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Assessment of some chemical residues in Egyptian raw milk and traditional cheese

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Abstract
Background: The assessment of risks related to food safety is becoming a challenge in developing countries with its consequent health hazards. Chemical risk assessment in dairy products is important to maintain consumer health locally and internationally. Since milk and dairy products are essential foods for a wide range of customers, mostly children, patients, and pregnant women, it is very important to estimate the risks of some chemical residues, such as pesticides, some heavy metals, and aflatoxins.
Aim: This work aims to determine the levels of chemical contamination in milk and traditional Egyptian cheese.
Methods: Heavy metals were determined in samples by atomic absorption spectrometry. GC-mass spectrometry (MS)/MS and LC-MS/MS were also used for measuring pesticide residues. The Aflatoxin M1 was determined by enzyme-linked immune-sorbent assay.
Results: Raw milk samples were tested and showed elevated concentrations of lead and cadmium, (46% and 4%, respectively). The heavy metals detected in the Egyptian cheese samples were variable depending on the type of cheese. Moreover, p.p.-DDE phenofose was present in 45% and 29% of raw milk and Ras cheese samples, respectively. For Aflatoxin M1, only 7% of milk samples and 2.9% of Ras cheese samples exceeded the acceptable limits.
Conclusion: More surveying and risk assessment of chemical residues in milk and milk products are essential for controlling health risks to consumers.
Keywords: Raw milk, Egyptian cheese, Heavy metals, Pesticides, Aflatoxin M1.

Introduction

Milk has important elements such as proteins, essential fatty acids, lactose, vitamins, and minerals in proportionate amounts, so it is regarded as a complete diet besides being one of the most necessary products in the food supply chain (Boudalia *et al.*, 2016). However, raw milk has the potential to contain chemical hazards and other pollutants that may threaten the health of consumers (Girma *et al.*, 2014). Dairy farms must therefore maintain high levels of hygienic and safety practices.

There are many environmental contaminants that can get into milk as residues of antibiotics, pesticides, or herbicides. Also, contaminants in milk can be from the equipment during and/or after milking. Antibiotic resistance increases as a result of livestock practices that heavily utilize antibiotics, which pose a risk to human health (Hosain *et al.,*2021).

Pesticides are divided into four groups: organophosphorus pesticides (OPPs), organochlorine pesticides (OCPs), pyrethroid pesticides, and carbamate pesticides (Eissa *et al.*, 2020). OCPs are broadly used in agriculture as insecticides (Abubakar *et al*., 2020). Also, OPPs are primarily still used as insecticides. All organochlorine insecticides exhibit environmental persistence and, hence, extended potency. However, these substances accumulate in the biosphere due to their lipophilicity and resistance to biodegradation as a result detectable levels may be found in milk and milk products (Nag, 2010). OCPs are recognized to be more stable and persistent than OPPs Salas *et al.* (2003). No doubt replacing OCPs with OPPs in agriculture activities, OPPs can accumulate during the food chain and affect public health. The studies on the binding OPPs in milk became essential (Pagliuca *et al.,* 2006).

Despite the fact that heavy metals are known to have a number of detrimental effects on health, contamination

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by heavy metals persists and is rising in many parts of the world and these effects can endure for a long time (Jaishankar *et al.*, 2014a). According to Jaishankar *et al.* (2014b), heavy metals are significant contaminants, and their toxicity has a harmful impact on the environmental and nutritional levels. Chromium, lead, arsenic, nickel, zinc, copper, and cadmium pose concerns for the environment and human health (Lambert *et al.*, 2000). Heavy metals such as cadmium and lead are carcinogens and linked to a number of illnesses affecting many body systems especially neurological and cardiovascular systems (Zhuang *et al.*, 2009).

Previous studies described that the main source of worldwide milk contamination with heavy metals and pesticide residues is animal consumption of contaminated water, feed, and grass or corn silage (Gill *et al.,* 2020; Boudebbouz *et al.,* 2021, 2023). On the other hand, raw milk can be contaminated by aflatoxin through the contaminated feeding of lactating animals or directly because of intentional or accidental cross-contamination with toxin-producing molds during and after processing (Creppy, 2002; Kokkonen *et al.* 2005). AFM1 is Aflatoxin B1 predominant metabolite (4-hydroxy), that is classified as class 2b (possibly carcinogenic), while B1 is classified as class 1 (carcinogenic) according to World Health Organization (WHO) (1993) and the International Agency for Research on Cancer (IARC).

Traditional dairy products, especially those produced from raw milk under low hygienic conditions and uncontrolled treatments, are potential vehicles for the transfer of different chemical hazards and contaminants. In Egypt, Domiati, Karish, and Ras cheeses are the most popular traditional dairy products. When using contaminated milk in cheese making, pesticide residue levels are affected by processing steps (Yigit and Velioglu, 2019).On the other hand, Aflatoxin M1 (AFM1) is heat stable and could be transferred to cheese (Galvano *et al.,*1996)

The maximum residue level (MRL) of different contaminants in dairy products for pesticides ranges from 0.8 to 2,000 g/kg (European Commission (EU), 2019) or from 0.4 to 1,000 g/kg (World Health Organization (WHO) and Food and Agriculture Organization (FAO), 1995). For AFM1, the concentration should not exceed 50 ng/kg (World Health Organization (WHO) and Food and Agriculture Organization (FAO), 2001; European Commission (EU), 2006). Therefore, the development of sensitive analytical techniques for the monitoring of these compounds becomes crucial when considering the maximum residue limits set by various regulatory agencies for these contaminants de Faria *et al*. (2021). In this context, antibiotic residues were modified and extracted from milk samples to enhance extraction recoveries while also getting close to the European Union's lower residue limitations according to Elbalkiny and Yehia (2022) and Mohamed and Elbalkiny (2023). Atomic absorption spectroscopy is considered an efficient, fast and sensitive method was developed for the determination of heavy metals in cheese products (Christophoros *et al.,* 2019). Nowadays, using mass spectrometry (MS) is increasingly common for pesticide residue detection and other contaminants, particularly in food (Pérez-Fernández *et al.,* 2017).

Therefore, to maintain the safety and acceptability of milk and its products for human consumption, effective sanitary management of milk and its products across the food chain is crucial. This study intends to evaluate the present threat posed by aflatoxin M1, heavy metals, and pesticide residues in raw milk and various Egyptian cheeses.

Materials and Methods

Materials and reagents

Nitric acid 65% and hydrogen peroxide 30% were purchased from Panreac Quimica, Barcelona, Spain. Heavy metal standard solutions (1,000 mg/l) of mercury as $Hg(NO_3)_2$ in HNO_3 0.5 mol/l, cadmium as $Cd(NO_3)_2$ in HNO_3 0.5 mol/l, copper as $Cu(NO_3)_2$ in HNO_3 0.5 mol/l, iron as $Fe(NO_3)_2$ in HNO_3 0.5 mol/l, arsenic as $As(NO_3)_2$ in HNO_3 0.5 mol/l, lead as $Pb(NO_3)_2$ in HNO_3 0.5 mol/l, and zinc as $Zn(NO_3)_2$ in HNO_3 0.5 mol/l were Sigma-Aldrich, Darmstad, Germany.

OCP standards were dichloro-diphenyltrichloroethane (DDT), DDE (1, 1-(dichloro 2,2 bis (4-clorophenyl) ethane), aldrin, heptachlor, cypermethrin, and lindane (γ-HCH). Standard solutions were prepared in hexane. Organophosphorus, carbamate, pyrethroids, and triazoles pesticide standards were dimethoate, malathion, chlorpyriphos, diazinon, monocrotofos, tetramethrin, aldicarb, profenofose, propiconazole, and carbofuran. Standard solutions were prepared in ethyl acetate. All the used standards in this study were purchased from Dr. Ehrenstorfer, LGC Ltd. (Germany). The purity of all used standards was more than 95%.

The organic solvents used were acetone, ethanol, ethyl acetate, methanol, methylene chloride, and n-hexane, analytical grade for residue analysis, anhydrous sodium sulfate, and Florisil-PR grade, (60–100 mesh) for column chromatography, obtained from Merck Company, Germany.

The enzyme-linked immunosorbent assay (ELISA) commercial kit (Helica) was purchased from Hygiena company, USA: Aflatoxin M1 (high sensitivity, low matrix; Cat. No. 961AFLM01C-UL TRA).

Collection of samples

A total of 200 random individual samples of raw milk and Egyptian cheeses were collected; (One hundred raw milk samples were collected from small dairy sectors in Alexandria and Cairo in the winter of 2021 and one hundred samples of Karish, Domiati, and Ras cheese were collected from Alexandria, El Behira, El Gharbia, and Cairo governorates). Samples were kept refrigerated until arrival at the laboratory for analysis.

Determination of heavy metals

Mercury, cadmium lead, iron, zinc, arsenic, and copper were determined in cheese samples by atomic absorption spectrometry (AA500AFG/SSN: 19071508) using microwave (ETHOS EASY/SSN: 18043065). Acid digestion of the samples occurred by microwave using HNO_3 (65%) and H_2O_2 (30%), then the solution was filtered and diluted with deionized water. The accuracy of instruments and analytical procedures was checked with standard solutions (1,000 mg /l) of mercury as $Hg(NO_3)_2$ in HNO_3 0.5 mol/l, cadmium as $Cd(NO_3)_2$ in HNO_3 0.5 mol/l, copper as Cu(NO3)2 in HNO_3 0.5 mol/l, iron as $Fe(NO_3)_2$ in HNO_3 0.5 mol/l, arsenic as $As(NO_3)_2$ in HNO_3 0.5 mol/l, lead as $Pb(NO_3)_2$ in HNO_3 0.5 mol/l, and zinc as $Zn(NO_3)_2$ in HNO_3 0.5 mol/l (Sigma-Aldrich, Darmstadt, Germany).

After obtaining the optimized microwave digestion conditions, the method was validated for linearity, detection limits, accuracy, and precision. The precision of the method was expressed as %RSD of seven replicates in two different concentration levels. Intra-day repeatability of the method was also assessed. The limit of detection (LOD) was calculated by using the standard deviation obtained by seven replicates of the lowest standard solution concentration. The correlation coefficient (R^2) of the reference curve was 0.9990 for Pb, 0.9980 for Cd, 0.9991 for Fe, 0.9993 for Zn, 0.9996 for Cu, 0.9972 for As, and 0.9996 for Hg.

The reliability of the analytical method was examined by fortifying the tested samples with known quantities of trace elements under the study following the same procedures of determination. The percentage rate of recovery was 100% for Pb, 97.5% for Cd, 91% for Fe, 89% for Zn, 97.3%for Cu, 109% for As, and 82% for Hg.

Determination of pesticide residues

Pesticide residues were determined by GC-MS/MS (Agilent 7010 B, SN: US1841v101) and LC-MS/MS (QTRAP 6500, SN: BL24151405) after acetonitrile extraction, partitioning, and clean up by dispersive SP Modular QuEChERS-method (EN 15662:2018).

Stock standard solutions were prepared separately in a concentration of 1,000 µg/ml, by accurately weighing individual analytical standards into volumetric flasks, followed by dissolving and diluting them to volume with toluene or toluene-methanol or toluene-acetone mixtures, and stored in the freezer at −20°C. An intermediate solution mixture of 10 µg/ml was prepared by diluting all stock standard solutions in toluene. This mixture was stored in the refrigerator at 4°C to be used as a spiking solution mixture and to prepare the calibration mixtures. The calibration solutions for LC-MS/MS and GC-MS/MS were prepared by diluting the spiking solution mixture at concentrations of 0.01, 0.05, 0.1, and 0.5 µg/ml and stored in the refrigerator at 4°C till usage. The organic solvents used were acetone, ethanol, ethyl acetate, methanol, methylene chloride, and n-hexane, analytical grade for residue analysis, anhydrous sodium sulfate, and Florisil-PR grade, (60–100 mesh) for column chromatography, obtained from Merck Company, Germany.

Extraction, clean up, and gas chromatograph

Pesticide residues were determined by GC-MS/MS (Agilent 7010 B, SN: US1841v101) and LC-MS/MS (QTRAP 6500, SN: BL24151405) after acetonitrile extraction, partitioning, and clean up by dispersive SP Modular QuEChERS-method according to the European Standard Method (EN 15662:2018). The measurement uncertainty expressed as "expanded uncertainty" (at a 95% confidence level) was within the range of 50%.

Validity of analytical method

The following parameters were observed during routine analysis; peak selection (retention time and qualifier ratios), spiking recovery, and blank samples to ensure the specificity of the method. The reliability of the analytical method was examined by fortifying the tested samples with known quantities of tested pesticides following the same procedures of extraction, partitioning, clean up, and analysis. The percentage rate of recovery of OCPs ranged from 83.4% to 92.1%; while, in organophosphorus, carbamate, pyrethroids, and triazoles the percentage rate of recovery varied from 85.17% to 97.95%.

Calibration, qualitative, and quantitative analysis were carried out in addition to recovery experiments through a limit of quantification (LOQ) of 0.01 mg\kg for all pesticides under the study.

Determination of aflatoxin M1

Aflatoxin M1 was detected using the ELISA commercial kit (Helica, Hygiena), USA: Aflatoxin M1 (high sensitivity, low matrix; Cat. No. 961AFLM01C-UL TRA). The kit contained AFM1 standard solutions in milk buffer for the calibration curve at levels of 0, 5,10, 20, 40, and 80 ng/l. All samples were prepared and defatted using the method outlined in the ELISA kit. The optical density was taken at the recommended wavelength (450 nm) in a specific Microplate reader (Readwell TOUCH, Roboic, Indian) using the Excel sheet (provided with the ELISA kit) was used to create a standard curve, and the final concentration of aflatoxin M1 was calculated by putting the absorbance of the samples against the standard curve. According to the kit's instruction, the dilution factor for the calculation of final AFM1 was 1, and the LOQ was 0.001 µg/kg for all examined samples with a recovery rate of 80%–113% where the coefficient of variation average (CV%) was 3.32. and cross reactivity less than 0.1%.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) program software version 21 was used for analysis of data variance.

Ethical approval

Not required for this study.

Results

The concentrations of heavy metals in milk and cheese samples

Table 1 showed that lead (Pb) concentrations ranged from 0 to 45.3 mg/l, while cadmium (Cd), concentrations ranged from 0 to 9.4 mg/l in raw milk samples.

For traditional Egyptian cheese, lead was detected in 60% of the examined samples. Where, the average concentration of Pb in different cheese samples was 0.0186 mg/l (Karish cheese), 0.0288 mg/l (Domiati cheese), and 0.0215 mg/l (Ras cheese) (Table 1). The lead content of the Domiati and Ras cheese samples was thus higher than that recommended by the Egyptian Organization for Standards and Quality (EOSQ) (2010) Egyptian Standard (ES 7136:2010). Lead and cadmium concentrations in examined milk samples were compared with the maximum permissible limits set by the International Dairy Federation, Egyptian standard (EC 7136:2010), European Commission, and Codex Alimentarius Commission. The comparison showed that lead and cadmium concentrations in examined raw milk samples (Pb: 3.02 mg\l and Cd: 1.67 mg\l) exceeded the accepted limits based on the mentioned standards. In addition, only 4% of raw milk samples had lead concentrations higher than the allowed limits, and all of them were collected from Alexandria governorate (Table 2 and Fig. 1). Also, most milk samples collected from Alexandria have a higher Cd content than the

Table 1. The concentrations (mg/kg) of heavy metals in milk and cheese samples.

Element mg/Kg	Raw milk *n* = 100	Karish cheese *n* = 33	Domiati cheese *n* = 33	Ras cheese *n* = 34
Lead (Pb)				
Min	ND*	ND	ND	ND
Max	45.3	0.056	0.079	0.055
Mean ± SE	3.02 ± 0.02	0.0186 ± 0.0002	0.0288 ± 0.001	0.0215 ± 0.001
% of samples exceed the limit	4	50	40	40
Permissible limit	0.020**[1,2]			
LOD	0.006			
Cadmium (Cd)				
Min	ND	ND	ND	ND
Max	9.74	ND	ND	ND
Mean ± SE	1.67±0.17	ND	ND	ND
% of samples exceed the limit	4			
Permissible limit	0.2[3]			
LOD	0.01			
Iron (Fe)				
Min	0.46	ND	0.46	0.84
Max	6.15	4.88	6.49	37.08
Mean ± SE	5.249 ± 0.19	2.40833 ± 0.0035	2.554 ± 0.102	9.503 ± 0.201
Permissible limit	0.7[4,5]			
LOD	0.041			
Zinc (Zn)				
Min	2.72	2.72	6.68	19.44
Max	58.2	13.69	19.48	58.2
Mean ± SE	18.91±0.90	6.747±0.412	12.882±0.905	36.934±0.856
Permissible limit	3-5 [6]			
LOD	0.033			
Copper (Cu)				
Min	ND	ND	ND	0.09

(Continued)

Element mg/Kg	Raw milk n = 100	Karish cheese n = 33	Domiati cheese n = 33	Ras cheese n = 34
Max	1.58	1	0.88	0.27
Mean ± SE	1.01 ± 0.01	0.60556 ± 0.11	0.217 ± 0.01	0.185 ± 0.01
Permissible limit	0.4 [3]			
LOD	0.010			
Arsenic (As)				
Min	ND	ND	ND	ND
Max	ND	ND	ND	ND
Mean ± SE	ND	ND	ND	ND
LOD	0.01			
Permissible limit	0.02			
Mercury (Hg)				
Min	ND	ND	ND	ND
Max	0.02	ND	ND	ND
Mean ± SE	0.01±0.002	ND	ND	ND
LOD	0.005			
Permissible limit	0.03			

*ND: not detected, (1) (European Commission (EU), 2006), (2) World Health Organization (WHO) and Food and Agriculture Organization (FAO) (1995), (3) European Commission (EU) (2001), (4) Storelli *et al.* (2007), (5) Safonov (2020), (6) World Health Organization (WHO) (1996).

Table 2. Percentage of milk samples containing higher heavy metal contents than the normal range.

Location	Milk samples containing higher heavy metals than the normal range (%) (number)			
	Copper	Cadmium	Lead	Iron
Alexandria (n = 50)	24 (n = 12)	84 (n = 42)	8 (n = 4)	20 (n = 10)
Cairo (n = 50)	84 (n = 42)	8 (n = 4)	0 (n = 0)	12 (n = 6)
Total (%)	54%	46%	4%	16%

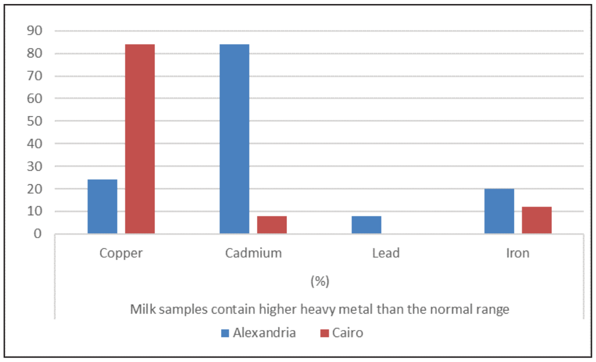

Fig. 1. Comparison between raw milk samples containing heavy metals above the maximum limit in two governorates Cairo and Alexandria in Egypt.

normally accepted range. The obtained results revealed that 46% of milk samples had a high level of cadmium, although cadmium was not detected in all traditional Egyptian cheese samples. Lead was not detected in 40% of Domiati cheese, where the maximum level was 0.079 mg/kg and the average was 0.0288 mg/kg, while lead was not detected in 50% of Karish cheese samples, where the maximum level was 0.056 mg/kg and the average was 0.0186 mg/kg. Lead was not detected in 40% of Ras cheese samples; the maximum level was 0.055 mg/kg, and the average was 0.0215 mg/kg.

Moreover, the concentrations of iron, zinc, and copper in examined milk samples were from 0.46 to 6.15 (Fe), 2.72 to 58.2 (Zn), and 0 to 1.58 mg/l (Cu) (Table 1).

Regarding cheese samples, the iron and zinc contents of Ras cheese (9.503 and 36.934 mg/kg, respectively)

Table 3. Percentage of pesticide residues in raw milk and traditional Egyptian cheeses collected from different areas.

Area	Raw milk (*n* = 100)	Karish (*n* = 33)	Domiatta (*n* = 33)	Ras cheese (*n* = 34)
Alexandria (%) (no.)	22 (11)	0 (0)	40 (6)	11 (3)
Cairo (%) (no.)	68 (34)	0 (0)	0 (0)	0 (0)
El-Behira (%) (no.)	0 (0)	100 (3)	0 (0)	0 (0)
El-Gharbia (%) (no.)	0 (0)	0 (0)	100 (6)	100 (6)
Total (%) (no.)	45 (45)	9 (3)	36 (12)	26 (9)

Table 4. Pesticide residues (mg/Kg) detected in raw milk and traditional Egyptian cheese.

Product type	No. of samples	Pesticide residue	Average	% of contaminated samples	MRL *	% of samples above MRLs
Raw milk	100	p.p-DDE Profenofose**	<LOQ***	45	0.02[1]	0
Karish cheese	33	Malathion	0.01	15	0.02[2]	0
Domiatta cheese	33	Chlorpyrifos	0.01	10	0.01[3]	0
		Malathion	0.03	15	0.02	10
Ras cheese	34	Cypermethrin	0.01	10	0.05[4]	0
		p.p-DDE Profenofose	<LOQ	10	0.02[1]	0
		Tetramethrin	0.03	10		
		Propiconazole	0.16	6	0.01[5]	10
		Piperonyl butoxide	0.04	3		

*MRL: Maximum Residue Levels, **DDE: dichloro diphenyl dichloro ethylene, ***LOQ: Limit of Quantities (1) World Health Organization (WHO) (1997), (2) European Commission (EU) (2015), (3) European Commission (EU) (2020), (4) European Commission (EU) (2016), (5) European Commission (EU) (2021).

were greater than those in the milk samples. Furthermore, it was above the permissible limit. While Karish and Domiati cheeses were lower in their content of iron and zinc than Ras cheese. On the other hand, the copper concentration in Karish cheese (0.605 mg/kg) was greater than its level in Domiati cheese (0.217 mg/kg) and Ras cheese (0.185 mg/kg).

Arsenic content was not detectable in any of the raw milk and traditional Egyptian cheese samples. The mean mercury content concentration in raw milk was 0.010002 mg; however, it is still below the accepted limit (0.03 mg/l) and was not detected in Egyptian cheese.

In this study, the determination of heavy metals was conducted by using atomic absorption spectrometry which is considered an efficient, fast, and sensitive method developed for the determination of heavy metals in cheese products (Christophoros *et al*., 2019). In our study, for all analyzed metals, low LODs, good linearity ($R^2 > 0.997$), and high recovery (82%–109%) have been achieved. The developed analytical method was successfully applied to the analysis of heavy metals in all samples under study.

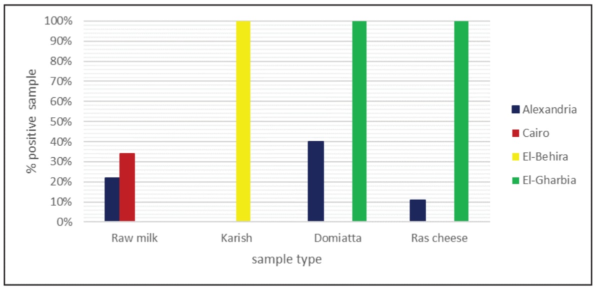

Fig. 2. Percentage of contaminated milk and cheese samples by pesticide residues.

Pesticide residues in milk and cheese samples

The estimated residual pesticide concentrations in raw marketed milk and three types of cheese (Domiati cheese, Karish cheese, and Ras cheese) in Egypt are shown in Tables 3 and 4. Most of the positive raw milk samples were from Cairo. All the Domiati cheese and Ras cheese samples from El Gharbia were positive (Fig. 2). The results revealed that 45% of raw milk samples were contaminated with P.P.-Dichlorodiphenyldichloroethylene (p.p.-DDE) profenofose, while this percentage was only 29% in Ras cheese. All collected samples were free from DDTs.

Malathion residue was found in 45% of Karish cheese and Domiati cheese. 10% of Domiati cheese exceeded the permissible limits by EU: 399/2015; however, all Karish cheese samples contained concentrations below the permissible limit.

Chlorpyrifos, as an organophosphate insecticide, was detected in Domiati cheese, and the highest level was 0.01 mg/kg, while the permissible limit was 0.01 mg/kg, according to EU: 1085/2020.

Propiconazole (fungicide) was detected in Ras cheese (0.16 mg/kg). It was greater than the permissible limit (0.01 mg/kg) according to EU: 155/2021. Insecticides such as cypermethrin and tetramethrin were detected in Ras cheese at low concentrations (0.01 and 0.03 mg/kg, respectively).

Pesticide screening of foodstuffs by both LC/MS and GC/MS analysis provides faster analysis of a wide range of pesticide classes. Chromatography/MS is irreplaceable in pesticide analysis. Nowadays, international bodies consider MS as the method of choice for pesticide analysis and quantitative methods using GC/MS and LC /MS. The key characteristics of these methods in pesticide analysis are their selectivity and sensitivity. Besides, its great advantage in identification is that it gives a mass spectrum that can be easily and quickly compared online with a library of more than 200,000 mass spectra. Specific pesticide libraries are available with hundreds of compounds. Mass spectra can be obtained with just a few pictograms of the compound injected.

Aflatoxins M1 (AFM1) in milk and cheese samples

AFM1 was assayed in a total of 200 samples (100 raw milk, 33 Domiati cheese, 33 Karish cheese, and 34 Ras cheese) (Table 5). 80% of raw milk samples were contaminated by AFM1, ranging from lower than the detection limit to 0.105 g/kg. Seven positive samples exceeded the European Commission Regulations (EC) No 1881/2006 limit.

Table 5 showed that 21.2% (7 of 33) of Domiati cheese samples were contaminated by AFM1, ranging from lower than the detection limit to 0.099 g/kg. AFM1 level in six positive samples was exceeding 0.05 g/kg. in contrast, all Karish cheese samples were positive for AFM1 ranging from below the detection limit to 0.183 g/kg. All Domiati and Karish cheese samples were below the detection limit. From the 34 Ras cheese samples, 33 ranged below the detection limit of 0.25 g/kg, with one sample exceeding the EC's limit. AFM1 levels in raw milk and cheese samples (Karish and Ras cheese) were compared, and it was found that the AFM1 level in cheese is greater than it is in milk.

ELISA method can be considered as an alternative to HPLC, owing to its advantages (rapid, easy to use, sensitive, and so on). It can be used for screening in laboratories in which HPLC is not available or when a large number of samples have to be analyzed. Also, this method is suitable and reliable for surveys of AFM1 in milk and dairy products. Kaniou-Grigoriadou *et al*. (2005) reported that the determination of aflatoxins M1 by ELISA is quick, reliable, and cost effective for the estimation of AFM1.

Discussion

The development in industrial and agricultural activities has resulted in the notable discharge of various waste materials that contain elevated levels of contaminants, such as heavy metals. Heavy metal compounds may become more concentrated in the air, water, and soil as a result of activities, and they may also find their way into grazing animals' tissues and milk (Maas *et al.,* 2011). Dairy products are one way that the majority of metals absorbed by plants and animals can subsequently find their way into the food chain (Ogabiela *et al*., 2011). Lead and cadmium are regarded as highly toxic substances that are detrimental to human health (Zhuang *et al.*, 2009; Zhu *et al.*, 2011). Lead is one of the most harmful metals for humans, plants, and animals. It is also one of the most metals commonly found in the environment. The main sources of lead in the environment are lead mines, coal combustion, wastewater applications, industrial waste, farmyard manure, and vehicle exhausts (Reilly, 2008). High cadmium concentration in milk indicates that the animals may have consumed tainted water or been fed cadmium-laden food (Eleboudy *et al.*, 2016).

Table 5. Occurrence of AFM1 in milk and Egyptian traditional cheese.

Sample type	Samples number	Positive samples number	Mean ± SD (µg/Kg)	AFM1 concentration range (µg/Kg)	No. of samples above 0.05 µg/kg	No. of samples above EC limit (0.05 µg/kg; Raw milk) (0.25 µg/Kg; Cheese)
Raw milk	100	80(80%)	0.048 ± 0.01	BDL-0.105	7 (7%)	7 (7%)
Karish cheese	33	33(100%)	0.115 ± 0.025	BDL-0.183	24 (73%)	0
Domiatta cheese	33	7(21%)	0.023 ± 0.001	BDL-0.099	6 (18%)	0
Ras cheese	34	28(82%)	0.073 ± 0.007	BDL-0.25	17 (50%)	1 (2.9%)

BDL: Below detection limit.

The difference in heavy metal content between different cheese types may be due to the variant methods used for cheese production, as the production process may have an impact on the levels of heavy metals, with the exception of lead, where the increase in mature cheese was likely caused by environmental contamination since ripening operation had no effect on the metal levels. The metals' levels are also related to the cheeses' dry matter, as the average was low in Karish cheese (a very soft cheese) and high in Ras cheese (a hard cheese). Egyptian cheeses tested in this study were heavy metal-free because most samples contained low levels of heavy metals. The level of lead in some cheese samples exceeded the permissible limit (0.02 mg/kg) (European Commission (EU), 2006). Lead ranks as the heavy metal with the most dispersion into the atmosphere due to its extensive usage in industrial operations.

A lot of research focused on removing heavy metal pollution from many sources by using smectite and Palygorsctite to absorb heavy metals (Farrah and Pickering 1977). This method can also be used in milk and dairy products. To absorb low levels of cadmium in aquatic environments, another study used a modified rice husk with varying sodium bicarbonate concentrations (Shahmohammadi *et al*., 2008). According to Penaud *et al*. (2006), lactobacilli, a probiotic agent, could absorb heavy metals from yogurt. The ability of lactobacilli to remove heavy metals refers to the binding between heavy metals and lactobacilli-specific proteins (LAB).

Some corrective actions must be taken to reduce heavy metal pollution for milk and its products during the milk production chain and the continuous regular inspection of drinking water for cattle animals. Heavy metal determination should be monitored during the handling, processing, and storage of milk and dairy, regard should be taken and at these levels by responsible organizations. Animal herds and land used for animal feeding should be far away from industrial and heavy-traffic regions.

Heavy metal determination should be monitored during the handling, processing, and storage of milk and dairy by responsible organizations. Ziarati *et al*. (2018) suggested that improving the operational efficiency and financial sustainability of milk and dairy products should be done.

In the current study, the positive samples (raw milk and Ras cheese) for p.p.-DDE profenofose were below those identified by Raslan *et al.*, who found 75% positive samples in the examined cow, buffalo, and goat milk samples collected from Zagazig, Egypt (Raslan *et al.*, 2018). All positive samples for p.p.-DDE phenofose in the current study were below the MRL.

The recorded concentrations of p.p.-DDE profenofose (LOQ) in this study were much lower than those obtained from cow milk and buffalo milk (317.83 ± 34.11 and 605 ± 50.54, respectively) in Egypt by Raslan *et al.* (2018). Although all collected samples were free from DDTs, this agreed with the results published by Shaker and Elsharkawy (2015). Its metabolite, pp-DDE, was found in 45% and 29% of the examined raw milk and Ras cheese, respectively.

Also, Hasan and Ismail (2007) reported that all collected Karish cheese samples from Kafr El-Sheikh, Egypt, was contaminated with OCPs, but much lower than the maximum residue limit. Nath *et al.* (2013) found that raw cow's milk was contaminated with Malathion in five regions, including Egypt, India, and Brazil. One percentage of the samples was above MRLs, while high concentration was reported in samples collected from the Panta region (India) (20.20 ± 5.89 ng/g). Pesticide residue levels were below the MRL except for 30% of Domiatta and Ras cheese samples. Malathion residue level was increased in 30.3% of Domiatta cheese while Propiconazole residue level was increased in 29.4% of Ras cheese. These findings refer to increasing awareness of dairy farmer owners regarding the avoidance of pesticide residues in milk from the previous status. To avoid pesticide residue contamination, it should be continuous monitoring program established for raw milk to improve dairy safety.

The percentage of contaminated milk samples by AFM1 was (80%) which is considered greater than that illustrated by Amer and Ibrahim (2010) (38%); Ismaiel *et al.* (2020) (21.6% first year and 18.3% the second year), El-Hofi and Abo El-Naga (2021) (20%) and lower than that reported by Shaker and Elsharkawy (2014) (100%) (Amer and Ibrahim, 2010; Shaker and Elsharkawy, 2014; Ismaiel *et al.*, 2020; El-Hofi and Abo El-Naga, 2021). Our results showed that only 7% of examined milk samples contaminated by Aflatoxins M1 were higher than the EC limit, which was lower than those reported by Ismaiel *et al.* (2020).

Several factors contribute to cheese contamination by AFM1. Aflatoxins (G1, G2, B1, and B2) synthesis is aided by growing microorganisms such as *Aspergillus flavus* and *Aspergillus parasiticus*; adding AMF1 contaminated powdered milk during cheese making and AFM1-casein micelles interaction (Colak, 2007).

Comparing AFM1 levels detected in raw milk and cheese samples (Karish and Ras cheese), it was shown that the AFM1 level was greater in cheese than in milk. This is reflected in the stronger association between AFM1 and casein found in cheese versus milk. Therefore, cheese curd contained a higher level of AFM1. Also, elevated AFM1 level is related to the type of cheese, the steps applied, and the degree of water loss through cheese processing (Iqbal and Asi, 2013). There are differences among countries in the AFM1 limits on cheese. The AFM1 limit in Italy is 450 ng/kg (Fallah, 2010; Duraković *et al.*, 2012; Cavallarin *et al.*, 2014; Škrbić *et al.*, 2015). In countries such as Switzerland, Austria, France, and Iran, the limit is 250 ng/kg and the Netherlands has a limit of 200 ng/kg. However, other countries such as Egypt and Romania shall apply a "zero-tolerance" strategy to ensure high consumer protection (Anfossi *et al.*, 2011). Finally, aflatoxins enter the dairy production chain by using contaminated feeding as well as contamination during the process by mold that produces these toxins. In Egypt, strict observation of aflatoxins detection in raw milk should be done. An urgent need to amend current dairy regulations in Egypt to include aflatoxins as potent contaminants in cheese.

Conclusion

Milk is a nutrient-rich food that benefits human health in several ways. Traditional Egyptian cheese is made by traditional techniques using raw milk. During milk production, it is exposed to many hazards and contaminants, which reflect on the quality of its products. The current study gives important data on heavy metals levels, pesticide residue, and aflatoxin M1 in milk and some traditional Egyptian cheese. The iron and copper levels in milk (16% and 54%, respectively) and cheese samples were higher than the permissible limit. Lead was found in 60% of cheese samples and in only 4% of milk samples. On the other hand, cadmium was detected in 46 samples out of 100, while it was not detected in any cheese samples.

All raw milk and Karish cheese samples collected were below the MRL for pesticide residue. Malathion residue was detected in 10% of Domiati samples above the MRL. A fungicide such as propiconazole was found in Ras cheese at a concentration of 0.16 mg/kg, which was higher than the MRL (0.01 mg/kg). The positive samples (raw milk and Ras cheese) for p.p.-DDE phenofose were lower than those detected by other studies in Egypt.

Aflatoxin M1 was detected in milk (80%), Karish cheese (100%), Domiati cheese (21%), and Ras cheese (82%). Only 7% of milk samples (0.05 g/kg) exceeded the acceptable limit, while 2.9% (one sample) of Ras cheese samples exceeded the acceptable limit (0.25 g/kg). In general, positive milk samples for AFM1 under this study were higher than those recorded in previous studies conducted in Egypt and other countries. While AFM1 levels in milk samples were lower than those. Thus, milk production in Egypt must be rigorously monitored to safeguard the public's health. Moreover, data verification is necessary for controlling the hazards surrounding milk production.

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Authors contributions

A. Ibrahim and S. Awad designed the research; all authors conducted the research; data analysis by A. Ibrahim; Manuscript writing by A. Ibrahim. Manuscript revision by S. Awad and M. Elsenduony. The final version is the responsibility of A. Ibrahim. All authors reviewed and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Data availability

All data supporting the findings of this study are available within the manuscript.

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