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Assessment of Quality and Safety of Ras Cheese in Egyptian Delta Governorates

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ABSTRACT

Ras cheese is manufactured from only raw milk and it is ripening in unhygienic conditions that caused grow the pathogenic bacteria, molds, and yeasts. Therefore, in this survey study, Some chemical, Physiochemical and microbiological analysis were conducted of Ras cheese in Egyptian Delta Governorates. The average of moisture content was 35.57% In addition; the average of Fat and protein % in all samples were 33.53 % and 23.27% respectively with no significant differences. Ash and salt content of all samples were 4.23 and 3.56% respectively. Physical hazards were also measured and listed. Moreover, Biogenic amines including Histamine, Tyramine, Putrescine, and Cadaverine are 50.2, 757.9, 56.38, and 70.4 mg/kg respectively with highly significant different between the tested samples and high level than limits. Aflatoxin M1 average was 51.46 ng/kg in 60 % of samples, these values were higher than The European Union limit (25 ng/kg). Heavy metals, Pesticides and progesterone residues were detected, and appeared higher values in most samples than limits. The microbial analysis of TBC, yeast & mold, and Coliform were higher than Egyptian standards. In most, samples some pathogenic bacteria including Bacillus cereus, Clostridium sp., Salmonella sp., Campylobacter sp., Staphylococcus aureus, and Listeria monocytogenes were determined with unallowable numbers. Finally, we recommended using of HACCP and Good Manufacturing and hygiene practices to reduce hazards and produce safe Ras cheese.

1. INTRODUCTION

Ras cheese is one of the most important hard cheeses in Egypt and it is favorable due to its taste and rich nutrient values. Ras cheese makes up about 20% of cheese production in Egypt approximately 75000 tons. This type of cheese made of collection mix of cow's and buffalo's milk or only raw cow's milk. Recently, the increasing of consumer request for quality and safe foods; require form manufacturers to follow HACCP (Hazard Analysis Critical Control Point) system. The most essential system in food safety and quality assurance programs is the HACCP system, and it is a factory-specific and product-specific safety system. The most Ras cheese product in Egypt lac to quality and safety assurance. It is needs to control the microbial, chemical (biogenic amines, aflatoxins, veterinary drugs, pesticides residues, and heavy metals), and physical hazards (**FDA, 2001.,** and **FDA, 2011).**

This study's primary objective was to evaluate the quality and safety of Ras cheese. Furthermore, the Egyptian Organization for Standardization and Quality Control reported that to reduce the harmful microbial content in Ras cheese; milk must be thermal treated, whereas the pasteurization eliminates the natural microflora in raw milk **(Egyptian standards, 2005)**. Therefore, the application of this system plan in a small cheese factory was achieved to determine microbiological hazards and (CCPs) and the major hazard was raw milk **(Nasr, 2018).** The most essential hazards in most dairy products in Egypt are microbiological hazards. Thus, many microbiological tests should be check and verify about raw materials and select monitoring to accepted suppliers. Total yeast and mold counts, coliforms count and total count are used to know the pointers for the quality and safety of cheese **(McMeekin, 2003).** Microbiological hazards were existed more than physical or chemical hazards in dairy products, when the comparing was made in different food hazards **(Nasr, 2018).** Pathogenic Bacteria including Bacillus cereus, Clostridium sp., Salmonella sp., Campylobacter sp., Staphylococcus aureus, Listeria monocytogenes and Escherichia coli **(Teng et al., 2004 and Asselt et al., 2017).**

Biogenic amines (BAs) are low molecular weight organic bases that have biological activity, also they are created in food during the decarboxylation of amino acids by diverse micro-organisms. Biogenic amines configuration is imperative in fermented food products like cheese **(Erim 2013).** Biogenic amines in dairy products reflect the public worry about the concentration of BAs in those products for the harmful health effects, especially in allergic patients or histamine sensitive **(Moniente, 2022).** Ras cheese needs a long ripening period, therefore the biochemical changing, i.e., proteolysis and different microorganisms decompose milk protein to free amino acids. Moreover, the most popular Biogenic amines in Ras cheese are cadaverine, histamine, putrescine, and tyramine, and these amines are biogenic. Some of those microorganisms are Lactobacillus, Streptococcus, Enterobacteriaceae, Clostridium, Pseudomonas, and Micrococcus create biogenic amines **(Shalaby, 2016).**

One of the main natural chemical compositions that cause a lot of concern around the world is Aflatoxin, from animal feeds and dairy products **(Sasan, 2020).** The aflatoxin B1 is metabolized in the liver to form

AFM1, which is expressed in cow's milk **(Maria Helena, 2013).** The level of progesterone increases in high-fat dairy products, so there are variations between progesterone concentrations in milk and dairy products. The effect of Progesterone on hormonal activity in humans is a carcinogenic effect **(FAO/WHO 2012., and FDA 2015).**

Another hazard critical factor is heavy metals in milk products, which come from feeds, soil, grass, and environmental pollution **(Licata, 2004 and Pilarczyk, 2013).** In addition, pesticide residue is one of the most human health harmful can also be secreted in milk and present in dairy products **(Nag, 2010 and Asselt, 2017).** Physical hazards are foreign materials or strange objects not usually found in dairy and food products, also those can cause illness, psychological trauma, and injury to the consumers who intake them **(Aguiar et al., 2018).**

This study aimed to survey Hazards in Ras cheese in Gharbia and Kafr El sheikh governorates and develop an HACCP plan as a preventive approach for microbiological, chemical and physical control.

2. MATERIALS AND METHODS

2.1. samples.

Ras cheeses samples (1 kg of each) were collected from 20 small factories, 10 samples from Gharbia and 10 from Kafr El Sheikh Governorates. Production date of Tested samples was from **September** to **December 2021**. Cheese samples were transported and stored at 4°C tell analysis. All other reagents and chemicals were used in analytical grade.

2.2. Methods.

2.2.1. Chemical analysis.

Chemical and physical analysis of Ras cheese samples (moisture, protein, fat, salt, ash, and pH contents) were determined as described by (AOAC, 2012). pH values were determined using bench–top pH meters, HANNA Model HI – 9321. salt content was determined using Volhard method.

2.2.2. Detection of Physical hazards.

Ras cheese samples were examined for some physical hazards by optical examination including fine stones, sand, and other foreign materials such as plastic, nails steel, Metal filings, Broken glass, Wooden splinters, and hair from salt and raw materials (**Aguiar 2018).**

2.2.3. Detection of chemical hazards:

Ras cheese samples were examined for some chemical hazards including (progesterone hormone by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) according to **Romero *et al.*, (2007).** Biogenic amines were determined by high-performance liquid chromatography (HPLC) according to **Krause *et al.*, (1995).** Aflatoxin M1 was determined by high-performance liquid chromatography (HPLC) **Wang *et al.*, (2012).** Pesticides Residues was determined by LC-MSMS, GC-MSD. **EN 15662:2008.** Heavy metals were determined by Atomic absorption spectrometry after high-pressure microwave digestion. **Sepe *et al.*, (2003).**

2.2.5. Microbiological Analyses Methods.

Total bacterial count (TBC) and Total Coliform (TC) counts were determined according to **(APHA, 2012).** *Lactococcus* sp. and *Lactobacillus* sp. were determined according to **Fornasari *et al.*, (2006).** Yeast and Molds were determined according to

Hantke, (1972). *B.cereus* was determined according to **Mossel *et al*., (1967).** Clostridium numbers was detected according to **Fryer T. F. and Halligan A. C. (1976).** *Salmonella* sp. was used according to **Andrews *et al*., (1995).** *Campylobacter* sp. according to **(ISO-10272-1-2006).** *Staphylococcus aureus* ss *aureus* was determined as described by **(FDA, 2001).** *L. monocytogenes* was detected according to **(ISO 112090-2006).**

2.2.6. Statistical Analysis:

The results of chemical and microbiological analyses were statistically evaluated by SPS**S 25**, (SPSS Inc., Chicago, IL, USA), and are presented as the means ±SD. Significance among means was carried out by using Duncan's multiple tests at $p \leq 0.05$. and Significant differences between treatments were tested by ANOVA.

3. RESULTS AND DISCUSSION

3.1. Physiochemical analysis of Ras Cheese samples.

Moisture content in samples from Gharbia was 35.13± 0.4% and from Kafr El Sheikh was 36.00± 0.4%. Data in Table (1) shows that there was no significant different between moisture content in the two governorates. The average moisture content of all 20 samples was 35.57 ± 0.4%. Our findings are agreed with the Egyptian standards (1007-5/2005) adopt that the Moisture of Ras cheese should be below 40%. On the other hand, moisture average in our results was higher than those mentioned by **Abbas et. al., (2017)** that found the moisture contents of Ras cheeses made from raw milk was 30.95%.

Fat and Protein content: data presented in Table (1) indicates that no significant differences in fat and protein average in both governorates. Whereas fat average was 33.43± 0.3% in Gharbia samples and 33.62± 0.3% in Kafr El Sheikh samples. On the other hand, protein average was in the tested samples from Gharbia was 23.16±0.2 % and from Kafr El Sheikh was 23.38±0.2 %. The Egyptian standards (1007-5/2005) established that the Fat percentage in Ras cheese should be 27% and protein should be 24% at least. In general, the average of Fat and protein in the all tested samples of the two governorates were 33.53 ± 0.3% and 23.27± 0.2% respectively.

Comparing with results of other articles, our results are higher than those recorded by **Hammam *et al*., (2018)** who found that the fat content of Ras cheese was 30.66 %, while were lower than those mentioned by **(Abbas, 2017)** was 36.39 %. Also, these results are below those accorded by **Hofi *et al*., (1970),** who found the total protein of Ras cheese made from raw milk at three months of age was 26.25%. However, total protein will rise because of the loss of moisture after the ripening period for more time. Furthermore, protein plays an essential role in cheese firmness due to increasing protein trigger a harder texture, as a result, protein constitutes the only continuing solid phase in Ras cheese.

As the Ash content principally reflects the salt content of Ras cheese due to the Ash is the compound of minerals and salts. Table (1) represent the average of Ash in samples from Gharbia is 4.27± 0.052% and from Kafr El Sheikh is 4.18± 0.052%. In addition, Table 1 shows the average of salt in samples from Gharbia is 3.61±0.043 % and from Kafr El Sheikh is 3.51±0.043 % and there is no

different significant between the means of salt content in the two governorates and the average Ash and salt content of all 20 samples is 4.23± 0.052 and 3.56±0.043 % respectively. he last measured parameter in Table (1) is the pH value. The average pH in samples from Gharbia was 5.46±0.05 and from Kafr El Sheikh 5.47±0.05, that indicates no different significant between the two governorates in the mean of pH values, and the average pH of all 20 samples is 5.47±0.05.

The Ras cheese made from raw milk had natural microflora, which caused the increasing in acidity. Hence, the pH has major importance to the safety and quality of the final product.

Table 1. Physiochemical Composition of Ras cheese samples collected from 20 factories in two different governates: Gov1 = Gharbia and Gov2 = Kafr El Sheikh

property	Governates	samples										means Gov	average
		1	2	3	4	5	6	7	8	9	10		
Moisture%	Gov1	37.4^{a} ± 0.409	35.9^{a} ^{b}± 0.409	34.8^{a} ^{b}± 0.409	34.8^{a} ^{b}± 0.409	32.9^{b} ± 0.409	33.9^{a} ^{b}± 0.409	33.8^{a} ^{b}± 0.409	36.7^{a} ^{b}± 0.409	35.7^{a} ^{b}± 0.409	34.9^{a} ^{b}± 0.409	35.13^{a} ± 0.409	35.57± 0.409
	Gov2	36.6^{a} ^{b}± 0.409	36.2^{a} ^{b}± 0.409	36.5^{a} ^{b}± 0.409	36.6^{a} ^{b}± 0.409	34.8^{a} ^{b}± 0.409	36.7^{a} ^{b}± 0.409	35.6^{a} ^{b}± 0.409	36.5^{a} ^{b}± 0.409	33.8^{a} ^{b}± 0.409	36.3^{a} ^{b}± 0.409	36.00^{a} ± 0.409	
Fat%	Gov1	33.743^{a}± 0.318	33.34 3^{a}± 0.318	33.24 3^{a}± 0.318	33.14 3^{a}± 0.318	34.14 3^{a}± 0.318	3343^{a} ± 0.318	33.34 3^{a}± 0.318	33.44 3^{a}± 0.318	33.34 3^{a}± 0.318	33.54 3^{a}± 0.318	33.43^{a} ± 0.318	33.53± 0.318
	Gov2	33.64 3^{a}± 0.318	33.64 3^{a}± 0.318	33.54 3^{a}± 0.318	33.44 3^{a}± 0.318	33.44 3^{a}± 0.318	33.54 3^{a}± 0.318	33.74 3^{a}± 0.318	33.54 3^{a}± 0.318	33.84 3^{a}± 0.318	33.84 3^{a}± 0.318	33.62^{a} ± 0.318	
Protein%	Gov1	22.7^{a} ± 0.233	23.1^{a} ± 0.233	23.2^{a} ± 0.233	22.8^{a} ± 0.233	24.2^{a} ± 0.233	23.1^{a} ± 0.233	23.3^{a} ± 0.233	22.6^{a} ± 0.233	22.7^{a} ± 0.233	23.6^{a} ± 0.233	23.16^{a} ± 0.233	23.27± 0.233
	Gov2	22.9^{a} ± 0.233	23^{a}± 0.233	22.9^{a} ± 0.233	23.2^{a} ± 0.233	23.4^{a} ± 0.233	23.9^{a} ± 0.233	23.4^{a} ± 0.233	23.2^{a} ± 0.233	24^{a}± 0.233	23.6^{a} ± 0.233	23.38^{a} ± 0.233	
Ash%	Gov1	4.35^{b} ^{c}± 0.052	4.14^{c} ± 0.052	4.07^{c} ± 0.052	4.28^{b} ^{c}± 0.052	4.77^{a} ± 0.052	4.14^{c} ± 0.052	4.17^{c} ± 0.052	4.38^{b} ^{c}± 0.052	4.26^{b} ^{c}± 0.052	4.15^{c} ± 0.052	4.27^{a}± 0.052	4.23± 0.052
	Gov2	4.12^{c} ± 0.052	4.14^{c} ± 0.052	4.07^{c} ± 0.052	4.04^{c} ± 0.052	4.15^{c} ± 0.052	4.34^{b} ^{c}± 0.052	4.07^{c} ± 0.052	4.16^{c} ± 0.052	4.63^{a} ^{b}± 0.052	4.15^{c} ± 0.052	4.18^{a}± 0.052	
Salt%	Gov1	3.7^{ab} ± 0.043	3.5^{bc} ± 0.043	3.4^{c}± 0.043	3.6^{bc} ± 0.043	4^{a}± 0.043	3.6^{bc} ± 0.043	3.5^{bc} ± 0.043	3.7^{ab} ± 0.043	3.6^{bc} ± 0.043	3.5^{bc} ± 0.043	3.61^{a}± 0.043	3.56± 0.043
	Gov2	3.4^{c}± 0.043	3.5^{bc} ± 0.043	3.4^{c}± 0.043	3.4^{c}± 0.043	3.5^{bc} ± 0.043	3.6^{bc} ± 0.043	3.4^{c}± 0.043	3.5^{bc} ± 0.043	3.9^{ab} ± 0.043	3.5^{bc} ± 0.043	3.51^{a}± 0.043	
pH	Gov1	5.4^{a}± 0.051	5.5^{a}± 0.051	5.4^{a}± 0.051	5.5^{a}± 0.051	5.4^{a}± 0.051	5.5^{a}± 0.051	5.4^{a}± 0.051	5.5^{a}± 0.051	5.4^{a}± 0.051	5.5^{a}± 0.051	5.46^{a}± 0.051	5.47±0.051
	Gov2	5.5^{a}± 0.051	5.5^{a}± 0.051	5.4^{a}± 0.051	5.4^{a}± 0.051	5.5^{a}± 0.051	5.5^{a}± 0.051	5.5^{a}± 0.051	5.4^{a}± 0.051	5.5^{a}± 0.051	5.3^{a}± 0.051	5.47^{a}± 0.051	

3.2. Physical hazards of Ras cheese

Physical Contaminants found in Ras cheese samples by optical examination, as they are show in **Table (2)**. All samples were polluted with foreign materials, such as metals, plastics, insects, hair, wooden splinters, stones and sand. Thus, high-grade salt must be use and Filter milk correctly (**Aguiar 2018).**

Table 2. Physical hazards in Ras cheese samples collected from 20 factories in two different governorates.

Physical hazards	Govern-orates	samples									
		1	2	3	4	5	6	7	8	9	10
Metals	Gov1	ND	+	ND	ND	ND	ND	+	ND	ND	ND
	Gov2	ND	ND	ND	+	ND	ND	ND	ND	ND	+
plastics	Gov1	+	ND	ND	ND	ND	ND	ND	+	ND	+
	Gov2	ND	ND	ND	ND	ND	ND	ND	+	+	ND
insects	Gov1	ND	+	+	ND	ND	+	+	ND	ND	+
	Gov2	ND	ND	ND	+	+	ND	ND	ND	+	+
Hair	Gov1	ND	ND	+	ND	ND	+	+	+	+	ND
	Gov2	+	+	+	+	+	+	+	+	+	+
wooden splinters	Gov1	+	+	ND	+	ND	ND	ND	ND	ND	ND
	Gov2	ND	+	ND	ND	ND	ND	ND	ND	ND	ND
Stones & Building materials	Gov1	+	+	+	+	+	+	ND	+	+	+
	Gov2	+	+	+	+	+	ND	+	+	+	+
sand	Gov1	+	+	+	+	+	+	ND	+	+	+
	Gov2	+	+	+	+	+	ND	+	+	+	+

3.3. Chemical hazards of Ras cheese

Biogenic amines (BAs) Contaminants in Ras cheese samples:

The determined Biogenic amines i.e., Histamine, Tyramine, Putrescine and Cadaverine in Ras cheese samples are show in T**able (3).** The averages of Histamine, Tyramine, Putrescine, and Cadaverine content of Ras cheese samples are 50.2± 0.05, 757.9± 0.05, 56.38± 0.05, and 70.4 ± 0.05 mg/kg respectively with highly significant different (P<0.0001).

These results reported that the detected Histamine level in our samples is very high regarding to the U.S. Food and Drug Administration standers, which set histamine limits in food in general at 50 mg/kg. **(FDA,2018)[1]and (FDA,2018)[2]**. Our results are higher than those mentioned by (**Shalaby, 2016)** who found that the Histamine, Putrescine, and cadaverine in Ras cheese was 9.6, 40.8, and 67.4 mg/kg respectively, but below the tyramine content in Ras cheese was 930.8 mg/kg. While are lower than those mentioned by **(Pesqueira, 2018),** who found that the Histamine, Putrescine, and cadaverine in cheese were 53.22, 260.32, and 88.28 mg/kg respectively and upper tyramine value was 384.6 mg/kg.

Egyptian Standards (1007-5/2005) did not define the limits for biogenic amines in Ras cheese or any food, moreover, legislation selected the limits for one single biogenic amine that is histamine due to it being the main essential Amine from a toxicological, without creating limits for other amines, particularly tyramine, that have hard toxic effects **(Capillas,2019).** The configuration of biogenic amines in cheeses is based on milk pasteurization, pH, time and temperature of the ripening period, salt concentration, and of starter cultures **(Linares *et al.* 2012).**

Aflatoxin M1 (AFM1) Contaminants in Ras cheese samples:

Aflatoxin M1 determined in our Ras cheese samples is listed in **Table (3)**. The AFM1 levels in Ras cheese samples are ranging from 4.35 ± 0.05 to 95.71 ± 0.05 ng/kg, with an average of 51.464 ± 0.05 ng/kg, which is highly different significant (P<0.0001). These result is lower than this mentioned by **Aiad, (2013)** who found that AFM1in Ras cheese ranged from 7.40 to 111.50 ng/kg, with an average of 56.048 ng/kg. On the other hand, Aflatoxin M1 in 60 % of our samples was higher than The European Union limit for AFM1 in dairy products (25 ng/kg) **EC, (2010) and** the **Egyptian Regulations, (1990)** which pointed that milk and dairy products should be free from AFM1.

AFM1 is carcinogenic and hepatotoxic. Therefore, It's an urgent need to control Aflatoxins in animal feeds. Duo to the presence of AFM1 in dairy products is the presence of AFB1 in the animal feed then Aflatoxin B1 transformed to M1 in the liver of animal mammals, and the creation of AFB1, B2, G1, and G2 by fungi that grow on cheese such as Aspergillus sp. **(Andreia Vaz, 2020).** Consequently, the concentration of AFM1 in hard cheese is five-fold greater than in milk, and in soft cheese is three-fold greater than in milk. **(Sasan, *2020*).**

Veterinary Drugs and hormones Contaminants in Ras cheese:

Progesterone detected in all tested Ras cheese samples is shown in **Table (3)**. Progesterone levels were ranging from 4.2 ± 0.05 to 25 ± 0.05 ng/g with an average of 16.84 ± 0.05 ng/g, with highly significant different (P<0.0001). These results are not complied with the **Egyptian standard (1007-5/2005),** which establishes that Ras cheese should be free of hormones and veterinary drugs residuals. The possible source of progesterone and veterinary compounds is the bad use of GVP (Good veterinary practices) by not following withdrawal periods, so the

farmers should wait a particular interval of time after giving animals veterinary drugs to can sell them products, **Asselt, (2017).** The level of progesterone increases in high-fat dairy products, so there are variations between progesterone concentrations in milk and dairy products. **(Malekinejad, 2015).**

Residuals of pesticides Contaminants in Ras cheese samples: While **Egyptian standard (1007-5/2005),** establishes that Ras cheese should be free of pesticides and the CODEX Maximum Residue Limit (MRL) of 0.1 mg/kg on a fat basis; **all tested Ras cheese samples** were contaminated with Residuals of the most determined pesticides as shown in T**able (3).** Imidoclopride and Diazinon were not detected in all cheese samples. The results showed that 40% of collected samples were contaminated with Fluazifop-P- Butyl. In addition, 60% of samples were contaminated with Chlorfluazuron and Ortho-Phenyl-Phenol. Moreover, 80 % of the tested samples were contaminated with Deltamethrin. Finally, all samples are contaminated with Malathion and Chlopyrifos. The lack of awareness of the danger of pesticides among Egyptian farmers, also the absence of product control are the main reason to such results.

Table 3. Chemical hazards in Ras cheese samples.

chemical hazards		samples					average	limits
		1	2	3	4	5		
biogenic amines (mg/kg)	histamine	10.6^{e}±0.057	36.0^{c}±0.057	61.0^{b}±0.057	11.4^{d}±0.057	132^{a}±0.057	50.2±0.057	50
	tyramine	893.3^{a}±0.057	785.6^{d}±0.057	457.9^{e}±0.057	812.4^{c}±0.057	843.3^{b}±0.057	757.9±0.057	80
	putrescine	35.8^{e}±0.057	49.4^{d}±0.057	55.1^{c}±0.057	72.7^{a}±0.057	68.9^{b}±0.057	56.38±0.057	50
	cadavraine	58.2^{e}±0.057	61.3^{d}±0.057	77.5^{b}±0.057	70.1^{c}±0.057	84.9^{a}±0.057	70.4±0.057	50
Veterinary Drugs Residual (hormones) ng/g	progesterone	20.0^{b}±0.057	18.5^{c}±0.057	16.5^{d}±0.057	4.2^{e}±0.057	25.0^{a}±0.057	16.84±0.057	2.5
mycotoxins (ng/g)	aflatoxin M1	4.35^{e}±0.057	62.93^{c}±0.057	7.05^{d}±0.057	87.28^{b}±0.057	95.71^{a}±0.057	51.464±0.057	0 - 25
Pesticides Residuals (mg/kg)	Malathion	0.03	0.02	0.05	0.02	0.04	_	0.02
	Deltamethrin	0.01	0.16	0.08	ND	0.18	_	0.05
	Chlorfluazuron	ND	0.02	ND	0.021	0.01	_	0.01
	Chlopyrifos	0.52	0.35	0.14	0.83	0.02	_	0.01
	Fluazifop-P-Butyl	ND	ND	ND	0.01	0.02	_	0.01
	Diazinon	ND	ND	ND	ND	ND	_	0.01
	Ortho-Phenyl-Phenol	0.02	0.02	ND	0.01	ND	_	0.01
	Imidocloprid	ND	ND	ND	ND	ND	_	0.01

Heavy metals contaminants in Ras cheese samples:

Tables (4) shows heavy metals contaminants of the tested Ras cheese samples; the major pollution with iron and zinc. As well as 25% of samples were contaminated with aluminum and about 15% of samples with copper and lead. In addition, nickel and manganese were found in only one sample. These Results show that the majority of cheese samples comply with the **Egyptian standard (1007-5/2005),** and the International Dairy Federation **(IDF 1979)** registered the limits of the Heavy metal in dairy products should be 0.10, 0.049, 0.025, 0.026, 0.37, and 3.28 mg/kg milk for Cu, Pb, Mn, Ni, Fe, and Zn, respectively.

Górska-Warsewicz et al., (2019) pointed to the normal concentration of organic iron and zinc (nutrients) in milk was 1.23 and 4.38 mg/kg, respectively and in cheese were 0.31 and 1.3 mg/kg respectively. The average Iron and Zinc contamination in samples from Gharbia were 9.65± 0.005 and 19.14± 0.005 mg/Kg higher than Kafr El Sheikh were 4.70± 0.005 and 15.68± 0.005 mg/Kg respectively. with significant different ($P<0.0001$) between the means of Iron and Zinc contamination content in the two governorates, and the averages Iron and Zinc of all 20 samples are 5.47±0.005 and 18.05±0.005 mg/Kg respectively.

Our analysis results of Fe and Zn were lower than the obtained by **Grzegorz Zwierzchowski, (2018)** who found the iron content of raw milk was 9.09 mg/Kg and the zinc content of raw milk was 38.68 mg/Kg. The high values of iron because the cheese may contaminate with iron during cheese making, milk production, or adding salt.

In general, minerals in milk are higher than those in cheese due to the intense whey drainage that led to the loss of the metals which are soluble in whey. **Fresno et al., (1995).** Contrarily, the contact between the milk and manufacturing equipment could play an essential role in increasing the number of heavy metals such as Cu, Pb, Mn, Ni, Fe, and Zn in the final product **(Coni et al., 1994).**

Table 4. Heavy metals concentration (mg/kg) of Ras cheese samples

property	Governates	samples										means GOV	limits mg/kg
		1	2	3	4	5	6	7	8	9	10		
Fe	Gov 1	19.02^{b}±0.005	0.65^{f} ±0.005	5.28^{e} ±0.005	18.67^{b}±0.005	15.78^{c}±0.005	0.92^{f} ±0.005	4.37^{e} ±0.005	9.02^{d} ±0.005	0.0^{g} ±0.005	21.75^{a}±0.005	9.56^{a} ±0.005	0.37
	Gov 2	1.70^{e} ±0.005	8.42^{d} ±0.005	0.65^{f} ±0.005	17.39^{b}±0.005	0.0^{g}± 0.005	0.0^{g}± 0.005	2.46^{e} ±0.005	15.78^{c}±0.005	0.0^{g} ±0.005	0.48^{f} ±0.005	4.70^{b} ±0.005	
Zn	Gov 1	4.81^{h} ±0.005	38.21^{c}±0.005	7.25^{g} ±0.005	7.90^{g} ±0.005	7.30^{g} ±0.005	43.10^{b}±0.005	4.08^{h} ±0.005	36.52^{c}±0.005	4.61^{h} ±0.005	37.34^{c}±0.005	19.14^{a}±0.005	3.28
	Gov 2	4.71^{h} ±0.005	7.68^{g} ±0.005	52.46^{a}±0.005	4.80^{h} ±0.005	16.5^{e} ±0.005	12.38^{f}±0.005	4.82^{h} ±0.005	7.61^{g} ±0.005	14.8^{e} ±0.005	30.82^{d}±0.005	15.68^{b}±0.005	
Al	Gov 1	11.24	ND	ND	27.14	ND	ND	ND	ND	22.78	ND	_	10
	Gov 2	ND	ND	ND	ND	ND	14.28	ND	ND	ND	12.93	_	
Pb	Gov 1	ND	ND	0.057	ND	ND	0.082	ND	ND	ND	ND	_	0.049
	Gov 2	ND	ND	0.063	ND	ND	ND	ND	ND	ND	ND	_	
Cu	Gov 1	ND	0.063	ND	ND	ND	ND	ND	ND	ND	0.026	_	0.1
	Gov 2	ND	ND	ND	ND	ND	ND	ND	0.038	ND	ND	_	
Ni	Gov 1	ND	ND	ND	ND	0.029	ND	ND	ND	ND	ND	_	0.026
	Gov 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	_	
Mn	Gov 1	ND	ND	ND	ND	ND	ND	ND	0.034	ND	ND	_	0.03
	Gov 2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	_	

3.4. Microbiological hazards of Ras cheese

Various microbial groups were detected in Ras cheese samples to assess their microbiological safety. Total Bacterial Count, Total Coliform, *Lactococcus* sp., *Lactobacillus* sp., Yeast and Molds, and Pathogenic Bacteria including *Bacillus cereus, Clostridium* sp., *Salmonella* sp., *Campylobacter* sp., *Staph. Aureus, and Listeria monocytogenes,* were assessed.

Some of spoilage microorganisms and Starter Lactic Acid Bacteria were detected in Ras cheese samples. **Tables (5)** shows significant different ($P<0.0001$) in Total Bacterial Count, Total Coliform, and *Lactobacillus* sp. content between the two governorates Gharbia (Gov1) and Kafr El Sheikh (Gov2) Ras cheese samples. Nonetheless, yeast & mold and *Lactococcus* sp. were indicate that no significant difference ($P > 0.05$) between the two governorates.

Total Bacterial Count in the tested samples from Gharbia is 7.86±0.16 log cfu/g lower than Kafr El Sheikh is 8.22±0.16 log cfu/g and the average Total Bacterial Count of all 20 samples is 8.04±0.16 log cfu/g. These results are lower than those mentioned by **(Hammam *et al.*, 2018)** who found that the total bacterial in 3-month-old Ras cheese was 13×10^6cfu/g.

Total Coliform count in the tested samples from Gharbia is 1.41±0.15 log cfu/g lower than Kafr El Sheikh is 1.59±0.15 log cfu/g, and the average Total Coliform of all 20 samples is 1.5±0.15 log cfu/g. total coliform was found in 85% of all samples, but it was not found in three samples. **Yeast & Mold average** in the tested samples from Gharbia is 5.27±0.17log cfu/g and from Kafr El Sheikh is 5.38±0.17 log cfu/g and the average yeast & mold of all 20 samples is 5.33±0.17 log cfu/g. These results are below those reported by **Hammam *et al.*, (2018)** who found total coliforms and yeast & molds counts in Ras cheese were 96×10^5 and 20×10^4cfu/g, respectively. but both results are compliant together, especially in they are high counts in Ras cheese due to poor hygienic practices during cheese manufacturing and handling, and the cheeses were made from raw milk. Moreover, these results do not correspond with the **Egyptian standards (1007-5/2005),** which select the maximum limits of coliform or *E. coli* count is lower than 10 cfu/g, also the limits of yeasts and molds must be under 100 cfu/g.

3.5. Lactic acid bacterial count

***Lactococcus* sp count** in the tested samples from Gharbia is 6.14±0.35 log cfu/g and from Kafr El Sheikh is 6.05±0.35 log cfu/g and the average *Lactococcus* sp of all 20 samples is 6.1±0.35 log cfu/g. ***Lactobacillus* sp. count** in the tested samples from Gharbia is 4.46±0.43 log cfu/g higher than Kafr El Sheikh is 4.13±0.43 log cfu/g and the average *Lactobacillus* sp. of all 20 samples is 4.3±0.43 log cfu/g. As well as, starter lactic acid bacteria have an essential role in raw milk Ras cheese making, particularly, in some process's steps, such as cheese ripening and curd cooking. In addition, these results are higher than those accorded by **Abdullah *et al.*, (2006)** who detected Lactococcus and Lactobacillus count12-month-old Ras cheese, therefore, the numbers of those bacteria adopted on ripening and manufacturing circumstances.

Table 5. Spoilage microbial & starter lactic acid bacterial counts of Ras cheese samples collected from 20 factories in two different governates: Gov1 = Gharbia and Gov2 = Kafr El Sheikh

property	Governorate	samples										means GOV	average
		1	2	3	4	5	6	7	8	9	10		
TBC	Gov 1	6.54^{i} ±0.161	8.05^{defg}±0.161	7.37^{h}±0.161	7.92^{efg}±0.161	7.24^{hi}±0.161	8.64^{cd}±0.161	8.23^{def}±0.161	8.51^{cd}±0.161	6.94^{i}±0.161	9.07^{ab}±0.161	7.86^{b}±0.161	8.04 ±0.161
	Gov 2	7.52^{fgh}±0.161	7.68^{fgh}±0.161	8.06^{efg}±0.161	8.42^{cde}±0.161	8.67^{cd}±0.161	7.38^{h}±0.161	8.47^{cde}±0.161	7.29^{hi}±0.161	8.94^{bc}±0.161	9.67^{a}±0.161	8.22^{a}±0.161	
Y&M	Gov 1	4.02^{g} ±0.175	6.08^{b} ±0.175	5.23^{ef}±0.175	4.62^{f} ±0.175	4.93^{ef}±0.175	5.96^{c}±0.175	5.42^{de}±0.175	6.38^{b} ±0.175	3.68^{g} ±0.175	6.34^{b}±0.175	5.27^{a}±0.175	5.33 ±0.175
	Gov 2	4.82^{f}±0.175	5.41^{de}±0.175	5.07^{ef}±0.175	4.79^{f}±0.175	5.48^{d}±0.175	4.03^{g} ±0.175	5.37^{de}±0.175	4.92^{ef}±0.175	6.07^{b} ±0.175	7.85^{a}±0.175	5.38^{a}±0.175	
T. Coliform	Gov 1	0.0^{k} ±0.158	1.52^{h}±0.158	1.05^{j} ±0.158	1.48^{hi}±0.158	1.3^{i}± 0.158	1.97^{ef}±0.158	1.81^{f} ±0.158	2.16^{c} ±0.158	0.0^{k} ±0.158	2.82^{a}±0.158	1.41^{b}±0.158	1.5 ±0.158
	Gov 2	1.42^{hi}±0.158	1.63^{g} ±0.158	2.03^{de}±0.158	1.85^{f}±0.158	1.83^{f} ±0.158	0.0^{k} ±0.158	1.41^{hi}±0.158	1.32^{i} ±0.158	2.1^{cd} ±0.158	2.35^{b}±0.158	1.59^{a}±0.158	
Lactobacillus sp.	Gov 1	1.27^{k} ±0.433	4.36^{g} ±0.433	3.02^{h} ±0.433	2.43^{i} ±0.433	5.18^{f} ±0.433	6.73^{b} ±0.433	6.07^{d} ±0.433	6.92^{b} ±0.433	0.92^{k} ±0.433	7.69^{a}±0.433	4.46^{a}±0.433	4.3 ±0.433
	Gov 2	3.06^{h} ±0.433	2.48^{i} ±0.433	2.68^{hi}±0.433	4.53^{g} ±0.433	6.31^{c} ±0.433	0.82^{k} ±0.433	5.71^{e} ±0.433	1.83^{j} ±0.433	6.02^{d} ±0.433	7.84^{a}±0.433	4.13^{b}±0.433	
Lactococcus sp.	Gov 1	3.42^{g} ±0.356	6.62^{cd}±0.356	5.74^{e} ±0.356	4.28^{f} ±0.356	4.34^{f} ±0.356	8.06^{a} ±0.356	8.12^{a} ±0.356	8.37^{a} ±0.356	4.13^{f} ±0.356	8.27^{a}±0.356	6.14^{a}±0.356	6.1 ±0.356
	Gov 2	3.75^{fg}±0.356	7.25^{bc}±0.356	6.28^{de}±0.356	6.47^{d} ±0.356	7.32^{b} ±0.356	2.03^{h} ±0.356	8.76^{a} ±0.356	3.52^{g} ±0.356	6.37^{de}±0.356	8.69^{a}±0.356	6.05^{a}±0.356	

Pathogenic bacteria in **Table (6)** shows that 90% of the samples were contaminated with *Clostridium* sp. and 60% of samples with *Salmonella* sp. Moreover, all Ras cheese samples contaminate with *Bacillus cereus* and *Staph. Aureus*. Besides, 40% of samples were contaminated with *Listeria monocytogenes,* but *Campylobacter* sp. attended in only 3 samples**.** These results have not corresponded with the **Egyptian standard (1007-5/2005),** which defined that Ras cheese must not have pathogenic bacteria. So that, these results show refer to absent the hygiene practices during handling, processes, and environmental conditions.

Data from **Tables (6),** showed that, there are different significant ($P<0.0001$) in *Bacillus cereus*, and *Staph. Aureus* of Ras Cheese Survey samples between the two governorates Gharbia (Gov1) and Kafr El Sheikh (Gov2).

***Bacillus cereus* average** in the tested samples from Gharbia is 3.88±0.303 log cfu/g lower than Kafr El Sheikh is 4.13±0.303 log cfu/g, and the average *Bacillus cereus* of all 20 samples is 4.01±0.303 log cfu/g.

***Staph. Aureus* average** in the tested samples from Gharbia is 3.88±0.266 log cfu/g lower than Kafr El Sheikh is 4.77±0.266 log cfu/g, and the average *Staph. Aureus* of all 20 samples is 4.33±0.266 log cfu/g.

Table 6. Pathogenic bacteria of Ras cheese samples collected from 20 factories in two different governates: Gov1 = Gharbia and Gov2 = Kafr El Sheikh

Pathogenic bacteria	Governorate	samples										means GOV	average
		1	2	3	4	5	6	7	8	9	10		
Bacillus cereus	Gov 1	1.6^{kl} ±0.303	2.9^{ji} ±0.303	3.5^{gh} ±0.303	1.4^{kl} ±0.303	4.73^{f} ±0.303	5.6^{de} ±0.303	5.2^{e} ±0.303	3.4^{gh} ±0.303	3.8^{g} ±0.303	6.7^{c} ±0.303	3.88^{b} ±0.303	4.01 ±0.303
	Gov 2	2.5^{j}± 0.303	1.3^{l}± 0.303	1.8^{k}± 0.303	4.71^{f} ±0.303	3.8^{g} ±0.303	2.5^{j}± 0.303	5.7^{d} ±0.303	3.1^{hi} ±0.303	7.3^{b} ±0.303	8.6^{a} ±0.303	4.13^{a} ±0.303	
Staph. Aures	Gov 1	1.8^{k} ±0.266	5.3^{cd} ±0.266	4.7^{efg} ±0.266	4.2^{gh} ±0.266	3.1^{j}± 0.266	3.7^{hi} ±0.266	4.5^{fg} ±0.266	3.3^{ij} ±0.266	1.7^{k} ±0.266	6.5^{ab} ±0.266	3.88^{b} ±0.266	4.33 ±0.266
	Gov 2	5.7^{c} ±0.266	3.9^{h} ±0.266	5.7^{c}± 0.266	5.1^{de} ±0.266	3.7^{hi} ±0.266	1.6^{k} ±0.266	4.8^{ef} ±0.266	4.2^{gh} ±0.266	6.2^{b} ±0.266	6.8^{a} ±0.266	4.77^{a} ±0.266	
Salmonella sp	Gov 1	ND	+	+	ND	ND	+	+	+	ND	+	_	_
	Gov 2	+	ND	+	+	ND	ND	+	ND	+	+	_	
Clostridium sp	Gov 1	ND	+	+	+	+	+	+	+	ND	+	_	_
	Gov 2	+	+	+	+	+	+	+	+	+	+	_	
Listeria monocytogenes	Gov 1	+	+	ND	ND	ND	+	ND	ND	ND	+	_	_
	Gov 2	ND	ND	ND	+	ND	ND	+	ND	+	+	_	
Campylobacter Sp	Gov 1	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	_	_
	Gov 2	ND	ND	ND	ND	+	ND	ND	ND	ND	+	_	

Finally, samples do not correspond with the Egyptian standards (1007-5/2005). All samples were contaminated with foreign materials, biogenic amines (CAD – HIS – PUT - TYR), Aflatoxin M1, progesterone, zinc, Malathion, Chlopyrifos, *Bacillus cereus,* and *Staphylococcus aureus.* Besides, 90% of the samples were polluted with *Clostridium* sp. Also, 80% of the samples were contaminated with iron and Deltamethrin. Moreover, 60% of the samples were contained *Salmonella* sp, Chlorfluazuron and Ortho-Phenyl-Phenol. As 40% of the samples have been polluted with Fluazifop-P- Butyl and *Listeria monocytogenes.* However, 25% of the samples were contaminated with aluminum.

In addition, the total protein is 23.25%, which is lower than the requirements of the Egyptian Standards (1007-5/2005), which should be 24% at least. On the other hand, 15% were contained copper, lead, and *Campylobacter* sp (3 samples). And 5% for nickel and manganese (one sample). Also, Imidoclopride and Diazinon were not detected. Hence, these results and total solids, moisture, and fat corresponded with the requirements of the Egyptian standard (1007-5/2005).

There are no significant differences ($P>0.05$) in all the Physiochemical content, yeast & mold, and *Lactococcus* of Ras Cheese Survey samples between the two governorates Gharbia (Gov1) and Kafr El Sheikh (Gov2). There are a different significant ($P<0.0001$) in all the microorganisms and heavy metals content of Ras Cheese Survey samples between the two governorates Gharbia (Gov1) and Kafr El Sheikh (Gov2), also, Gov1 higher than Gov2 in *Lactobacillus*, iron, and Zinc, however, Gov2 is highest in TBC, Total Coliform, *Bacillus cereus*, and *Staph. Aureus.*

4. CONCLUSION AND RECOMMENDATION

From these results of the survey, it had concluded that the Ras cheese has several issues especially, which was those made from raw milk and unhealthy ripening conditions, and that is necessary to know the problems and try to solve them, by avoiding sources, that were caused risks to consumer health. Thus, the recommendation to improve its overall quality and safety could be via following Good Hygienic Practices (GHP), Good Manufacturing Practices (GMP), and the Hazard Analysis and Critical Control Point (HACCP) system.

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تقييم جودة وسلامة جبن الراس في محافظات الدلتا المصريه

عبدالله مدحت الديناري , مصطفي عبدالله حسن , دينا عامر , اشرف شعبان بكر

مجلة العلوم الزراعية والبيئية المستدامة

الملخص

يتم تصنيع جبنة الراس من الحليب الخام فقط وتنضج في ظروف غير صحية تسببت في نمو البكتيريا المسببة للأمراض والعفن والخمائر. لذلك ، في هذه الدراسة المسحيه ، تم إجراء بعض التحاليل الكميائيه و الفيزيوكيميائية والميكروبيولوجية لجبن الرأس في محافظات الدلتا المصرية. كما بلغ معدل المحتوى الرطوبه 35.57٪. كان متوسط النسبة المئوية للدهون والبروتين في جميع العينات 33.53٪ و 23.27٪ على التوالي مع عدم وجود فروق معنوية. كان محتوى الرماد والملح لجميع العينات 4.23 و 3.56٪ على التوالي.

أيضًا تم قياس المخاطر الفيزيائية وتدوينها. علاوة على ذلك ، فإن الأمينات الحيوية بما في ذلك الهيستامين والتيرامين والبوتريسين والكادافيرين هي 50.2 و 757.9 و 56.38 و 70.4 مجم / كجم على التوالي مع اختلاف معنوي كبير بين العينات المختبرة ومستوى مرتفع عن الحدود.

. وعند قياس الأفلاتوكسين بلغ متوسطها نوع الـ M1 منها 51.46 نانوغرام / كجم في 60٪ من العينات، وكانت هذه القيم أعلى من حدM1 الاتحاد الأوروبي (25 نانوغرام / كجم). تم الكشف عن المعادن الثقيلة والمبيدات وبقايا البروجسترون ، وظهرت قيم أعلى في معظم العينات من الحدود. كانت التحاليل الميكروبية لكل من TBC والخميرة والعفن والكوليفورم أعلى من المواصفات القياسية المصرية. في معظم العينات ، تم تقدير بعض أنواع البكتيريا الممرضة بما في ذلك Bacillus cereus و Clostridium sp. و Salmonella sp. و Campylobacter sp. و Staphylococcus aureus و Listeria monocytogenes بأعداد غير مسموح بها. أخيرًا ، أوصينا باستخدام نظام تحليل المخاطر ونقاط التحكم الحرجة وممارسات التصنيع الجيدة والنظافة لتقليل المخاطر وإنتاج جبن راس آمن.