J. Food and Dairy Sci., Mansoura Univ., Vol. 3 (12): 725 - 733, 2012

ASSESSMENT OF TOXIC HEAVY METAL RESIDUES IN SOME TYPES OF CHEESE BY USING ICP-OES

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ABSTRACT

A total of 52 random samples including white soft cheese (Fiume cheese, Double cream, Mallawi, Mature sheep, Pramely with pepper, Pramely, Talaga and Plant double cream cheese), Ras and processed cheese were collected from different locations in Assiut Governorate. The collected samples were evaluated for the presence of lead (Pb), cadmium (Cd), iron (Fe), selenium (Se), manganese (Mn) and mercury (Hg) by using inductively coupled plasma optical emission spectrometry (ICP-OES). All the examined soft, Ras and processed cheeses were contaminated with investigated trace elements with different values except Cd, Se and Hg were not detected in some types of cheese. The concentration ranges in the investigated samples were found to be 0.0145- 0.0429, 0.0000-0.0008, 0.4632- 4.9452, 0.0000-0.0038, 0.0267-0.1377 and 0.0000-0.0144 ppm for lead, cadmium, iron, selenium, manganese and mercury, respectively. High trace metal and mineral accumulation levels in the samples were found in Mallawi and Ras cheese for Pb, Fiume and Ras cheese for Cd, Fiume, processed and Ras cheese for Fe, Pramely, Double cream and Ras cheese for Se, Ras, Pramely and Fiume cheese for Mn and Fiume and double cream cheeses for Hg, respectively. Also, levels correlations between elements were tested.

Keywords: Heavy metals, Soft cheese, Ras cheese, Processed cheese, ICP-OES.
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INTRODUCTION

Cheese is very nutritive food stuff, not only for its protein and fat contents, (Renner, 1987) and also an important source of several nutritionally important elements, including calcium, phosphorus, and magnesium (O'Connor and O'Brien, 2000).

Many dangerous elements or compounds, such as metals and metalloids, accumulate along the food chain. Furthermore their concentrations in the environment grow with the increase of urban, agricultural, and industrial emissions. The almost ubiquitous presence of some metal pollutants, especially Cd, Pb and Hg, facilitates their entry into the food chain and thus increases the possibility of them having toxic effects on humans and animals. While post-milking contamination from processing equipment's, reagents, accidental contamination during storage and milking and leaching from containers (El–Kewaiey *et al.*, 2009)

Many of the dairy products produced from cow milk may expose to trace metal contamination. Several trace metals in dairy products may cause severe health problems in animals and human beings. Therefore, limitations on trace metal contents in foods are included in recent food legislation and regulations (Simsek *et al.*, 2000). According to the notifications in the Turkish

Food Codex (TFC) and European Communities regarding the determination of the maximum levels of contaminants in food products, Pb and Cd amounts should not exceed, 0.02 (milk)-0.20 (milk powder and cheese), 0.01–0.50 mg/kg, respectively. No limitation was imposed for elemental Se (TFC, 2002).

Food safety and quality start at the farm and continue throughout processing, distribution chain, storage and the final preparation (FAO, 1992). Both of cheese types and packaging materials (plastic and tin containers) played a key role in the content of trace metal contents (Bakircioglu *et al.*, 2011).

Heavy metals are persistent as contaminants in the environment and come to the fore front of dangerous substances causing healthy hazard in human. Lead, cadmium, mercury and tin are among the most important of these elements. Industrial and agricultural processes have resulted in an increased concentration of heavy metals in air, water, soil and subsequently, these metals are taken by plants or animals and take their ways into food chain (Ahmad, 2002).

Cadmium considered to be the most problematic of the heavy metals. It was a cumulative toxic element that was mainly deposited in the liver and kidneys (Niemi *et al.,* 1991). Cadmium level increased throughout the life span due to its long biological half-life which reached 10-30 years (Klaassen, 1985).

Consequently, this study was done to determine the levels of some toxic trace elements contaminant in some types of cheese, sold in major supermarket chains in Assiut province, in addition to determine the percentage of samples exceed the maximum permissible limits of the mentioned elements in cheeses, by using inductively coupled plasma atomic emission spectrometry (ICP-OES) in ppm.

MATERIALS AND METHODS

1-Collection of samples:

A total of 53 random cheese samples including white soft cheese (Fiume cheese, Double cream, Mallawi, Mature sheep, Pramely with pepper, Pramely, Talaga and Plant double cream cheese) in addition to processed and Ras cheese were collected from different local markets in Assiut Governorate. All samples of cheese were collected in polyethylene bags and taken to the laboratory without delay. Each sample was labeled to identify the source of cheese samples. Delayed samples were stored in ice bag. Sampling procedure was performed according to Ayar *et al.* (2009).

2. Chemical analysis

2.1. Estimation of certain elements content in cheese.

The levels in mg/kg of Pb, Cd, Fe, Se, Mn and Hg in all cheese samples had been estimated according to the method described by James (1995) in Analytical Chemistry of Foods. A weight of 5.0 g of cheese samples were ashed in muffle furnace at 550˚C over night .The obtained ash was dissolved in 5 ml HCl (36.6%) and the volume was completed to 50.0 ml by distilled water, then dilutions were applied to estimate the levels of investigated

elements using inductively coupled plasma optical emission spectrometry (ICP-OES) (ICAP6200) in the central laboratory, Faculty of Agriculture, Assiut University. The working conditions for ICP-OES are given in Table 1.

Table 1. Operating conditions for the instrument parameter.

Parameter	Setting
Pump tubing	Tygon orange/white
Pumb speed	45rpm
Nebulizer	Standard concentric
Nebulizer argon Flow	0.6L/min
Spray champer	Standard cyclonic
Center tube	1.5mm
RF Forward power	1150W
Purge gase	Argon
Coolant gase flow	12L/min
Auxiliary gase flow	0.5L/min

3. Statistical analysis

Results were evaluated statistically by using the software program; the SAS system for windows, release 8.02 TS level 02M0, SAS Institute Inc., Cary, NC, USA (SAS, 1999).

RESULTS AND DISCUSSION

The lead element was found in different concentrations in all types of examined cheeses. It is clear from obtained data presented in Tables 2, 3 and 4 that the mean values of lead (Pb) was the highest in Mallwai cheese with an average of 0.0429 ppm followed by Fiume with an average of 0.0270 ppm and the lowest value was found in Pramely with pepper which gives 0.0145 ppm.

The obtained levels of Pb content in investigated soft cheese were lower than that recorded for Domiati cheese by El-Baradie (1994) (1.2-1.58 mg/kg); Abdou and Korashy (2001), (0.77, 1.2 and 0.67 ppm for soft cheese, fresh Kareish and pickled Kareish, respectively), Ibrahim (2004) Aly *et al.* (2010) are in nearly agreement with El-malt (2001) (0.115 and 0.212 ppm for two Domiati cheese samples). On the other hand, IDF (1992) reported that the acceptable Pb limits in cheese were 0.5, 0.2, 1.0, 0.25, 0.3 and 0.5 ppm in Australia, Denmark, Czechoslovakia, Germany, Netherland and Poland respectively, which the present level was lower than these limits.

The levels showed for cadmium (Cd) were the highest contents in Fiume cheese with an average of 0.0008, while the lowest value was in Parmely with pepper cheese 0.00002 ppm. On the other side, the lead content was not detected in Mature sheep and Talaga cheeses.

These findings were lowest than those obtained by Abou-Elenein (1998) and El-Sharkawy *et al.* (2007) who indicated that pickled cheese and Kareish cheese contain Cd with levels of 0.415 and 0.500 ppm respectively, while the data of this study are in agreement with those obtained by Favretto

and Marletto (1984) who recorded higher values with 0.003 ppm for soft cheese and Abdou and Korashy (2001) reported that both of soft and Kareish cheese did not show any contamination with Cd. Ayar *et al.* (2009) found that the Cd content was varied between 0.022-0.058 in white cheese. The contents of Cd in the results of this study were lower than that reported by Ibrahim (2004) and Aly *et al.* (2010). As reported by IDF (1992) present levels of Cd in examined cheeses were lower than those acceptable levels for cheese in Australia and Germany (0.05 mg/kg) and in Netherland (0.1mg/kg).

Table 2. Residue concentration of some heavy metals (ppm) in examined white soft cheese.

Elements		Pb	Cd	Fe	Se	Mn	Hg
Fiume cheese n*=4	Min	0 .0205	0 .0001	0.7917	0.0000	0.0358	0.0019
	Max	0 .0407	0.0017	16.5900	0.0019	0.1214	0.0493
	Mean ±S.E	0. 0270 ± 0.0047	0.0008 ±0.0004	4.9452 ±3.8862	0.0008 ±0.0005	0.0806 ±0.0224	0.0144 ±0.0116
Double cream cheese n=3	Min	0.0184	0.0000	0.4378	0.0000	0.0611	0.0000
	Max	0.0235	0.0005	6.0020	0.0054	0.0709	0.0083
	Mean ±S.E	0.0209 ±0.0026	0.0002 ±.00024	3.2199 ±2.7821	0.0027 ±0.0027	0.0661 ±0.0049	0.0042 ±0.0042
Mallawi cheese n=4	Min	0.0243	0.0000	0.7203	0.0000	0.0391	ND
	Max	0.0871	0.0006	4.2360	0.0038	0.1298	
	Mean ±S.E	0.0429 ±0.0148	0.0002 ±0.0001	1.6443 ±0.8648	0.0009 ±0.0009	0.0754 ±0.0195	
Mature sheep cheese n=3	Min	0.0149	ND**	0.6970	ND	0.03792	ND
	Max	0.0149		0.6970		0.03793	
	Mean ±S.E	0.0149 ±0.0000		0.6970 ±0.0000		0.0379 ±0.0000	

*N: Number of Samples ** ND: Not Detected

Fiume cheese gives the highest levels of Iron (Fe) with an average of 4.9452 ppm followed by double cream cheeses with an average of 3.2199 ppm, respectively. On the other hand, both of Pramely with pepper and Plant double cream were the lowest value of Fe with an average of 0.5585 and 0.4632 ppm, respectively. These results are in harmony with those reported by Moreno-Rojas *et al.* (1994) who showed a very low level of Fe (0.007 mg/kg) of soft cheese, the same levels were found by Abou-Arab (1991), (5.38 ppm.) El-Baradie (1994) (ranged from 3.87 to 4.21 ppm), Coni *et al.* (1996) (ranged from 2.01 to 4.06 ppm.), and Ayar *et al.* (2009) showed that white cheese contains 0.60-2.20 ppm and Aly *et al.* (2010) ranged from 0.14 to 1.14 in white cheese. On the other hand, high levels were reported by Abou-El-Enein (1998) which varied between 28.5 ppm and 138.2 ppm.

The selenium element was found in different concentrations in all types of examined cheeses except Pramely with pepper, Talage and Mature sheep. From obtained data presented in Table 2, 3 and 4 it has been found that the mean values of selenium (Se) was highest in Pramely with an average of 0.0038 ppm followed by Double cream cheese with an average of 0.0027 ppm and the lowest value was found in Fiume cheese with an average of 0.0008 ppm. These results was lower than that reported by Ayar *et al.* (2009) who showed that the Se content in white cheese ranged from 0.09 to 0.55 ppm.

Food and milk selenium concentrations vary considerably from country to country and in different areas of the same country, due, primarily, to a variation in soil Se levels. Selenium enters the food chain through plants, but levels of the element in the plant do not necessarily reflect the soil level, because some plants can accumulate selenium against a concentration gradient and other factors, notably soil pH, affect very markedly the ability of plants to take up selenium (Yanardğ and Orak, 1999).

The levels recorded for manganese (Mn) were the highest in Ras cheese with an average of 0.1377 ppm followed by Pramely cheese with an average of 0.0965 ppm and Fiume cheese with an average of 0.0806, while the lowest value was in Talage cheese with an average of 0.0267 ppm. These findings were lower than those obtained by Sayed *et al.* (2012).

Table 3. Residues concentration of some heavy metals (ppm) in soft cheese.

Elements		Pb	Cd	Fe	Se	Mn	Hg
Pramely with pepper n=3	Min	0.0138	0.0000	0.5459	ND*	0.0332	ND
	Max	0.0153	0.0001	0.5710		0.0591	
	Mean ±S.E	0.0145 ±0.0007	0.00002 ±0.00002	0.5585 ±0.0125		0.0461 ±0.0129	
Pramely n=6	Min	0.0174	0.0000	0.4399	0.0000	0.0289	0.0000
	Max	0.0419	0.0014	1.1530	0.0157	0.1928	0.0051
	Mean ±S.E	0.0226 ±0.0039	0.0006 ±0.0002	0.7338 ±0.1005	0.0038 ±0.0025	0.0965 ±0.0220	0.0012 ±0.0008
Talaga cheese n=3	Min	0.0180	ND	0.7676	ND	0.0269	ND
	Max	0.01805		0.7676		0.0269	
	Mean ±S.E	0.0180 ±0.00001		0.7676 ±0.00001		0.0267 ±0.000001	
Plant double cream n=3	Min	0.0178	0.00013	0.4632	0.0018	0.0386	ND
	Max	0.0178	0.00013	0.4632	0.0018	0.0386	
	Mean ±S.E	0.0178 ±0.0000001	0.00013 ±0.00001	0.4632 ±0.00001	0.0018 ±0.0000002	0.0386 ±0.000006	

* **ND: Not Detected**

Mallawi, Mature sheep, Pramely with papper, Talaga, and Plant double cream cheeses were not contaminated by Hg. Data in Table 2, 3 and 4 cleared that the mean values of mercury (Hg) was the highest in Fiume cheese with an average of 0.0144 ppm followed by Double cream cheese with an average of 0.0042 ppm and the lowest value was found in Ras cheese with an average of 0.00005 ppm. Amer *et al.* (2005) and Janssen (1997) recorded Hg concentration in high level in cheese. Meanwhile, lower concentration was recorded by Simsek *et al.* (2000).

Table 4. Residue concentration of some heavy metals (ppm) in Ras and processes cheese.

Elements		Pb	Cd	Fe	Se	Mn	Hg
Plant double cream n=3	Min	0.0178	0.00013	0.4632	0.0018	0.0386	ND*
	Max	0.0178	0.00013	0.4632	0.0018	0.0386	
	Mean ±S.E	0.0178 ±0.0000001	0.00013 ±0.00001	0.4632 ±0.00001	0.0018 ±0.0000002	0.0386 ±0.000006	
Ras cheese n=5	Min	0.0143	0.0001	0.5817	0.0000	0.0632	0.0000
	Max	0.0299	0.0022	1.9490	0.0101	0.3860	0.0003
	Mean ±S.E	0.0229 ±0.0027	0.0007 ±0.0004	1.1394 ±0.2322	0.0023 ±0.0019	0.1378 ±0.0622	0.0001 ±0.00005
Processed cheese n=19	Min	0.0081	0.0000	0.2667	0.0000	0.0157	0.0000
	Max	0.0302	0.0037	17.4300	0.0069	0.1436	0.0095
	Mean ±S.E	0.0161 ±0.0009	0.0007 ±0.0002	1.4423 ±0.0393	0.0013 ±0.0004	0.0386 ±0.0012	0.0009 ±0.0003

* **ND: Not Detected**

From the previously mentioned results, it is evident that the trace elements content of cheese are variable because of many factors such as characteristics of the manufacturing procedures and possible contamination from the equipment during processing, packaging, and storage. So, it is necessary to control the manufacturing process at each step, in order to determine the source and levels of contamination and to ensure the desired product quality (Ayar *et al.*, 2009).

Correlation

Data in Table (5) presented the correlation between the trace elements in cheeses. The results showed that cadmium had a positive correlation with highly significantly differed ($p<0.01$) with selenium element.

Table 5. The correlations between heavy metals in cheese samples.

Correlations						
Elements	**Pb**	**Cd**	**Fe**	**Se**	**Mn**	**Hg**
Pb	1	0.144	0.127	0.265	0.060	-0.004
Cd		1	-0.101	0.391**	-0.117	0.017
Fe			1	-0.029	0.236	0.001
Se				1	-0.112	0.058
Mn					1	0.087
Hg						1

* **Correlation is significant at the 0.05 level**
** **Correlation is significant at the 0.01 level**

CONCLUSIONS

The present study concluded that all of the examined cheese samples were contaminated with investigated trace elements with different levels except Cd, Se and Hg were not detected in some types of cheese. The trace elements level not attributed only to raw milk but also to other ingredients used in its manufacturing process including addition of salt,

rennet, milk powder and emulsifier as well as, water supply, equipment. Furthermore, Egyptian Standards must be updated to include all acceptable levels of heavy metal residues in cheese and other dairy products. Additionally, quality control should be increased on cheese to produce safe cheese to consumers.

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تقييم متبقيات العناصر المعدنية السامة في بعض انواع من الجبن باستخدام جهاز انبعاث الطيف بالبلازما

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أجري هذا البحث لتقييم تواجد بقايا المعادن الثقيلة في بعض أنواع من الجبن، حيث تم تجميع عدد 52 عينة عشوائية من الجبن الأبيض الطري (الفيومي ، جبن بالقشدة، جبن ملوي، جبن الضاني، جبن براميلي، جبن براميلي بالفلفل، جبن المصانع بالقشدة) ، جبن الراس والجبن المطبوخة من مناطق مختلفة في محافظة اسيوط و بعد ذلك تم تجهيزها و هضمها ثم قياسها باستخدام جهاز انبعاث الطيف بالبلازما (نظام الحث الكهربي الحراري المزدوج للبلازما).

اشارت الدراسة الي أن انواع الجبن المختلفة موضع الدراسة تحتوي علي الرصاص، الكادميوم، الحديد، السيلنيوم، المنجنيز والزئبق بتركيزات مختلفة ما عدا عناصر الكادميوم ، السيلينوم والزئبق لم يتم اكشتافها في بعض انواع من الجبن.

واظهرت الدراسة ان محتوي هذه الجبن يتراوح من 0.0145-0.0429 ، 0.000-0.0008 ، 0.4632-4.9452 ، 0.000- 0.038 ، 0.0267-0.1377، 0.000- 0.0144 جزء في المليون لكل من الرصاص والكادميوم والحديد والسيلنيوم والمنجنيز والزئبق علي التوالي.

ايضا اشارت النتائج الي ان أعلي التركيزات للعناصر الثقيلة في العينات موضع الدراسه وجدت في جبن ملوي والراس لعنصر الرصاص، جبن الفيومي والراس لعنصر الكادميوم، الفيومي، الجبن المطبوخ وجبن الراس لعنصر الحديد، جبن البراميلي والجبن بالقشدة والجبن الراس لعنصر السيلنيوم ، جبن الراس، جبن البراميلي، والجبن الفيومي لعنصر المنجنيز بينما في الجبن الفيومي والجبن بالقشدة لعنصر الزئبق. ايضا تم مناقشة الأرتباط بين تواجد هذه العناصر المعدنية السامة في الجبن تحت الدراسه.

قام بتحكيم البحث

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