cheese .

Cheese samples	+ve samples No.	+ve samples %	Min	Max	Mean ± S.E.M.
Kareish	12	48	5.3×10^2	4.6×10^4	$8.56 \times10^3 \pm 3.20\times10^3$
Tallaga	6	24	2.7×10^2	$5.8\ 10^3$	$4.30 \times10^2 \pm 2.62\times10^2$
Ras(romy)	5	20	2.4×10^2	3.4×10^3	$4.86 \times10^2 \pm 2.24\times10^2$

Table (6) shows the statistical analysis findings for *Staph. aureus* count/g of several varieties of cheese.

Cheese samples	+ve samples No.	+ve samples %	Min	Max	Mean ± S.E.M.
Kareish	11	44	2.5×10^2	6.7×10^4	$7.78 \times10^3\pm3.62 \times10^3$
Tallaga	8	32	2.3×10^2	3.5×10^4	$4.15 \times10^2\pm 1.90\times10^3$
Ras(romy)	5	20	3.3×10^2	5.3×10^3	$4.03 \times10^3\pm2.67 \times10^2$

Table (7) shows the statistical analysis findings of *E. coli* count/g of several varieties of cheese

Cheese samples	N	No of +ve samples	% of +ve samples
Kareish	25	10	40 %
Tallaga	25	8	32%
Ras (roomy)	25	7	28%

Table (8) shows the statistical analysis findings for yeast and mould count per gram of different varieties of cheese.

Cheese samples	+ve samples No.	+ve samples %	Min	Max	Mean ± S.E.M.
Kareish	11	44	2.5×10^2	5.2×10^4	$3.84\times10^3\pm2.42 \times10^3$
Tallaga	8	32	3.6×10^2	2.5×10^4	$1.76\times10^3 \pm 1.04 \times10^3$
Ras	10	40	2.5×10^2	3.9×10^4	$3.18\times10^3 \pm1.88 \times10^3$

Table (9) compares the findings of coliforms, The microbial quality and safety, yeast, and moulds in different investigated soft cheese with the Egyptian standard, 2005.

Cheese samples	Number of examined samples	Egyptian Standard, (2005)	Comply with Egyptian Standards NO	Comply with Egyptian Standards %	Not comply with Egyptian Standards NO	Not comply with Egyptian Standards %
Coliforms count						
Kareish	25	10 cfu / g	13	52	12	48
Tallaga	25	10 cfu / g	19	76	6	24
Ras(romy)	25	10 cfu / g	20	80	5	20
Staphylococcus aureus						
Kareish	25	free	14	56	11	44
Tallaga	25	free	17	68	8	32
Ras(romy)	25	free	20	80	5	20
E.coli						
Kareish	25	free	10	40	15	60
Tallaga	25	free	8	32	17	68
Ras(romy)	25	free	7	28	18	72
Yeast and moulds						
Kareish	25	Not more than 10 cfu/g	14	56	11	44
Tallaga	25	Not more than 10 cfu/g	17	68	8	32
Ras(romy)	25	Not more than 10 cfu/g	15	60	10	40

The result of PCR identified several virulent genes in isolated *E. coil* and *Staph. aureus* strains.

The stx1(614 bp), stx2 (779 bp), eaeA (890 bp) and hlyA (165 bp) genes were found in Enteropathogenic *E. coli* isolates (Fig. 1). While the sea (120 bp), seb (478 bp), sec (257 bp) and sed (317 bp) enterotoxin genes were identified *Staph. aureus* isolates (Fig. 2)

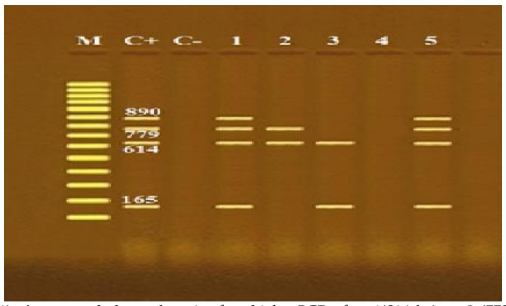

Fig (1): Agarose gel electrophoresis of multiplex PCR of *stx1*(614 bp), *stx2* (779 bp), *eaeA* (890 bp) and *hlyA* (165 bp) genes for characterization of Enteropathogenic *E. coli.*

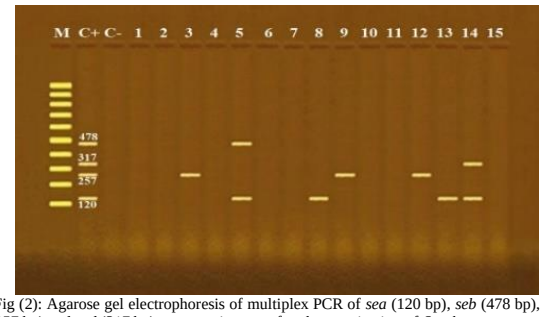

Fig (2): Agarose gel electrophoresis of multiplex PCR of *sea* (120 bp), *seb* (478 bp), *sec* (257 bp) and *sed* (317 bp) enterotoxin genes for characterization of *Staph. aureus.*

4. DISCUSSION

Cheese is the most popular dairy product, manufactured in a wide range of types across the world due to its delicious taste and variety of flavors. It is a dairy product with the highest nutritional content and health-care function. However, many processed cheeses are derived from lower-quality cheeses that may have been manufactured under poor conditions.

In the present study, table 3 displays the findings of the chemical analysis of the cheese samples. The mean fat/dry matter values for Kareish, Tallaga, and Ras(romy) cheese samples are nearly similar. Meanwhile, total solid % values were highest in Ras (romy) cheese, followed by Tallaga cheese samples, and lowest in Kareish cheese samples. In contrast, Kareish cheese had the greatest moisture level, followed by Ras (romy) and Tallaga cheese samples. Approximately similar results were reported by (Salwa and Galal, 2002) and Ismail *et al.,* (2011), Difference of moisture of cheese may be due to temperature variance during cooling or storage, salt percent

According to the Egyptian standard for soft cheese (1008 - 3 / 2005), moisture content must not exceed 60%, salt content must be within 9%, protein content at least 10%, and fat to dry matter ratio must be 45% or less than 60%.

In this work, table 4 showed that, kareish cheese samples had a highest mean titratable acidity value than the ras and tallaga cheese samples. Meanwhile, ras cheese samples had the greatest average salt percentage, followed by tallaga, while kareish cheese samples had the lowest salt percentage. The highest acidity% in the examined Kareish cheese samples may be attributed to the method of its manufacture, allowing lactic acid bacteria or other bacteria to grow and produce acid (Amer 2002). The reduced relative variability in acidity% across the investigated cheese samples might be due to differences in production method, ripening times, and storage temperature. In general, delayed manufacturing processes, lengthy ripening periods, and heated storage temperatures enhance the acidity of cheese being delayed can lead to increased microbial activity, especially from spoilage organisms and pathogens. (Mohamed, 2016)

Contamination of fermented milk products with bacteria has major consequences for both end product safety and foodborne pathogen transmission to consumers (Owusu-Kwarteng et al.,2020).

The microbial quality and safety of cheese is a serious concern for both producers and consumers. It is determined by organisms acquired from raw milk, the processing efficiency, and the sanitary practices used in the dairy facility. Milk handling in cheese production contributes significantly to the expansion of microbial flora, rendering the product unsuitable for human consumption *(*Yousef *et al*. 2001 and Leuschner and Boughtflower, 2002).

In this study the findings of coliform counts in cheese samples showed that, the highest mean percentage of positive samples and mean count of coliform was reported in Kareish, followed by tallaga and ras cheese samples.

Similarly, the results of *Staph. aureus* and *E. coli* counts revealed that the % positive samples of *Staph. aureus* and *E. coli* counts in kareish were higher than those of tallaga cheese followed by ras samples. According to the results of coliforms counts, the higher results were shown by Ahmed *et al*., (2018). The Egyptian Standard (2005) requires a coliform level of no more than 10 cfu/g. However, 48% of Kareish, 24% of Tallaga, and 20% of Ras cheese were rejected owing to higher coliform counts than this limit.

Staphylococcus aureus is a major pathogen in food poisoning due to its widespread distribution and the capacity of various strains to produce one or more enterotoxins (Normanno et al., 2012). The incidence of *Staph. aureus* was not comparable to that reported by Ghada et al., (2002); *Staph. aureus* was isolated from just one of the tested home or farmer-made kareish cheese (2%). Furthermore, the obtained incidence was lower than that found by Abo El-Makarem (2018) and Sayed-Ahmed (2020).

Staphylococcus aureus was detected in 32% of tallaga cheese samples, that were lesser than which mentioned by Ibrahim *et al.* (2015) and Eltokhy, and Abdelsamei (2021), and higher than those of Hassan and Gomaa (2016). All isolates *Staph. aureus* detected by PCR tested positive for sea, seb, sec, and sed primers.

The presence of *E. coli* in dairy products is utilized as an indicator of manufacturing cleanliness, water quality, poor heat treatment of raw milk used in cheese production, or post-pasteurization contamination during transit, handling, and sale (Metz et al., 2020). Table (7) shows that *E. coli* was detected in 40% of Kareish cheese. Similar results were obtained by Mohammed (2020) and Sayed-Ahmed (2020), while Meshref and Hassan (2009), Basha et al., (2012), Elbagory et al., (2015), Salem et al., (2016), and Kamal et al., (2017) reported higher incidences of 56, 86, 37.5, 48, and 45%, respectively. *E. coli* was found in 32% of the tested white cheese samples, which was greater than the records of Sallam et al. (2016) and Elzhraa et al. (2021), and in 28% of the analyzed Ras cheese samples, which was higher than the records of Mohamed et al. (2020). All isolated *E. coli* identified by PCR all are positive for *stx1*, *stx2*, *eaeA* and *hlyA* primers.

In Kareish, tallga, and ras cheese, the mean yeast and mold count was lesser than that mentioned by Ahmed et al., (2018) and more than those carried out by Hakim et al., (2013). According to EOSQC (2005), the acceptable yeast count in Kareish cheese is 10 cfu/g or below. Approximately 44, 32, and 40% of Kareish, Tallaga, and Ras cheese samples were over the acceptable limit.

Mislivec et al. (1992) found that yeast and mold development in cheese can cause defects such as off-flavor, color, and rot.

5.CONCLUSIONS

Non-heated milk and its products have a dangerous aspect in the spread of harmful germs as *E. coli* and *S. aureus* to humans. Heat treatment of milk and cheese-making components is preferred because it helps to reduce bacterial, yeast, and mould development and mycotoxin generation. PCR-based detection of food-borne bacteria is an essential technology for accurately, quickly, and precisely detecting contamination of milk and dairy product with food poisoning bacteria. Tallaga cheese had the lowest incidence and contamination for total staphylococci, yeast, and coliform count, whereas mold contamination was highest soft cheese, white cheese, and ras cheese.

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