Chemical and Microbial Safety Criteria for Egyptian Ras Cheeses

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ABSTRACT

Until now, most of the Ras cheese has been produced by small dairy sectors in Egypt using raw milk. This research aimed to determine the pathogens and undesirable microorganisms in traditional Egyptian Ras cheese using classical methods and real-time PCR. Forty samples of Ras cheese were analyzed for chemical and microbiological properties. The results revealed a wider range in the chemical composition of the collected samples. Most of the Ras cheese samples were within the Egyptian standard for chemical compositions. There were only two samples of Ras cheese that had more moisture than the Egyptian Standard recommended. The results of the microbiological analysis of the Ras cheese showed that the coliform count ranged between 1 and 5.20 log CFU/g, with an average of 1.70 log CFU/g. Anaerobic spore-forming bacteria (*Clostridium perfringens*) were found in 29 out of 40 Ras cheese samples. All Egyptian cheese samples contained more yeast and mold than the Egyptian Standard recommends. All cheese samples were free of *Listeria monocytogenes* and *Salmonella* spp. The real-time PCR showed that 5% of samples were positive for methicillin-resistant *Staphylococcus aureus*, and all the analyzed samples were free of *E. coli* O157:H7. According to this study's findings, most Ras cheese samples contained higher levels of *Staphylococcus aureus*, molds, and yeasts than what Egyptian standards recommended. The results of this study should be used to develop specific procedures for risk management along the milk production chain.

Key words: Ras cheese, chemical composition, *Listeria monocytogenes*, *Salmonella*, methicillin-resistant *Staphylococcus aureus*, *E. coli* O157:H7.

INTRODUCTION

In Egypt, Ras cheese is the most widely consumed hard cheese. It resembles the Greek cultivar "Kefalotyri" (Phelan *et al.,* 1993). Ras cheese is traditionally made in small dairy sectors located in Egyptian villages. It is manufactured without the use of starter cultures from raw cow's milk or raw cow's and buffalo's milk mixtures (Awad *et al.*, 2003 and El-Hamshary *et al.*, 2022). Native bacteria from the environment and raw milk are responsible for fermentation during manufacturing. Additionally, Ras cheese is typically kept in wet, unclean conditions that encourage the development of mold and yeast. As a result, the actions of all these components will have an impact on the ultimate flavor and texture (Ayad *et al.*, 2004).

Ras cheese is a traditional hard type of cheese that is frequently manufactured from raw milk. It is traditionally made by rennet coagulation, and the natural flora are responsible for the acidity developing as well as the formation of the cheese's flavor and texture. Ras cheese's moisture content shouldn't be lower than 40% (Egyptian Standard ES: 1007-P5/2005).

Traditional fermented dairy products have complex and incompletely described microbial populations. Traditional cheeses' sensory qualities are thought to be mostly dependent on microbial diversity. But some individuals in these microbial ecosystems might also pose a health risk (Montel *et al.,* 2014).

Pathogenic bacteria and spoiling microorganisms are the two main categories of unwanted milk microbes. Spore formers, Coiform bacteria, as well as a variety of yeasts and molds could be contaminated raw milk from the environment or from infected udders (Dubey *et al.*, 2022). It is occasionally possible to find harmful bacteria in raw milk, such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella spp.*, *Mycobacterium bovis*, and *Corynebacterium bovis* (Bramley & Dodd, 1984 and Bramley *et al.*, 1990).

Ras cheese is typically made in an artisanal manner with raw milk that hasn't been heat-treated. As previously reported, the complex ecology of traditional Ras cheese is made up of a variety of microorganisms, including yeasts, molds, *Staphylococcus* ssp, Coliform, fecal enterococci, and *Enterobacteriaceae*, all of which *were* found in high numbers (Hassan *et al.*, 2019).

Most traditional dairy products do not meet microbiological quality standards because of the unsanitary circumstances of manufacturing, storage, and handling (Lotfy *et al.*, 2023). Cheeses with a high vulnerability to microbial contamination cause financial loss and health hazards. Additionally, food spoilage from microbial sources can result in off flavors and odors through enzymatic pathways, producing non-acceptable cheese for consumers (El-Fadaly *et al.*,

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2015). During the ripening of Ras cheese, fungus growth on the cheese surface causes economic losses and quality problems (Sharaf *et al.*, 2019).

The purpose of this study was to determine the situation of the quality and safety of traditional Egyptian Ras cheeses. The microbiological safety assessments were carried out using classical methods and real-time PCR.

MATERIAL AND METHODS

Forty samples of Ras cheese were collected from different geographical locations in Egypt: Beheira (6 sample), Alexandria (12 samples), Damietta (3 samples), Gharbia (6 samples), Cairo (10 samples), and Monufia (3 samples). The samples were collected in retail packages and transferred in an ice chest to the laboratory for analysis within 24 hours.

Physicochemical analysis of cheese

The pH value of samples was determined at room temperature using a laboratory pH meter model (Basic 20 pH). The dry matter was determined using oven drying at 102 °C, fat was determined by Gerber method, and sodium chloride was determined by Volhard's method (AOAC, 2020). Protein was determined by Kjeldahl method and convert the nitrogen to protein using 6.38 factor according to the ISO 8968-1 (2014).

Fatty acid profile analysis

Fat content was extracted from cheese samples by organic solvents, and the fatty acid methyl esters were prepared according to the procedures of El-Nabawy *et al.* (2023). Gas chromatographic analysis was carried out using the ACME model 6100GC (Young Lin Instrument Co., Korea), fitted with a split injector and FID detector. The procedure published by El-Nabawy *et al.* (2023) was followed to determine the individual fatty acids in cheese fat.

Microbiological analysis

Ten grams of each sample were homogenized with 90 ml of sterile 2% sodium citrate solution using stomacher equipment. Serial dilutions were prepared according to ISO 6887-1 (2017): Microbiology 16b (ISO 6887-5: 2020).

Enumeration of Coiform bacteria

The enumerations of coliform bacteria were carried out on Violet-red bile lactose agar medium (Oxoid, England). Plates were incubated at 30 °C for 24 h (ISO 4832: 2006).

Enumeration of beta-glucuronidase-positive *Escherichia coli*

Appropriate dilutions were prepared in peptone saline solution (0.85%) then plated on selective medium; Tryptone Bile X-Glucuronide agar (TBX, Biolife, Italy). All plates were incubated at 44 °C for 24 hours. After the period of incubation, blue or blue green colonies were calculated per gram according to ISO 16649-2 (2001).

Enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species)

A specified quantity of decimal dilutions was suspended on the plates' surface of Baird Parker agar (LAB, United Kingdom). The plates were incubated under aerobic condition at 37 °C and examined after 48 hours. Typical colonies on Baird Parker medium were counted which had black to gray, brilliant and convex (1 to 1.5 mm in diameter after 24 hours of incubation and 1.5 to 2.5 mm after 48 hours of incubation and surrounded by a clear zone (it can be partially opaque) then confirmed by coagulase test using dry-spot staphytect (Oxoid, England). After purification, the colonies were inoculated in 10 ml of brain heart infusion broth (Biolife, Italy) then purified in Baird Parker agar before coagulase test was performed according to ISO 6888-1(2021).

Detection of *Salmonella* spp.

The detection and enumeration of *Salmonella* spp. in Ras cheese samples were carried out according to ISO 6579-1: 2017/Amd 1 (2020**).** Biochemical testing (Triple Sugar Iron Agar, urea agar and L-lysine decarboxylation medium, β-galactosidase and indole reaction), and serological testing O-antigen, Vi-antigen and H-antigen, were used to confirm the *Salmonella* spp.

Detection of *Listeria monocytogenes* and *Listeria* spp.

The detection and enumeration of *Listeria* spp. in cheese samples were carried out according to ISO 11290-1 (2017). Primary enrichment in selective half-Fraser broth (Biolife, Italy) with reduced concentration of selective agents (lithium chloride, acriflavin and nalidixic acid) was inoculated with the food sample (1g) then incubated at 30°C for 24 hours. Secondary enrichment in selective Fraser broth (Biolife, Italy). Cultivation on selective media**:** Cultures from the primary and secondary enrichment were streaked over two selective media; Oxford Agar (Biolife, Italy) and Agar Listeria. The agar plates were incubated at 37 °C for 24 hours, if necessary, at 48 hours for presence of characteristic colonies that may be *Listeria monocytogenes*. Typical colonies of *Listeria monocytogenes* on Oxford agar after 24 hours of incubation are small (1mm) and of gray/black color with black halo. After 48 hours, the colonies are black with a black halo. Typical colonies of *L. monocytogenes* on ALOA agar were green-blue and have zone of lipolysis around them (opaque halo or even no halo at all in case of stress, especially of acid stress). Confirmation**:** The presumptive colonies were streaked onto tryptone soya

yeast extract broth (Biolife, Italy) then incubated at 37°C for 24 typical colonies were confirmed with appropriate morphological test (Gram positive slim and short rods) and some biochemical tests such as catalase test (3%), the positive catalase reaction was indicated to be *L. monocytogenes*.

Count of *Clostridium perfringens*

The procedure of bacterial counting on Sulfite-Cycloserine agar (SC, Biolife, Italy) to detect *Clostridium perfringens* was carried out according to ISO 7937 (2004).

Count of yeasts and molds in Ras cheese

Yeast and molds enumeration were carried out on Oxytetracycline Glucose Yeast Extract agar (O.G.Y.E agar, Oxid, United Kingdom) according to ISO 6611 (2004). Appropriate dilutions were prepared in peptone saline solution (0.85%) then plated on oxytetracycline glucose yeast extract agar and incubated at 25°C for five days in aerobic conditions.

Detection of *methicillin*-resistant *Staphylococcus aureus* in Egyptian Ras cheese

Methicillin-resistant *Staphylococcus aureus* is tested in Ras cheese samples using the Biotium PMA Real-Time PCR Bacterial Viability Kit (*Staphylococcus aureus* methicillin-resistance gene *mecA).* DNA was extracted from the pure colonies of confirmed *Staphylococcus aureus* coagulase-positive *Staphylococcus aureus* using the kits and protocol recommended by Biotium, USA [PMA Real-Time PCR Bacterial Viability Kit – Staphylococcus aureus (nuc) The positive control reference strain, MRSA (methicillin-resistant *Staphylococcus aureus*), isolated by the Animal Health Research Institute, Ministry of Agriculture, Alexandria, was used as a reference strain.

Detection of *Escherichia coli* O157:H7 in Egyptian Ras cheese

The detection of *Escherichia coli* O157:H7 in all samples was carried out by the PMA Real-Time PCR Bacterial Viability Kit for *E. coli* O157:H7 (Z3276). DNA was extracted using the kits and protocol recommended by Biotium, USA (PMA Real-Time PCR Bacterial Viability Kit – E. coli O157:H7 (Z3276). A kit contains a viability PCR dye (PMAxx™ or PMA) and primers to amplify the Z3276 gene of *E. coli* O157:H7.

RESULTS AND DISCUSSION

Forty samples of Egyptian Ras cheese were analyzed for physical properties (pH) and chemical compositions (moisture, fat, salt, and proteins). The pH value of all analyzed samples was in the range of 4.81–5.58, with an average of 5.3 ± 0.18 (Table 1). The acidity was in the range of 1.3–3%, with an average of 1.88% ± 0.48. The average moisture content was 31.48% ± 3.73; it was recorded in a range of 23.65 to 41.84%. The Egyptian standard[4] recommended that the moisture in Ras cheese should not exceed 40%; only 2 samples of Ras cheese (out of 40) had a moisture content higher than 40%. The average moisture contents of the examined cheese samples were in agreement with those found by some researchers (Awad *et al.,* 2003; Ayad *et al.,* 2004; Awad, 2006 and Awad *et al.,* 2007). Since the samples were collected from local markets, the moisture in fresh Ras cheese is about 40%, but it decreased gradually during the storage period due to evaporation of non-packed cheese, so the low moisture level is usually in old samples (Awad *et al.,* 2003).

The fat in dry matter (F/D.M. %) should not be less than 45% in Ras cheese **(**Egyptian Standard ES: 1007-P5/2005). The obtained data showed that the fat in dry matter ranged from 45.38 to 61.41%, with an average of 55.15± 4.9. The F/D.M. % of all Ras samples is higher than that (45%), which meets the recommended level by the Egyptian Standard ES: 1007-P5 (2005). There is a large variation in the percentages of fat in the dry matter of the collected samples, depending on the fat content of the raw milk and/or the type of milk (cow or a mixture of cow and buffalo) used to make the cheese (Awad *et al.,* 2003). The same conclusion was also reported by Amer *et al.* (2023).

Table 1. Physiochemical properties of Ras cheese*

Sample	pH	Acidity%	Salt %	Salt / moisture %	Moisture %	Fat%	Fat/DM	Protein%
Minimum level	4.81	1.3	4.68	14.49	23.65	27	45.38	17.05
Maximum level	5.58	3	7.86	27.39	41.84	43	61.41	32.91
Average	5.30	1.89	6.47	20.89	31.48	37.92	55.15	25.64
SD	0.18	0.48	0.95	4.09	3.73	4.35	4.91	2.78

*The analyzed samples were 40, each sample was analyzed 3 times.

The salt level was in the range of 4.68 to 7.89%. The Egyptian Standard ES: 1007-P5 (2005). does not recommend the salt levels in Ras cheese. These values were higher than those reported by Osman *et al.* (2011) for cheese samples that were collected from Assiut, as well as higher than those in samples collected from Alexandria (Awad *et al.*, 2003). This demonstrated that Ras cheese now contains more salt than it did in previous decades.

The protein in traditional Egyptian Ras cheese was in the range of 17.05 to 32.91%, with an average of 25.63% ± 2.78; it was lower in 4 samples (10% of samples) than that recommended by Egyptian Standard ES: 1007-P5 (2005) which should be about 24%. The protein percentages in the samples that have been collected vary greatly, and this also depends on the type of milk that was used to manufacture the cheese and/or the protein concentration of the raw milk.

Detection of replacing milk fat with vegetable oil in Ras cheese

Table (2) shows that five samples of Ras cheese out of 40 contain palm oil, as the levels of short-chain fatty acids (C4-C10) and C14:0 myristic acid are lower and C16:0 palmitic acid is higher than in butter oil. Results of the current study agree with Elaaser (2017), who found that milk fat samples from local Cairo markets had an apparent increment in the palmitic (C16:0) and a decrease or absence in some other fatty acids, such as (C4:0), (C6:0), (C8:0), and (C10:0), or fatty acids that were found in low content, such as (C12:0) and (C14:0), and concluded that this sample characterized shortening palm oil. Additionally, Calvo *et al.* (2007) found that the fatty acid profile of the cheese fat was significantly altered by the replacement of vegetable oils for milk fat in many processed varieties of cheese sold in Egypt..

Table 2. Detection of replacing milk fat with vegetable oil of Ras cheese

	Standard		**Normal samples\35 samples**		**Non-normal samples/ 5 samples**	
Fatty acids	**Palm oil**	**Butter oil**	**Average**	**SD**	**Average**	**SD**
Saturated fats (% of total fatty acids)						
Butyric acid, C4:0	0.00±0	2.12±0.52	2.06	0.06	1.61	0.18
Caproic, C6:0	0.00±0	1.32±0.21	1.28	0.09	0.83	0.06
Caprylic acid, C8:0	0.27±0.1	0.91±0.11	1.08	0.49	0.67	0.11
Caproic acid, C10:0	0.00±0	2.03±0.12	2.48	0.32	1.16	0.03
Lauric acid, C12:0	0.28±0.12	2.25±.21	2.97	0.47	1.33	0.06
Myristic acid, C14:0	1.52±0.25	11.65±0.91	12.04	0.64	7.92	0.16
Palmitic acid, C16:0	49.36±1.75	32.05±1.21	31.48	0.62	38.61	0.06
Heptadecanoic acid, C17:0	0.18±0.06	1.31±0.24	1.00	0.21	0.36	0.09
Stearic acid, C18:0	5.37±0.45	12.11±0.75	12.27	0.31	8.57	0.27
Arachidic acid, C20:0	0.4±0	0.64±0.07	0.35	0.26	0.00	0.00
Monounsaturated fats (% of total fatty acids)						
Myristoleic acid, C14:1n9c	0.00±0	0.80±0.32	1.63	0.34	0.77	0.08
Palmitoleic acid, C16:1n9c	0.29±0.13	1.72±0.12	1.82	1.07	0.41	0.23
Oleic acid, C18:1n9c	41.84±1.24	26.76±1.05	26.86	0.83	33.60	0.70
Polyunsaturated fats (% of total fatty acids)						
Linoleic acid, C18:2n9c,12c	0.12±0.01	2.22±0.1	1.12	0.97	2.89	0.14
α-Linolenic acid, C18:3n3	0.0±0	0.74±0.09	0.32	0.30	0.00	0.00
Trans fatty acids (% of total fatty acids)						
Elaidic acid, C18:1n9t	0.13±0.01	0.15±0.1	0.19	0.02	0.25	0.08
Vaccenic acid, C18:1n7t	0 ±0.0	1.58 ±0.52	1.23	0.24	1.78	0.31

Table 3. Microbiological assessment of Ras cheese samples*

	Coliform		***E. coli***		***Staphylococcus* Spp. coagulase positive**		***Staphylococcus* Spp. coagulase negative**		***Clostridium* spp.**		**Yeast**		**Mold**	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Log CFU/g	<1	5.20	<1	5.06	<1	7.12	<1	7.41	<0.3	>110	<1	5.71	<1	6.22
Guide line *	<1**	free	free	-----	<2	<1	<1**	free	free	-----	<2	<1	<1**	free

*Analyzed samples were 40.

Microbiological analysis

Egyptian Standard ES: 1007-P5 (2005) stated that the microbial standards of Ras cheese require that the final product be free from pathogenic microorganisms. Coliforms, yeasts, and molds must not exceed 1, 2, and 1 log CFU/g of cheese, respectively. Tables (3, 4) and Fig. (1) show the microbiological evaluation of Ras cheese samples collected from different geographical locations in Egypt.

Data reveals that the coliform content of Ras cheese samples ranged from 1 to 5.20 log CFU/g, with an average of 1.70 log CFU/g. These results are nearly similar to those found by other researchers (Awad *et al.*, 2003 and Ibrahim *et al.*, 2015). They found that the coliform bacteria had an average of 1.69 log CFU/g, but were lower than those found by Al-Gamal *et al.* (2019), as they found the coliform bacteria were at an average of 2.1± 1.41 log CFU/g. *E. coli* was detected in 37.5% of Ras cheese samples (15 out of 40 samples), ranging between 1.39 and 5.06 log CFU/g. This finding was higher than that reported by Al-Gamal *et al.* (2019). They found *E. coli* in 2 out of 15 Ras cheese samples, which accounted for 13.3%. The presence of *E. coli* in about one-third of Ras cheese samples may be related to low hygiene and using the raw milk in cheese processing.

The values of *staphylococci ranged* from 0.1 to 7.12 log CFU/g, and *Staphylococcus coagulase* positivity was confirmed in 22.5% of examined Ras cheese samples. Comparably high counts were also found in other artisanal cheeses, including Canastra, Serro, and Campo das Vertentes (Johler *et al.*, 2015). These high counts could be attributed to two factors: the sequential horizontal transfer from cheese vats and cheese cloths to the next, and clinical or subclinical mastitis in the animals that supply the raw milk for cheese production (Cretenet *et al.*, 2011). Further testing for the primary staphylococcal enterotoxins could have provided additional information on the safety of the cheeses, as the counting of *S. aureus* was done only to indicate hygiene condition and meeting the requirement national and international standard.

All examined samples were free of *Listeria monocytogenes*, while *Salmonella spp*. was detected in only one sample of the 40 examined. The study of El-Baz *et al.* (2017) confirmed the presence of *Salmonella* in some cheese samples collected from Mansoura, Egypt. In a study by Awad (2016), all Egyptian Karish cheese samples were free from *Listeria monocytogenes.* So, the *Listeria monocytogenes* was not detected in most Egyptian dairy products.

Ras samples have mold loads in the range of 1 to 6.22 log CFU/g and 1 to 5.77 log CFU/g for yeasts. Egyptian Standard ES: 1007-P5 (2005) refer to the count of yeasts and molds in Ras cheese as not exceeding 2 log CFU/g (100 CFU/g) and 1 log CFU/g (10 CFU/g), respectively. This means that only 17.5% (Fig. 1) of tested samples were within the range of yeasts and molds counts recommended by Egyptian Standard ES: 1007-P5 (2005). There is a positive correlation between the yeast and mold count and the presence of staphylococci and *E. coli* in cheese samples. Most of the samples with low levels of the yeast and mold were free from *Staphylococci* and *E. coli*. This study can reveal unhygienic activities throughout the handling and collecting of milk, as well as during the manufacturing and ripening of cheese, and it required the need for understanding the good manufacturing practice in Ras cheese processing sectors. This finding was agreed with the results of Alnakip *et al.* (2023), they showed that the artisanal white soft cheese in Delta region, Egypt, have high levels of *S. aureus* and *E. coli* as these bacteria were observed in 66.66% and 36%, respectively.

Clostridium perfringens was found in 29 out of 40 samples of Ras cheese (Table 3). Spores of *C. perfringens* can germinate and grow at temperatures as high as 50 °C, with an optimal growth range of 15–55 °C (Rhodehamel and Harmon, 2001). Enterotoxigenic *C. perfringens* spores are highly resistant to pasteurization temperatures, and low temperatures do not affect their stability (Bhattacharya *et al.*, 2020). Ras cheese is ripened at a temperature of about 15–20 °C, which allows *C. perfringens* germination and growth, hence the high level of *C. perfringens* found in Ras raw

milk cheese. *C. perfringens* was detected in raw milk cheese, it is originating from the milk (Sakaridis *et al.,* 2022).

The results in Table (4) indicate the microbial quality of Ras cheese that was collected from different areas in Egypt. The result showed that Ras samples collected from Cairo and Alexandria markets had the highest count of coliform. While samples collected from the Bheira market have high counts of mold, all samples collected from Damietta were free of *Staphylococcus* coagulase. This may be related to hygiene levels in the dairy sector during the processing of cheese. Damietta has a long history of Ras cheese making, and the high quality and high cost of cheese are usually associated with cheese produced in Damietta.

Methicillin-resistant *Staphylococcus aureus* in Ras cheese

Given its capacity to contaminate food of animal origin, colonise, and infect both humans and animals, methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant public health problem (Petinaki and Spiliopoulou, 2012). Live MRSA was found in only two Ras cheese samples. These samples were collected from Behira and Cairo.

Methicillin-resistant *Staphylococcus aureus* (MRSA), which first appeared in humans in the 1960s in connection with healthcare facilities, is one of the deadliest antibiotic-resistant bacteria and a serious threat to human life (Hulme, 2017). In addition to hospital- and community-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA) strains, there is an increasing problem of MRSA colonisation in food-producing animals as a result of the widespread improper use of antibiotics in the veterinary field and their zoonotic transmission to people in contact with livestock (Goerge *et al.,* 2017 and Algammal *et al.,* 2020).

Table 4. Microbiological quality of Ras cheese samples collected from different areas of Egypt

Sampling of		Count (log CFU/g)					
		Coliform	*E.coli*	*Staphylococci* coagulase +	*Staphylococci* coagulase -	Yeasts	Mold
Cairo, 10 samples	Min	<1	<1	0	4.05	<1	<1
	Max	5.21	5.06	0.00	7.26	5.23	6.23
	Average	2.27	1.49	0.00	6.27	3.24	4.46
	SD	1.48	1.79	0.00	0.97	1.97	1.84
Alexandria, 12 samples	Min	<1	<1	<1	<1	<1	<1
	Max	4.51	4.12	6.24	6.63	5.91	6.16
	Average	1.44	1.07	1.62	4.39	2.94	3.16
	SD	1.69	1.46	2.47	2.03	1.93	2.27
Behira, 6 samples	Min	<1	<1	<1	4.77	3.73	3.31
	Max	3.28	2.09	5.39	7.42	5.04	6.01
	Average	1.07	0.35	1.52	6.28	4.17	4.70
	S	1.65	0.85	2.41	0.91	0.82	0.91
Gharbia, 6 samples	Min	<1	<1	<1	<1	<1	<1
	Max	4.36	3.67	7.12	7.06	6.18	5.86
	Average	2.64	1.21	1.19	5.16	4.94	3.97
	SD	1.60	1.87	2.91	2.71	1.40	2.19
Monufia, 3 samples	Min	0.00	0.00	0.00	0.00	0.00	0.00
	Max	1.60	0.00	5.12	0.00	5.71	0.00
	Average	0.80	0.00	2.56	0.00	2.86	0.00
	SD	0.65	0.00	2.09	0.00	2.33	0.00
Damietta, 3 samples	Min	<1	<1	<1	<1	<1	<1
	Max	2.53	1.50	<1	5.76	4.38	4.87
	Average	0.84	0.50	<1	2.88	3.13	3.07
	SD	1.46	0.87	0.00	3.33	2.37	2.43
Guide line *		<1**	free	free	-----	<2	<1

* Egyptian standards (Ras cheese) ES: 1007-P5/2005

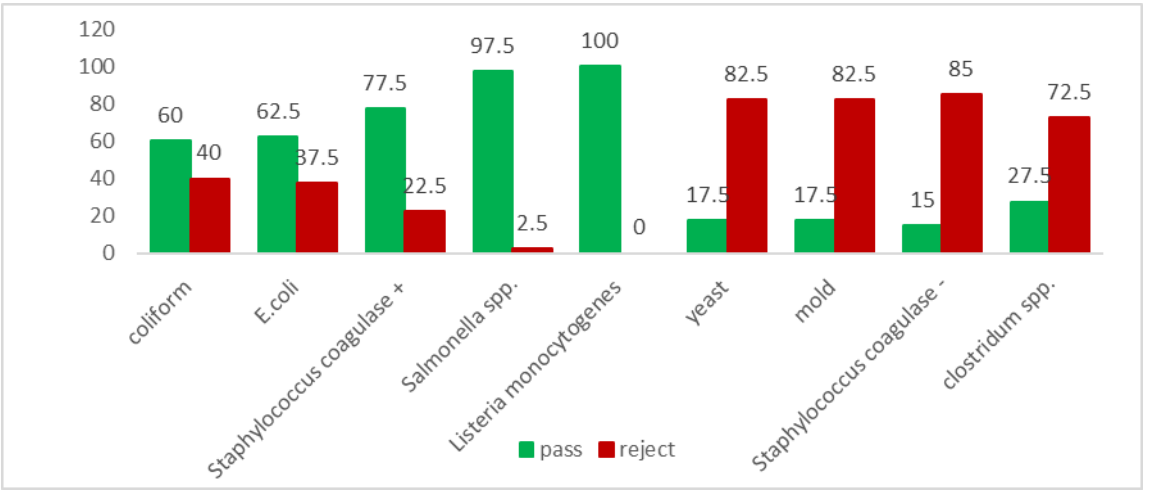

Fig. 1. Incidence of Microbes in examined Ras cheese

Pass / reject samples according to Egyptian standards (Ras cheese) ES: 1007-P5/2005

Viability of *E. coli* O157:H7 in Ras cheese

E. coli was detected in 37.5% of Ras cheese samples (15 out of 40 samples), but the viability of *E. coli* O157:H7 was not detected in all samples of traditional Egyptian cheeses. Consuming unpasteurized gouda cheese made at a dairy farm led to the reporting of 12 of the 13 outbreak cases after 2–8 days. Two of the 26 cheese samples produced by the accused producer had *E. coli* O157:H7. Despite having complied with legal microbiological and ageing criteria, the allegedly tainted cheese was discovered to contain *E. coli* O157:H7 104 days after it had been produced (Honish *et al.*, 2005). The absence of *E. coli* O157:H7 in Ras cheese samples may be related to low moisture, high salt, and long ripening time.

CONCLUSION

As most of the Ras cheese consumed in Egypt is made from raw milk, samples were collected from the Egyptian markets in some governorates for evaluation of the safety of the product. Most cheese samples are considered to be identical in chemical and physical standards, but the cheese contains huge numbers of undesirable microbes such as yeasts, molds, the coliform group, *Staphylococcus aureus*, and anaerobic spore-forming bacteria (Clostridium perfringens). These bacterial counts are higher in collected samples when compared with that recommended by Egyptian standard, but fortunately, most pathogenic microbes such as *Salmonella, Listeria, and Escherichia coli* O157:H7 were not detected in all tested samples. This is due to the high percentage of salt and acidity. The further study aims to determine the most suitable manufacturing and ripening conditions and the period required for ripening before consuming cheese when made from raw milk.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Data sharing

All data generated or analyzed during this study are included in this published article.

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الملخص العربي

معايير السلامة الكيميائية والميكروبية للجبن الراس

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حتى الآن، يتم إنتاج معظم جبن راس بواسطة قطاعات الألبان الصغيرة في مصر باستخدام اللبن الخام. يهدف هذا البحث إلى تحديد مسببات الأمراض والكائنات الحية الدقيقة غير المرغوب فيها في الجبن الراس المصري التقليدي باستخدام الطرق الكلاسيكية وتفاعل البوليميراز المتسلسل في الوقت الحقيقي. تم تحليل أربعين عينة من جبن راس للخصائص الكيميائية والميكروبيولوجية. كشفت النتائج عن مدي واسع في التركيب الكيميائي للعينات التي تم تحليلها. كانت معظم عينات جبن راس ضمن المواصفات القياسية المصرية للتركيب الكيميائي. كانت هناك عينتان فقط من جبن الراس تحتويان على رطوبة أكثر مما أوصى به المواصفات المصرية. تكشف نتائج التحليل الميكروبيولوجي لجبن الراس أن عدد بكتريا الكوليفورم يتراوح بين ١ و ٥,٢٠ لوغاريتم مستعمرة / جرام، بمتوسط ١,٧٠ لوغاريتم مستعمرة / جرام. تم العثور على البكتيريا المكونة للجراثيم اللاهوائية (Clostridium perfringens) في ٢٩ من أصل ٤٠ عينة جبن راس. تحتوي جميع عينات الجبن المصرية على خميرة وفطر أكثر مما توصي به المواصفة المصرية. كانت جميع عينات الجبن خالية من الليستريا والسالمونيلا وقد أظهرت نتائج اختبار تفاعل البوليميراز المتسلسل في الوقت الحقيقي أن ٥% من العينات كانت إيجابية لبكتيريا المكورات العنقودية الذهبية المقاومة للميثيسيلين، كما كانت جميع العينات التي تم تحليلها خالية من البكتيريا القولونية من النوع H7:٠١٥٧. ووفقاً لنتائج هذه الدراسة، فإن أغلب عينات جبن راس تحتوي على مستويات أعلى من المكورات العنقودية الذهبية والفطر والخميرة مقارنة بما أوصت به المعايير المصرية. وينبغي استخدام نتائج هذه الدراسة لتطوير إجراءات محددة لإدارة المخاطر على طول سلسلة إنتاج اللبن والجبن.