Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

ISSN NO. 2320-5407

Article DOI:10.21474/IJAR01/5669
DOI URL: http://dx.doi.org/10.21474/IJAR01/5669

RESEARCH ARTICLE

SCREENING OF SOME DAIRY PRODUCTS IN EGYPT FOR AFLATOXIN-M1 CONTAMINATION AND SOME HEAVY METAL RESIDUES.

Asmaa S.M.M[1], Walaa M. El-hady[2]and Eman N. Abdelfatah[3].

1. Food Control Department, Faculty of Veterinary Medicine, Zagazig Univeristy, Zagazig, Egypt.
2. Forensic Medicine and Toxicology Department, Faculty of Veterinary Medicine, Zagazig Univeristy, Zagazig, Egypt.
3. Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.

……………………………………………………………………………………………………....

Manuscript Info

…………………….

Manuscript History

Received: 20 August 2017
Final Accepted: 22 September 2017
Published: October 2017

Key words:-
Aflatoxins, Heavy metals, Cheese, Yoghurt.

Abstract

………………………………………………………………

This study sheds a light on the incidence of aflatoxin M1 and some heavy metals in ninety (90) samples of different types of dairy products marketed in Sharkia governorate including kariesh cheese, white soft cheese and plain yoghurt. The obtained result declared that aflatoxin M1 was highly concentrated in white soft cheese samples followed by kariesh cheese with mean values of 3.56± 0.37 and 2.78± 0.45 µg/Kg, respectively, while plain yoghurt samples recorded the least contamination level with a mean value 0.47± 0.05 µg/Kg. Concerning heavy metal results, The highest residual concentrations of heavy metals were found in kariesh cheese samples with mean values 7.03± 0.15, 0.98± 0.06, 1.67± 0.11 and 0.27± 0.04 ppm for iron, lead, copper and cadmium respectively, while the lowest values were detected in plain yoghurt samples with mean values 1.38± 0.063, 0.54± 0.03, 0.80± 0.05 and 0.18± 0.01 ppm for iron, lead, copper and cadmium respectively. The recorded data of the present study declared that, levels of aflatoxin M1 were higher than those set by Egyptian Standard and European Commission Regulation standards. Also, all the detected heavy metal residues exceeded the maximum permissible limits of the values recommended by international dairy federation standard and the Codex Alimentarius Commission. Finally, the results of our study indicate the importance of continuous and regular monitoring of chemical contaminant levels in different types of dairy products consumed in Sharkia Governorate, as our results show an alarming situation in these products with respect to all dairy product producers.

……………………………………………………………………………………………………....

Introduction:-

In the environment, there are many chemical agents that exert toxic effects on animals and humans, mycotoxins are considered the most prevalent type of them **(Bennett, and Klich, 2003).** These mycotoxins include a group of chemicals mainly produced by some species of aspergillus as *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* under particular conditions of temperature and humidity **(Hedayati et al., 2007).** These chemicals can be detected in milk and dairy products as Aflatoxin M1 (AFM1) which is a hydroxylated metabolite

Corresponding Author:- Walaa M. El-hady.
Address: Forensic Medicine and Toxicology Department, Faculty of Veterinary Medicine, Zagazig Univeristy, Zagazig, Egypt.

of aflatoxin B1 and it is produced from dairy cattle that have ingested feed contaminated with aflatoxin B1 **(Dashti et al., 2009).** The AFM1 has mutagenic, carcinogenic and teratogenic effects **(Sassahara et al., 2005)** also it is considered as major etiological factors for hepatocellular carcinoma so, its level in raw milk is of great concern **(IARC, 2002).** It is assumed that AFM1 toxin does not destroy neither by storage nor processing **(Tajkarimi et al., 2008)** and so it can be detected in dairy products submitted to heat treatment process and also in fermented products **(Picinin et al., 2013).**

The consumption of food containing heavy metal residues is another problem as the ubiquitous presence of some metal pollutants facilitates their entry into the animal ration and the food chain and thus increases the possibility of inducing toxic effects in humans and animals. Land application of sewage sludge, sewage water, and industrial wastes gradually increases the toxic metals in the soil environment, which is increasingly taking from plants and subsequently transferred into the food chain causing severe damage to both animal and human health **(Somasundaram et al. 2005).**

Some heavy metals such as copper (Cu) and iron (Fe) are necessary for a proper metabolic activity in living organisms; others, such as lead (Pb) and cadmium (Cd) are unnecessary and have no role in the biological process **(Ayar et al., 2009).** However, they can cause toxicity to living organisms, when their concentrations exceed the permissible limits **(Li et al, 2005).** They cause many health problems such as weakness, affects the kidney, heart failure and also induced cancer diseases **(McCally, 2002)**

This study was applied to determine the levels of aflatoxin-M1 and some heavy metal residues in some dairy products collected from different sites in Sharkia governorate, Egypt.

Material and methods:-

Collection of samples:-

A total of 90 samples of different types of dairy products which were locally manufactured, including kariesh cheese, white soft cheese and plain yoghurt (30 samples for each type) were used. These samples were randomly collected from different markets and dairy shops at Zagazig city, Egypt. The samples were stored at 4°C untill analysis, then each one was divided into two portions. The first portion of each type was used for quantitative determination of AFM1 and the second one was used for quantitative estimation of heavy metal.

Quantitative detection of AFM1:-

AFM1 was estimated in each sample by using the enzyme-linked immunosorbent assay (ELISA). Using Ridascreen AFM1 kits (R-Biopharm, Derm-Stadt, Germany), which contained Microtiter plates coated with specific antibodies to AFM1, AFM1 standard solution, peroxidase conjugated AFM1, together with substrate chromogen and stop solution. All samples were prepared and defatted using the method outlined in the ELISA kits, as briefly described **(Riedel de-Haen, 1997)**.

Quantitative detection of heavy metal residues:-

Quantitative determination of Fe, Pb, Cu, and Cd were measured by using atomic absorption spectrophotometer by adjusting the Cathode lamps at specific wavelengths. All glasswares were washed before use with detergent, then rinsed with tap water and soaked in nitric acid (30%), then rinsed with distilled water and air dried. 0.5 g of each milk sample was taken in 50 ml, pyrex digestion flak and digested with 5 ml of concentrated nitric acid and the contents of flask were heated on an electric hot plate at 80˚C for 2–3 hours, till the clear transparent digest was obtained. Then, the excess acid was evaporated to semi-dried mass on a heating plate. After cooling at room temperature, the final solution was diluted to 25 ml with 0.2 mol/L nitric acid and filtered through 0.45 ml Whatman filter paper in polyethylene flask for end determination **(Malhat et al., 2012).** The concentrations of metals (ppm) in the examined samples were calculated according to the following equation:

Concentration of metal in samples $= A \times B \div W$, where $A =$ metal concentration (ppm) in the prepared samples from the digital scale reading of atomic absorption spectrophotometer, $B =$ the final volume of the prepared samples, $W =$ weight of samples in gram **(Nasr et al., 2009)**

Data Analysis:-

Concentrations were expressed as a mean ± standard error, minimum and maximum values. Data were statistically evaluated by one-way analysis of variance (ANOVA). All statistical analyses were done using the statistical package for social sciences (SPSS 16.0) program.

Results And Discussion:-

The food contaminated with mycotoxins is an important source of food-borne illnesses **(WHO, 2002)**. One of the most common mycotoxins worldwide is AFM1. So, many countries have regulations for AFM1 limits in dairy products **(Van Egmond, 2004)**. Results of AFM1 of the examined samples are shown in table (1) and clear that cheese samples contain the highest concentrations of AFM1 with mean values of 3.56± 0.37 and 2.78± 0.45 µg/Kg in white soft cheese and kariesh cheese, respectively. These values may be due to the affinity of AFM1 for milk protein, mainly casein, therefore AFM1 is highly concentrated in cheese than in milk used in its production as the toxin is unevenly distributed between curd and whey **(Prandini et al., 2009)**. Moreover, the resistance of AFM1 to mild acidic conditions and heat treatment used in cheese manufacture and other dairy products has been accounted for the contamination of such products **(Colak, 2007).**

Awad et al. (2014) recorded that the range of AFM1 in the examined kariesh cheese samples was 1.95 to 6.11 with a mean value of 3.6 ppb. These results are similar to the results of our study. Higher foundings were reported by **Eman and Salem (2008)** who mentioned that the concentration of AFM1 in Kariesh cheese was 5 to 35 ppb with a mean value of 17.5±6.61 ppb, while a mean value of the examined soft cheese samples was 2.61µg/kg.

In other investigations, AFM1 was detected in cheese by 78, 64, 64.8 and 80% **(Elkak et al., 2012 and Tavakoli et al., 2012 and Duarte et al., 2013 and Mohajeri et al., 2013).**

For plain yoghurt, Aflatoxin M1 was detected with a mean value of 0.47± 0.05 µg/Kg and these results showed that the yoghurt contamination percentage was the least among all examined products. This decrease in AFM1 may be explained by the presence of *Lactobacillus acidophilus* and *L. Rhamnosus* (lactic acid bacteria) in yoghurt. These bacteria have the ability to make degradation of AFM1 and reduce its level by 18.5 - 49.6 % of yoghurt samples than its level in the original raw milk samples **(Pierides et al., 2000)**. Also, AFM1 may be associated with the milk protein during yoghurtproductiondue to thechange in casein structure causing occlusion of the toxin in the precipitate **(Brackett and Marth 1982)**. Lower results were recorded by **Akkaya et al. (2006)** and**Gürbay et al. (2006).** Other studies revealed that yoghurt samples are free from AFM1 **(Sylos et al., 1996 and Sarimehmetoglu et al., 2004).**

It is worth mentioning that there are several studies have been conducted regarding the effect of yoghurt manufacturing on AFM1 content. Some authors reported no influence on the aflatoxin M1 content **(Blanco et al., 1993).** In contrast, Bakirci **(Bakirci, 2001)** detected variable increases of AFM1 content in yogurt related to the milk.

On the other hand, results in (Table 2) showed that all the examined samples which were contaminated with AFM1 were exceeded the Egyptian regulation limits **(Egyptian Standard, 2007)** and the European Commission standards, EC **(European Commission Regulation, 2006)**, which recorded (0.25 µg/Kg) for cheese samples and (0.05 µg/Kg) for milk products.

Metals residues in milk are of a high concern because of the high consumption of milk by human especially infants **(Tripathi et al. 1999).** The concentrations of heavy metals in dairy products are presented in table (3) where the concentrations of Fe were varied from 1.38 to 7.03 ppm. The highest mean value was found in kariesh cheese samples, while the lowest one was found in plain yoghurt. The high concentration of Fe in milk and dairy products represents a problem in dairy technology due to its catalytic effect on lipid oxidation and its bounding proteins and membrane lipoproteins of the milk fat globule with the development of unpleasant smell **(Lant et al. 2006)**.

Table 5 showed that the average daily intake of Fe from consumption of 22g examined kariesh cheese, white soft cheese and 18g yoghurt samples were 0.155, 0.093 and 0.024 mg/day/person, respectively, which represents about 0.28, 0.16 and 0.04% of ADI recommended by **FAO/WHO (Codex, 2011).**

The mean concentration of Pb ranged from 0.54 to 0.98 ppm and observed to be the highest level in kariesh cheese, while plain yoghurt showed the lowest concentration. The high concentration of Pb in cheese samples is due to the characteristic Pb-binding to casein as the ripening process did not affect the level of Pb **(Coni et al. 2010)**. Pb concentration in dairy products has been determined by several studies in Egypt. **Ibrahim (2004)** and **Al-Ashmawy et al. (2008)** recorded Pb concentration as 0.13-0.98 µg/g and 0.03-0.2 µg/g respectively in kariesh cheese. These levels were lower than our results. The higher levels of Pb in our result may be attributed to the increased environmental pollutions which come from different sources (waste disposal, atmospheric deposition, urban effluent, vehicle exhausts etc.).

Table 5 showed that the average concentration levels of lead in the examined kariesh cheese, white soft cheese and yoghurt samples was 0.98, 0.94 and 0.54ppm for each type respectively, which gave daily intake of about 0.022, 0.020 and 0.012 mg/day/ person from consumption of 22g cheese or 18g yoghurt. This daily intake is 4.40, 4.00 and 2.40% of ADI recommended by **FAO/WHO (Codex, 2011).**

The concentrations of Cu were ranged from 1.14 to 2.13 µg/g., 0.90 to 1.72 µg/g., and 0.73 to 1.29 in kariesh cheese, white soft cheese and plain yoghurt respectively. These values of Cu were higher than those reported by **Guler (2007)** and **Pilarezyk et al. (2013)** who determined Cu contents in the range of < limit of detection to 0.738 mg/kg in different dairy products. The contamination of Cu in milk can occur from different sources as animal feed, high Cu content of water also from Cu bearing and Cu alloys used in equipment **(Mitchell 1981).**

On the other hand, table 5 showed that the average daily intake of Cu from consumption of 22g examined kariesh cheese, white soft cheese and 18g yoghurt samples was 0.039, 0.022 and 0.014mg/day/person which represents about 0.01, 0.06 and 0.04% of ADI recommended by **FAO/WHO (Codex, 2011).**

The Cd mean levels in the present study were ranged between 0.18 to 0.27ppm. These results were similar to that obtained by **Malhat et al. (2012)** who found Cd in a concentration ranged from 0.2 to 0.288 mg/kg in different dairy products, while our findings were higher than those reported by **Hafez and Kishk (2008)** (0.0018 mg/kg) for cow's milk; **El-Sayed et al. (2011)** (0.002–0.039 mg/kg) for milk collected from different areas in Egypt and by **Rahimi (2013)** (0.00951 mg/L) for milk. These higher values of Cd may be due to either anthropogenic or natural origin (deposition of Cd in soils from the atmosphere and fertilizers) **(Arafa et al. 2014)**. The values of Cd were not significantly different between all products of our milk product samples; this may be attributed to the close association of Cd to the soluble fraction and its dispersion between casein and components of low molecular mass (**Anastasio et al. 2006).**

Table 5 declared that the average concentration of Cd in the examined kariesh cheese, white soft cheese and yoghurt samples was 0.27, 0.23 and 0.18 ppm for each type respectively that gave daily intake of about 0.0051, 0.0049 and 0.0004 mg /day/person from consumption of 22g cheese and 18g of yoghurt that contributed about 7.29, 7.00 and 0.57% of ADI recommended by **FAO/WHO (Codex,2011).**

Table (4) showed that permissible limit of pb according to the **Codex Alimentarius Commission (2014)** was 0.02 ppm and all the examined dairy product samples are exceeding these permissible levels. On the other hand 100% of the examined dairy product samples were exceeded the permissible levels that recommended by and **IDF Standard (1979)** for Fe, Cu, and Cd which are 0.037, 0.01 and 0.0026 µg/g for each respectively. Higher levels of these metals in our dairy product samples may be attributed to the use of sewerage water for agriculture purposes that may be directly accessed by animals for drinking. Bioaccumulation of these metal residues in soil, fodder and vegetables may have a main role in the contamination of the milk composition **(Javed et al. 2009).**

Table (1):-Occurrence of AFM1 in dairy product samples collected from local markets in Sharkia.

Dairy products	Examined samples N.	Positive samples N (%)	Min–max (µg /Kg)	Mean ±SE (µg /Kg)
Kariesh cheese	30	15 (50 %)	0.98-5.92	2.78± 0.45
White soft cheese	30	12 (40%)	1.05-3.68	3.56± 0.37
Plain yoghurt	30	9 (30 %)	0.31-0.82	0.47± 0.05

Table (2):- Comparing the detected levels of AFM1 (µg/kg) in the examined dairy product samples to levels of the existing regulations

Dairy products	Positive samples	Exceeding Egyptian regulations (a) 0.0 µg//kg		Exceeding EC regulations (b) (0.25 µg/Kg for cheese) (0.05.µg/Kg) for milk products.	
		N (%)	Range	N (%)	Range
Kariesh cheese	15(50 %)	15(100 %)	0.98 -5.92	15(100 %)	0.98 -5.92
White soft cheese	12(40%)	12(100%)	1.05-3.68	12(100%)	1.05-3.68
Plain yoghurt	9 (30 %)	9 (100 %)	0.31-0.82	9 (100 %)	0.31-0.82

(a)- **Egyptian Standard, (2007)**. (b)- (**European Commission Regulation, 2006**

Table (3):- Mean levels of heavy metals in the examined dairy product samples:

Dairy products / Metals Mean±S.E		Kariesh cheese	White soft cheese	Plain yoghurt
Fe (ppm)	Min.	6.31	3.14	1.21
	Max	7.80	5.12	1.59
	Mean ±SE	7.03± 0.15	4.25± 0.34	1.38± 0.063
Pb (ppm)	Min.	0.89	0.76	0.35
	Max	1.34	1.46	0.57
	Mean ±SE	0.98± 0.06	0.94± 0.13	0.54± 0.03
Cu (ppm)	Min.	1.14	0.90	0.73
	Max	2.13	1.72	1.29
	Mean ±SE	1.67± 0.11	1.23± 0.22	0.80± 0.05
Cd (ppm)	Min.	0.22	0.19	0.14
	Max	0.31	0.31	0.36
	Mean ±SE	0.27± 0.04	0.23± 0.02	0.18± 0.01

Table (4):- The examined dairy product samples that comply with the permissible limits for the analyzed heavy metals:

Metals	Permissible limits (ppm)	Dairy product samples within permissible limit					
		Kariesh cheese		White soft cheese		Plain yoghurt	
		No.	%	No.	%	No.	%
Fe	0.037[a]	0	0	0	0	0	0
Pb	0.02[a]	0	0	0	0	0	0
Cu	0.01[a]	0	0	0	0	0	0
Cd	0.0026 [b]	0	0	0	0	0	0

a: IDF Standard (1979) **b: -** Codex Alimentarius Commission (2014)

Table (5):-Comparison of Acceptable Daily Intake (ADI) value of heavy metals with the calculated daily intake of the examined dairy products:

Metals	ADI mg /60kg person [a]	Mean of metals in total examined dairy product samples (ppm)		Calculated average daily intake of metals from consumption of 22g,22g and 18g /day [b]	
				mg /day/person	% [c]
Fe	**56**	Kariesh cheese	7.03	0.155	0.28
		White soft cheese	4.25	0.093	0.16
		Plain yoghurt	1.38	0.024	0.04
Pb	**0.5**	Kariesh cheese	0.98	0.022	4.40
		White soft cheese	0.94	0.020	4.00
		Plain yoghurt	0.54	0.012	2.40
Cu	**35**	Kariesh cheese	1.67	0.039	0.01

		White soft cheese	1.23	0.022	0.06
		Plain yoghurt	0.80	0.014	0.04
Cd	**0.07**	Kariesh cheese	0.27	0.0051	7.29
		White soft cheese	0.23	0.0049	7.00
		Plain yoghurt	0.18	0.0004	0.57

a: according to FAO/WHO (Codex, 2011).
b: Daily consumption for adult person according to (FAO, 2009 &Al-Ashmawy, 2011&Meshref et al, 2015)
c: Percentage calculated to ADI.

Conclusion:-

In conclusion, the present study revealed high contamination of Kariesh, white soft cheese and even plain yoghurt with AFM1 and toxic levels of heavy metals (Fe, Pb, Cu and Cd) which comprise a public health hazard. And so strict regular monitoring of heavy metals in milk and dairy products, storage of food under a condition which prevents mould growth, Implementing a food control system, such as the HACCP (*Hazard analysis and critical control points)* system in the food industries are recommended. Aflatoxin B1contaminated feeding for dairy cattle should be avoided.This seems to be the most practical way for decreasing the AFM1 contamination level in dairy products.

References:-

1. Akkaya, L., Birdane, Y. O., Oguz, H. and Cemek, M. (2006): Occurrence of aflatoxin M1 in yogurt samples from Afyonkarahisar, Turkey. Bulletin of the Veterinary Institute in Pulawy, 50, 517-519.
2. Al-Ashmawy, M.A.M., Hassan, G.M. and Abd-Elall, A.A. (2008): Toxic heavy metals in market raw milk and some dairy products. J. Egypt. Vet. Med. Assoc. 68(2), 211–224
3. Al-Ashmawy, M.A.M. (2011): Prevalence and public health significanceof aluminum residues in milk and some dairy products. J. FoodSci. 76(3), T73–T76 (2011)
4. Anastasioa, A. R. C., Macchiatoc, M., Catellani, P., Maria, R., Salvatore, P. and Cortesi, M.L. (2006): Heavy Metal Concentrations in Dairy Products from Sheep Milk Collected in Two Regions of Southern Italy. Acta vet. scand. 47, 69-74.
5. Arafa M. S. M., Walaa A. M. and Nour El-Houda Y. H. (2014): Heavy metals and trace elements levels in milk and milk products.Food Measure 8:381–388
6. Awad, E.I., Amer, I.H., Mansour, H and Ismail, S.Y. (2014): Qualitative and Quantitative Detection of Aflatoxin M1 Residues in White Soft Cheese. Alexandria Journal of Veterinary Sciences, 40: 119-123.
7. Ayar, A., Sert, D. and Akin, N. (2009): The trace metal levels in milk and dairy products consumed in middle Anatolia-Turkey, Environmental Monitoring Assessment, 152: 1-12.
8. Bakirci, I. (2001): A study on the occurrence of aflatoxin M1 in milk and milk products produced in Van province of Turkey. Food Control, 12: 45-51.
9. Bennett, J. W. and Klich, M. (2003): Mycotoxins. Clinical Microbiology Reviews.16, P.497-516.
10. Blanco, J.L., Carrion, B.A., Liria, N., Diaz, S., Garcia, M.E., Domingues, L. and Suarez, G. (1993): Behavior of aflatoxin during manufacture and storage yogurt. Milchwissenchaft, 48: 385-387.
11. Brackett, R.E. and Marth, E.H. (1982): Association of aflatoxin M1 with casein. Zeitschrift fur LebensmittelUntersuchung und Forschung, 174: 439-441
12. Codex Alimentarius Commission (2011): FAO/WHO, Joint Food Standards Programme, Codex Committee on Contaminants in Foods, Working document for information and use in discussions related to contaminants and toxins in the GSCTFF, List of Maximum Levels for Contaminants and Toxins in Foods, Part 1, March, CF/5 INF/1.
13. Codex Alimentarius Commission (2014): FAO/WHOJoint Food Standards Programme, Codex Committee on Contaminants in Foods, Eighth Session The Hague, The Netherlands, 31 March – 4 April 2014 (Prepared by Japan and the Netherlands.
14. Colak, H. (2007): Determination of Aflatoxin M1 Levels in Turkish White and Kashar Cheeses Made of Experimentally Contaminated Raw Milk Journal of Food and Drug Analysis, Vol. 15, No. 2, pp. 163-168 ISSN 10219498
15. Coni, E., Bocca, A., Coppolelli, P., Caroli, S., Cavallucci,C. , Trabalza and Marinucci M. (2010): Minor and trace element content in sheep and goat milk and dairy products. Food Chem; 57:253e60
16. Dashti, B., Al-Hamli, S., Alomirah, H., Al-Zenki, S., Bu Abbas, A. and Sawaya, W. (2009): Levels of aflatoxin M1 in milk, cheese consumed in Kuwait and occurrence of total aflatoxin in local and imported animal feed.

Food Control Journal. 20, P. 686–690.
17. Duarte, S.C., Almeida, A.M., Teixeira, A.S., Pereira, A.L., Falcão, A.C., Pena, A. and Lino, C.M. (2013): Aflatoxin M1 in marketed milk in Portugal: Assessment of human and animal Exposure. Food Control, 30: 411-417.
18. Egyptian Standard 1- 1875. (2007): "Maximum levels of mycotoxin for foods and feeds, part-1: Aflatoxins" Egyptian Organization for Standardization and Quality.
19. Elkak, A., El Atat, O., Habib, J. and Abbas, M. (2012): Occurrence of a flatoxin M1 in cheese processed and marketed in Lebanon. Food Control, 25: 140-143.
20. El Sayed, E.M., Hamed, A.M. ,Badran, S.M. and Mostafa, A.A. (2011): A survey of selected essential and heavy metals in milk from different regions of Egypt using ICP-AES. Food Addit.Contam. Part B 4(4), 294–298
21. Eman, M. El-Diasty and Salem, R. M. (2008): Incidence of lipolytic and proteolytic fungi in some dairy productsand their public health significance .Faculty of Veterinary Medicine, Omar El-Mokhtar University-Libya.
22. European Commission Regulation (EC). (2006): No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of European Union, 364:5-24. downloaded*from http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF* on 12/8/2014.
23. FAO (Food and Agriculture Organization) (2009) Food balancesheet, www.faostat3.fao.org/faostat-gateway/go/to/download/FB/*/E
24. Guler, Z. (2007): Levels of 24 minerals in local goat milk, its strained yoghurt and salted yoghurt (Tuzlu yogurt). Small Ruminant Res. 71, 130–137.
25. Gürbay, A., Engin, A. B., Çaglayan, A., and Sahin, G. (2006): Aflatoxin M1 levels in commonly consumed cheese and yogurt samples in Ankara, Turkey. Ecology of Food and Nutrition, 45, 449-459.
26. Hafez, L.M. and Kishk, A.M., (2008): Level of lead and cadmium in infant formulae and cow's milk. J. Egypt. Public Health Assoc. 83(3&4), 285–293.
27. Hedayati, M. T., Pasqualotto, A. C., Warn, P. A., Bowyer, P. and Denning, D. W.(2007): *Aspergillusflavus*: human pathogen, allergen and mycotoxin producer. Microbiology. 153, P. 1677-1692.
28. Ibrahim, E.M.A. (2004): Cadmium, copper and lead in some kinds of cheeses. Benha Vet. Med. J. 15(2), 55–64
29. International Agency for Research on Cancer (IARC)(2002): Some traditional herbal medicines: Some mycotoxins, naphthalene and styrene. IARC Monograph on the Evaluation of Carcinogenic Risks to Humans, World Health Organization, Lyon, France. 82, 2002, P.1–556.
30. International dairy federation standard (IDF) (1979): Metal contamination in milk and milk products. International Dairy Federation Bulletin. Document no. A. Doe 37.
31. Javed, I., Jan, A., Muhammad, F., Rahman, Z., Zargham Khan, M., Aslam, B. and Iqbal S. (2009): Heavy Metal Residues in the Milk of Cattle and Goats During Winter Season Bull Environ ContamToxicol. 82:616–620
32. Lant, G., Lomolino, G., Cagnin, M. and Spttoli, P. (2006): Content and characterisation of minerals in milk and in Crescenza and Squaacquerne Italian fresh cheese by ICP-OES. Food Control 17:229–233
33. Li, Y.; McCrory, D.F.; Powel, J. M.; Saam, H. and Jackson-Smith, D. (2005): A survey of selected heavy metal concentrations in Wisconsin Dairy Feeds. Journal Dairy Science, 88: 2911-2922.
34. Malhat, F., Hagag, M., Saber, A. and Fayz, A. (2012): Contamination of cows milk by heavy metal in Egypt. Bull. Environ. Contam.Toxicol. 88, 611–613
35. McCally, M. (2002): Human health and heavy metals exposure. The Environment and Human Health (Chapter 4).
36. Meshref, A.M.S, Walaa, A., Moselhy and Nour El-Houda, Y.H. (2015)**:** Aluminium Content in Milk and Milk Products and its Leachability from Dairy Utensil**s.** International Journal of Dairy Science 10 (5): 236-242, 201**5**
37. Mitchell, E. (1981): Trace metal level in Queensland dairy products. Aust J Dairy Technol 6:70–73.
38. Mohajeri, F.A., Ghalebi, S.A., Rezaeian, M., Gheisari, H.R., Azad, H.K., Zolfaghari, A., Aziz, A. and Fallah, A. (2013): aflatoxin M1contamination in white and Lighvan cheese marketed in Rafsanjan, Iran. Food Control, 33: 525-527.
39. Nasr, I.N., salah El-Dien, W.M. and Nabela I. Elsharkawy (2009): Evaluation of polychlorinated Biphenyls, Organochlorine Pesticide and Heavy metal levels in some meat products. J.Egypt.vet.med.Assoc 70,no.2:7
40. Picinin, L.C.A., Cerqueira, M.M.O.P., Vargas, E.A., Lana, A.M.Q., Toaldo, I.M. and Bordignon-Luiz, M.T. (2013): Influence of climate conditions on a flatoxin M1 contamination in raw milk from Minas Gerais State, Brazil Food Control Journal. 31, P.419-42.
41. Pierides, M., El-Nezami, H. and Peltonen K, et al. (2000): Ability of dairy strains of lactic acid bacteria to bind aflatoxin M1 in a food model. J Food Protect, 63: 645-650.

42. Pilarezyk, R., Wojcik, J., Czerniak, P., Sablik, P., Pilarezyk, B. and Tomza-Marciniak, A. (2013): Concentrations of toxic heavy metals and trace elements in raw milk of Simmental and Holstein-Friesian cows from organic farm. Environ. Monit. Assess. 185, 8383–8392
43. Prandini, A., Tansini, G., Sigolo, S., Filippi, L., Laporta, M., and Piva, G. (2009): On occurrence of aflatoxin M1 in milk and dairy products. Food and Chemical Toxicology, 47, 984-991.
44. Rahimi, E. (2013): Lead and cadmium concentrations in goat, cow, sheep and buffalo milks from different regions of Iran. Food Chemistry, 136: 389–391.
45. Riedel de-Haen, A.G. (1997): EnzymeimmunoassayZur quantitative bestimung von Aflatoxin-M1, ELIZA system, diagnostic cataloug, p.2.
46. Sarimehmetoglu, B., Kuplulu, O. and Celik T.H. (2004): Detection of aflatoxin M1 in cheese samples by ELISA. Food Cont. 2004, 15, 45–49.
47. Sassahara, M., Pontes, N. D. and Yanak, E. K. (2005): Aflatoxin occurrence in foodstuff supplied to dairy cattle and aflatoxin M1 in raw milk in the North of Parana state. Food and Chemical Toxicology. 43, 2005, P.981-984.
48. Somasundaram, J., Krishnasamy, R. and Savithri, P. (2005): Biotransfer of heavy metals in Jersey cows. Indian Journal Animal Science, 75:1257–1260.
49. Sylos, C.M., Rodriguez-Amaya, D.B. and Carvalho, P.R.N. (1996). Occurrence of aflatoxin M1 in milk and dairy products commercialized in Campinas, Brazil. Food Additives and Contaminants, 13, 169-172.
50. Tajkarimi, M., Aliabadi-Shojaee, F., Salah Nejad, A., Pursoltani, H., Motallebi, A. A. and Mahdavi, H. A. (2008): Aflatoxin M1 contamination in winter and summer milk in 14 states in Iran. Food Control. 19, P. 1033-1036.
51. Tavakoli, H.R., M. Riazipour, A. Kamkar, H.R. Shaldehi and A.S.M. Nejad, (2012): Occurrence of aflatoxin M1in white cheese samples from Tehran, Iran. Food Control, 23: 293-295.
52. Tripathi, M., Raghunath, R., Sastry, N. and Krishnamoorthy, M. (1999): Daily intake of heavy metals by infants through milk and milk products. Sci Total Environ 227:229–235.
53. Van Egmond, H. P. (2004): Worldwide regulations for mycotoxins in food and feed in 2003, FAO Food and Nutrition Paper 81. Rome, Italy: Food and Agriculture Organization of the United Nations
54. WHO, (2002): Global Strategy for Food Safety: safer food for better health. Food Safety Programme.World Health Organization (WHO), Geneva, Switzerland.