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ASSESSMENT OF SOME HEAVY METALS IN DOMIATI CHEESE IN EL-BOHEIRA GOVERNORATE

(With 4 Tables)

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تقييم بعض المعادن الثقيلة في الجبن الدمياطي في محافظة البحيرة

إبراهيم على القويعى ، فائزة عبد العزيز التداوي ، هناء فتحي فرج

أجرى هذا البحث لتقدير بقايا المعادن الثقيلة في الجبن الدمياطي الثلجة والخزين المعروضة للاستهلاك بمحافظة البحيرة وقد تم تجهيزها للقياس بواسطة جهاز الامتصاص الذرى الطيفي. وقد أظهرت نتائج فحص العينات عن تواجد الرصاص في الجبن الثلجة والخزين بنسب ٦٠% و ٢٥% وبمتوسطي 2.10 ± 0.40 و 2.28 ± 0.59 ملجم/كجم على التوالي. بينما لم يتم اكتشاف الكاديوم في جميع عينات الجبن الثلجة وتواجد في النوع الخزين بنسبة ٣٠% وبمتوسط 0.16 ± 0.02 ملجم/كجم. كما تواجد النحاس بنسب ٧٠% و ١٠٠% وبمتوسطي 0.16 ± 0.02 و 1.38 ± 0.19 ملجم/كجم على التوالي. وتم اكتشاف عنصر الزنك في جميع العينات المفحوصة من هذه الأجناب (١٠٠% و ١٠٠%) وبمتوسطي 12.77 ± 0.82 و 9.87 ± 0.47 ملجم/كجم على التوالي. كما أوضحت النتائج عن تواجد الرصاص في ٤٥% و ٨٠% من العينات بنسب مسموح بها حسب المواصفات القياسية المصرية لسنة ١٩٩٣ بينما كانت نظيراتها بالنسبة لتواجد الكاديوم كالاتي: ١٠٠% و ٧٠% على التوالي. بينما تواجد النحاس بنسب مسموح بها في جميع العينات المفحوصة من الجبن الثلجة وبنسب غير مسموح بها في جميع عينات الجبن الخزين وتواجد الزنك بنسب مسموح بها في ٩٠% و ١٠٠% من العينات ذاتها لنوعى الجبن الدمياطي. وقد تم مناقشة المخاطر الصحية من تناول الأغذية التي تحتوى على معدلات عالية من المعادن الثقيلة تتجاوز الحدود المسموح بها محليا ودوليا والإجراءات التي يجب أن تتخذ للاقلال من التلوث بها.

SUMMARY

Forty random samples of fresh and pickled Domiati cheese (20 of each) were collected from supermarkets in El-Boheira governorate and then analyzed for detection of heavy metals residues using Perkin-Elmer atomic absorption spectrophotometer. The obtained results revealed that, the mean values of lead in fresh and pickled samples of cheese were 2.10 ± 0.40 and 2.28 ± 0.59 ppm wet weight with an incidence of

60% and 25% respectively. While cadmium residues were not detected in fresh cheese samples, but were detected in 30% of pickled ones by a mean of 0.60 ± 0.16 ppm wet weight. Whereas such values for copper were 0.16 ± 0.02 and 1.38 ± 0.19 ppm with incidence rates of 70% and 100%, respectively, and for zinc which was detected in all examined samples were 12.77 ± 0.82 and 9.87 ± 0.47 ppm, respectively. The percentages of the examined fresh cheese samples which agreed with the permissible limits according to Egyptian Organization for Standardization and Quality Control "E.O.S.Q.C." (1993) were 45, 100, 100 and 90%, where-as for pickled ones were 80, 70, 0.0 and 100%. The public health importance, the hazardous toxic effects of these heavy metals and the suggestive recommendations to minimize pollution with heavy metals were discussed.

Key words: Cheese, heavy metals, lead, zinc, cadmium, copper.

INTRODUCTION

Cheese is a very nutritive foodstuff, not only for its protein and fat content, but also for its mineral content. One hundred grams of soft cheese provide 30-40% of the daily calcium (Ca) requirements and 12-20% of the daily phosphorus (P) requirements, while 100 g of hard cheese provide the total daily calcium requirements and 40-50 % of the daily phosphorus requirements (Renner, 1987).

Dommati cheese is the most popular type of local white soft cheese (either fresh or pickled) consumed in Egypt due to its nutritional value and good taste. Fully ripened Dommati cheese has a strong sharp flavour in addition to a smooth body and texture (Yousef *et al.*, 2001 and Kepary *et al.*, 2007). It is known to be made from different variable whole or partially skimmed raw, pasteurized or sub-pasteurized milks (buffalo, cow or combined milk mixes), which reflects on the composition of the resultant cheese by rennet coagulation of milk previously salted with 5-15 % sodium chloride. Full pasteurization of the milk should be employed instead of a long traditional method of making cheese from raw milk.

The toxic metals are naturally present in the environment, industrial processes have resulted in an increased concentration of heavy metals in air, water and soil, subsequently, these metals are taken-in by plants and animals and take their way into the food chain.

Heavy metals are from the most dangerous pollutants that have a tendency to accumulate in tissues and organs of animals as well as humans (Antoniou *et al.*, 1989) by the continuous exposure to low

concentration of metals that result in bioaccumulation. So, contamination of milk and milk products by heavy metals is one of the prime problems confronting public health. Ingestion of contaminated feeding materials have been considered as the main source of heavy metals residues in milk (Carl, 1991), while post-milking contamination from processing equipments, reagents, accidental contamination during storage and marketing and leaching from containers (Ukhun *et al.*, 1990).

Metals that cannot be metabolized (as cadmium and lead) persist in the body and combined with one or more reactive groups essential for normal physiological function, so, their inhibition is clearly manifested by cellular disturbances and clinical diseases (Friberg and Elinder, 1988 and Skerfving, 1988). Copper and zinc are essential for all plants and animals, but they may be detrimental if ingested in high concentrations (Murthy and Rhea, 1971). Therefore, the aim of this work was to determine the level of lead, cadmium, copper and zinc in Domiati cheese varieties to ensure their safety for human consumption.

MATERIALS and METHODS

A. Collection of samples:

Forty random Domiati cheese samples (20 each of fresh and pickled) each weighing 100 g were collected at the consumer level from different supermarkets in El-Boheira governorate and taken to the laboratory without delay. All samples were stored frozen in plastic bags prior to analysis for detection of some heavy metals which may gain access to such products.

B. Preparation of collected samples:

Each cheese sample was thoroughly mashed in clean and acid-washed mortar and a measured weight (2g) was transferred into clean and acid-washed screw-capped digestion tubes. All digestion tubes were identified for examination.

C. Digestion procedure:

Each prepared cheese sample was digested according to Tsoumbaris and Papadopoulou (1994). 10 ml solution of concentrated nitric and perchloric acids (1:1) were added to each sample. The tubes were tightly closed and the contents were vigorously shaken and allowed to stand overnight to be cold digested followed by mild increase in temperature till heating at 100 °C in a water bath for 3-4 hours to ensure complete digestion of samples. 4-5 drops of hydrogen peroxide 30% were added to each sample and heating continued till the brown nitrous

gases were expelled and specimens become clear. After cooling, each digest was diluted to 25 ml with deionized water and filtered through Whatman filter paper No. 42. The clear filtrate of each sample was kept in refrigerator to avoid evaporation. All samples (solutions and blank) were analysed by using Flame Atomic Absorption Spectrophotometry "AAS" (Perkin-Elmer Atomic Absorption Spectrophotometry model 2380, USA) for determination of lead, cadmium, copper and zinc.

D. Quantitative determination of heavy metals in the examined cheese samples:

The concentration of Pb, Cd, Cu and Zn in the examined samples were calculated according to the following equation:

$$C = R \times D/W$$

Where: C = Concentration of heavy metal (mg/kg) wet weight (ppm).

R = Reading of element concentration on digital scale of Atomic Absorption Spectrophotometer.

D = Final volume of prepared sample in mls.

W = Weight of the wet sample.

The concentration of absorbance values of heavy metals in blank samples were also calculated and subtracted from each analysed cheese sample.

RESULTS

Table 1: Residue concentration of some heavy metals (ppm) wet weight in examined Domiati cheese samples (n=20 of each)

Metal	Examined Domiati cheese samples			Minimum	Maximum	Mean ± SEM
	type	No. of positive	%			
Lead (Pb)	Fresh	12	60	0.25	4.00	2.10 ± 0.40 ^{ns}
	Pickled	5	25	0.25	3.63	2.28 ± 0.59
Cadmium (Cd)	Fresh	0	0	0.00	0.00	0.00
	Pickled	6	30	0.09	0.86	0.60 ± 0.16
Copper (Cu)	Fresh	14	70	0.04	0.30	0.16 ± 0.02 ⁺⁺⁺
	Pickled	20	100	0.31	3.33	1.38 ± 0.19
Zinc (Zn)	Fresh	20	100	6.48	21.90	12.77 ± 0.82 ^{**}
	Pickled	20	100	6.71	13.85	9.87 ± 0.47

- SEM = standard error of the mean
- * p < 0.05 ; ** P < 0.01 ; +++ P < 0.001; ns, not significant P > 0.05
- Negative samples were under detectable limit of Atomic Absorption Spectrophotometer (AAS).
- ppm = mg\Kg.

Table 2: Frequency distribution of heavy metals residues in Domiati cheese

Metal	Permissible limits mg/kg*	Fresh Domiati Cheese (20 samples)				Pickled Domiati Cheese (20 samples)			
		Within P.L.		Above P.L.		Within P.L.		Above P.L.	
		No. of samples	%	No. of samples	%	No. of samples	%	No. of samples	%
Lead (Pb)	0.3	9	45	11	55	16	80	4	20
Cadmium (Cd)	0.05	20	100	0	0	14	70	6	30
Copper (Cu)	0.3	20	100	0	0	0	0	20	100
Zinc (Zn)	20.0	18	90	2	10	20	100	0	0

- P.L. = Permissible limits. - * According to E.O.S.Q.C. (1993).

Table 3: Comparison of Acceptable Daily Intake (ADI) value of heavy metals with the calculated daily intake from fresh Domiati cheese.

Metal	ADI ^(a) ug/70kg person	Mean concentration of metal (ug/kg) in this study	Calculated daily intake of metals from consumption of 100g soft cheese per day ^(b)	
			Ug/day/person	%
Lead (Pb)	500	2100	210	42.0
Cadmium (Cd)	70	0	0	0.0
Copper (Cu)	35000	160	16	0.046
Zinc (Zn)	70000	12770	1277	1.824

- (a) FAO/WHO, Joint Expert Committee on Food Additives, World Health Organization "WHO" Technical Report Series, 1972, 1974, 1980, 1982, 1987 and 1989.
- (b) Daily consumption of soft cheese for adult person according to *Nutrition Institute (1996)*.
- ADI = Acceptable Daily Intake.
- Acceptable weekly intake of lead (AWI) (3 mg/person) according to WHO (1972).
- Acceptable daily intake of lead (0.3 mg/person) according to *Casarett and Doull (1975)*.

Table 4: Comparison of Acceptable Daily Intake (ADI) of heavy metals with the calculated daily intake from pickled Domiati cheese.

Metal	ADI ^(a) ug/70kg person	Mean concentration of metal (ug/kg) in this study	Calculated daily intake of metals from consumption of 80g pickled cheese per day ^(b)	
			Ug/day/person	%
Lead (Pb)	500	2280	182.4	36.5
Cadmium (Cd)	70	600	48.0	68.6
Copper (Cu)	35000	1380	110.4	0.32
Zinc (Zn)	70000	9870	789.6	1.13

- (a) FAO/WHO, Joint Expert Committee on Food Additives, World Health Organization "WHO" Technical Report Series, 1972, 1974, 1980, 1982, 1987 and 1989.
- (b) daily consumption of pickled cheese for adult person according to *Nutrition Institute (1996)*.
- ADI = Acceptable Daily Intake.

DISCUSSION

Public health hazard of pollution with heavy metals have a special interest all over the world in recent years.

Lead (Pb): It is considered as the most serious metal polluting the food as well as the cadmium where they accumulate in the body due to their low rate of elimination. Lead as an environmental contaminant is often combined with cadmium which has effects similar to that produced by lead, so their effects are additive (Neathery and Miller, 1975). The damage of the central nervous system is a marked and a common sign particularly in children due to their low lead tolerance (Johansen *et al.*, 2004). It inhibits the biosynthesis of haem groups of blood and thereby affects the membrane permeability of liver, kidney and brain cells, reducing their functions or complete damaging these organs (Ahmed and El-Boushy, 1998).

Table1 revealed that the concentrations of lead in fresh and pickled Domiati cheese ranged from 0.25 to 4.00 and 0.25 to 3.63 with mean values as 2.10 ± 0.40 and 2.28 ± 0.59 ppm wet weight with incidences of 60% and 25%, respectively. While the percentages of positive samples which were agree with the permissible limits according

to Egyptian Organization for Standardization and Quality Control "E.O.S.Q.C." (1993) were 45% and 80% respectively, Table (2).

Lower results of lead concentration (0.75, 1.10, 1.26, 1.20 and 1.58 mg/kg) were reported by El-Baradie (1994) in fresh Domiati cheese made from cow milk with different local salt levels (0, 4, 8, 12 and 16%). In another study, it ranged from 0.0047 to 0.2360 with an average of 0.0908 ± 0.0248 ppm by dry weight basis (Fouzy, 2000). Also, other lower results were recorded by Cimino *et al.* (1991), Bagni *et al.* (1992), Bulinski *et al.* (1993), Cabrera *et al.* (1995) and Coni *et al.* (1999).

Higher levels (4.568 ± 0.446 and 5.136 ± 0.761) were obtained by Nasef (2002) from Domiati and hard cheese and also from raw milk samples that collected from heavy traffic areas (Bhatia and Choudhri, 1996). Lead residue have been increased in cheese manufactured from polluted milk (Marletta and Favretto, 1983). So, Zidan *et al.* (1994) stated that 96.8% of Pb present in milk was retained in Domiati cheese made from the same milk. Cheese samples manufactured from milk collected from an area crossed by roads of heavy traffics or from an industrial area may be polluted by lead (Bagni *et al.*, 1992). Also, the presence of impurities in the added foodstuff may increase the level of lead in cheese (Ereifej and Gharaibah, 1993 and El-Baradie, 1994). In addition, lead pollution from soldered cans is an important source for milk products (Carl, 1991).

The WHO (1977) recommended that the maximum level of lead should not exceed 10 mg/kg for cheese in tinfoil, 0.2 mg/kg for milk or 2.0 mg/kg for milk and milk products in tinfoil containers. It also established a provisional tolerable weekly intake for adults of 3 mg of lead per person.

The results achieved in Table 2 concluded that the most examined samples of fresh Domiati cheese contained high levels of lead over the permissible limit (55%). This may be due to high lead level in the original raw milk, an additional pollution during manufacturing from salt impurities and/or from cheese cans. On the other hand, the most samples of pickled cheese type (80%) contained the permissible limit of lead according to "E.O.S.Q.C." (1993). These differences between the two cheese types may be justified by the difference in the intensity of whey drainage.

The mean values of lead residues in examined cheese samples (Table 1) changed from ppm (mg/kg) to $\mu\text{g}/100\text{g}$ and $\mu\text{g}/80\text{g}$ for fresh and pickled type cheese (Tables 3 and 4) as the human daily intakes from each cheese recommended by Nutrition Institute, (1996).

Results recorded in Tables 3 and 4 revealed that the calculated human daily intake of lead was 210 µg and 182.4 µg for adult person from consumption of fresh (100 g/day) and pickled (80 g/day) types of Domiati cheese, respectively. These calculations were representing 42.0% and 36.5% of acceptable daily intake recommended by FAO/WHO (1972, 1974, 1980, 1982, 1987 and 1989). Also, these calculations were within the permissible limits "3 mg/person weekly" and "0.3 mg/person daily" of WHO (1972) and Casarett and Doull (1975), respectively. In this respect, "E.O.S.Q.C." (1993) stated that the maximum weekly intake of lead in food by human is 0.05 mg/kg body weight. By calculation (Tables 3 and 4) the maximum weekly intakes of lead in Domiati cheese types were within the limits of "E.O.S.Q.C." (1993).

Cadmium (Cd): It is an element that accumulate inside the human body especially in the kidneys. The ingestion of food containing cadmium may result in acute gastroenteritis manifested by sudden onset of vomition, diarrhoea and abdominal pain (Buckler *et al.*, 1986).

The results recorded in Table 1 revealed that the cadmium residues were not detected in all fresh Domiati cheese samples, while detected only in 30 % of pickled Domiati cheese samples, ranging from 0.09 to 0.86 with a mean value of 0.60 ± 0.16 ppm wet weight.

Higher results were obtained by El-Baradie (1994) in fresh Domiati cheese made from cow milk with different salt levels (< 0.001 mg/kg by wet weight basis). While Fouzy (2000) reported that the cadmium level in market Domiati cheese samples ranged from 0.0167 to 1.2801 with a mean of 0.2157 ± 0.1531 ppm by dry weight basis.

Mata *et al.* (1995) explained the distribution of lead and cadmium added to cow's milk and whey. They found 97% of Pb and 89% of Cd recovered in the casein fraction obtained by enzymatic coagulation of the milk, while they found only 6 % of lead and 41 % of cadmium were found in the same fraction separated by acid coagulation indicating that the distribution was dependent on fractionation method.

Nasef (2002) detected cadmium in examined Domiati cheese in higher percentage (100%) with a mean value of 0.545 ± 0.070 ppm.

With respect to the pickled type of Domiati cheese, lower cadmium contamination levels were reported by Marletta and Favoretta (1983), Gartrell *et al.* (1986), Milhaud *et al.* (1998) and Coni *et al.* (1999).

Tables 3 and 4 recorded that the calculated daily intake of cadmium from fresh (100 g) and pickled (80 g) Domiati cheese types

were 0.0 and 48.0 µg for adult person, and these amounts contributed about 0.0 % and 68.6 % of permissible daily intake recommended by FAO/WHO (1972, 1974, 1980, 1982, 1987 and 1989).

From the results recorded in Table 1, it is obvious that the cadmium could neither be detected in all of the examined fresh Domiati cheese samples (100%) nor in 14 (70%) out of 20 samples of pickled ones. The other pickled cheese samples, 6 (30%), exhibited levels over the acceptable limit (0.05 ppm) recommended by "E.O.S.Q.C." (1993).

Milk and its products usually contain very low level of cadmium (El-Baradie, 1994) except when animals consumed polluted feeds (Cabrera *et al.*, 1995). Therefore, cheese manufactured from polluted milk may be produced with high concentration of cadmium or due to post-secretory contamination of milk during processing, as well as addition of polluted salt and/or from milk cantainers (Ereifej and Gharaibeh, 1993 and Cabrera *et al.*, 1995).

The high levels of cadmium in some examined samples may be due to the grazing of animals in polluted pasture with cadmium from industrial sources or due to fertilization with high concentrations of phosphate fertilizers and/or manure (Scoullou *et al.*, 2001).

Following absorption of cadmium, it is transported and bound to certain proteins of the plasma and red blood cells to other sites throughout the body. The metabolism of cadmium was antagonized with copper and iron leading to anemia (Underwood, 1977). Meanwhile, cadmium acts synergistically with other metals to increase toxicity. In this respect, a copper level at 1ppm or more substantially increase the toxicity of cadmium (Faust and Aly, 1981).

Copper (Cu): It is essential as a trace element at low concentrations but it is toxic at high level. So that, daily intake of an excessive dose of copper may lead to Wilson's disease which manifested chemically by destruction of nerve cells, liver cirrhosis, ascitis, oedema and hepatic failure (Gossel and Bricker, 1990).

The results reported in Table 1 showed that the copper concentration in fresh and pickled Domiati cheese samples ranged from 0.04 to 0.30 and from 0.31 to 3.33 ppm, respectively. While the mean values were 0.16 ± 0.02 and 1.38 ± 0.19 ppm, with incidence rates 70% and 100%, respectively.

Higher results were reported by El-Baradie (1994) as 0.10, 0.49, 0.53, 0.27 and 0.26 mg/kg in fresh Domiati cheese samples made from cow milk with different local salt levels.

Nasef (2002) detected copper in Domiati cheese in all samples (100%), ranging from 0.4 to 0.8 ppm with a mean value of 0.576 ± 0.044 ppm.

With respect to fresh Domiati cheese, higher values of other cheese varieties were recorded by Cimino *et al.* (1991), Rojas *et al.* (1994), Coni *et al.* (1999) but lower ones were obtained by Bulinski *et al.* (1993) from cottage cheese. On the other hand, higher results were obtained by Bottazzi *et al.* (2000) in comparison to that of pickled cheese.

Table 2 declared that all examined fresh Domiati cheese having copper content within the permissible limit (0.3 ppm). Meanwhile, all pickled cheese samples having over levels according to "E.O.S.Q.C." (1993). These results may be attributed to the difference in concentrations of total solids in these cheese varieties.

According to hygienic view, the high level of copper in milk products has an adverse effect on lactic acid bacteria and production of cheese with bad quality (Bottazzi *et al.*, 2000).

It is evident from Tables 3 and 4 that the calculated daily intake of copper from consumption of fresh and pickled Domiati cheese types were 16 and 110.4 μg for adult person, respectively, and these intakes through these types of cheese representing 0.046% and 0.32% of total copper intake for adult as recommended by FAO/WHO (1972, 1974, 1980, 1982, 1987 and 1989).

Zinc (Zn): Results recorded in Table 1 showed that zinc concentrations in fresh and pickled Domiati cheese samples ranged from 6.48 to 21.90 and from 6.71 to 13.85 ppm, with mean values of 12.77 ± 0.82 and 9.87 ± 0.47 ppm and with incidence rates of 100% and 90%, respectively.

Lower values were reported by El-Baradie (1994) in fresh Domiati cheese while Fayed *et al.* (1993) stated that zinc was the major contaminant in milk and milk products samples, followed by iron, but copper, lead, manganese and cadmium were found in lower concentrations.

Nasef (2002) stated that zinc concentrations in Domiati cheese ranged from 7.28 to 12.40 ppm with a mean value of 9.702 ± 0.451 ppm and with incidence rate 100%.

Nearly similar results were obtained by Gambelli *et al.* (1999) in soft cheese (10.6 mg/kg), while lower results were detected by Gartrell *et al.* (1986) in dairy products (4.45 ppm).

The differences between fresh and pickled Domiati cheese types in Zn concentration levels were due to the fact that these varieties are

manufactured using a predominating acid coagulation. The acid coagulation makes the minerals which are mainly found in the casein micelles (Ca, P and Zn) migrate to the soluble fraction (Argumosa *et al.*, 1992; Prieto *et al.*, 1994 and Lafuente *et al.*, 1995). At the low pH values which are reached during the coagulation in these cheeses, Zn migrates from the casein micelles to the soluble fraction and it is lost in greater quantities during whey drainage. These 2 varieties (as shown in Table 1) contained low Zn contents (12.77 ± 0.82 and 9.87 ± 0.47 ppm) in comparison with the Egyptian permissible limit (20.0 ppm) (E.O.S.Q.C., 1993), Table 2. According to Parkash and Jennes (1976), 85% of the Zn in the milk is associated to casein micelles and is only freed at acid pH values. The degree of ripening process plays a role in decreasing the trace element contents of cheese (Rojas *et al.*, 1992).

With respect to the human daily intakes of heavy metals in these examined cheeses, Tables 3 and 4 demonstrated that the calculated daily intakes of Zn were 1277.0 and 789.6 μg and these amounts were constituted about 1.82 and 1.13 % of acceptable daily intake recommended by FAO/WHO (1972, 1974, 1980, 1982, 1987 and 1989), respectively.

Finally, it could be concluded that the high levels of some heavy metals in some Domiati cheese samples were due to that the dairy animals were subjected to environmental pollution and due to the accumulative effect of such metals and/or directly during cheese manufacturing and canning. Therefore, the preventive measures for minimizing the pollution of of milk and milk products with such metals are of significant concern, including:

- 1- Prevention of environmental pollution and hygienic disposal of industrial effluents.
- 2- Animal feed in polluted areas as well as drinking water should be controlled. The permitted limits of metals (ppm) in cattle feeds were 5, 1, 35 and 250 for Pb, Cd, Cu and Zn, respectively (Nederlandse Statscourant, 1985 and 1988).
- 3- Regular examination of milk and dairy products and their load for heavy metals should be evaluated according to the international guide lines.
- 4- Minimizing the use of phosphates and sludge for land fertilization as possible.

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