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TRACE METALS CONCENTRATIONS IN CHEESE COLLECTED FROM KAFR-EL-SHEIKH GOVERNORATE, EGYPT

(With 2 Tables)

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تركيز العناصر الدقيقة في الجبن المجمع من محافظة كفر الشيخ - مصر

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أجريت هذه الدراسة على مائة عينة من الجبن [10عينات جبن شيدر، 10 عينات جبن فلمنك ، 10عينات جبن جودة، 10عينات جبن رومى ، 10 عينات جبن ريكفورت، 15 عينة جبن مطبوخ ،10 عينات جبن فيتا، 10عينات جبن دمياطى و 15 عينة جبن قريش] تم تجميعها من محال البقالة بمحافظة كفر الشيخ، تم حفظ العينات فى درجة التجميد لحين اختبارها. وقد تم فحص العينات قبل انتهاء تاريخ الصلاحية لتحديد كمية المعادن الضرورية مثل الحديد والنحاس والزنك والمعادن الثقيلة مثل الرصاص والكادميوم باستخدام جهاز المطياف الذرى (الأتوميك سبكتروفوتوميتر). وقد تم استخدام وحدة الجرافيت لتعيين كمية الرصاص والكادميوم ووحدة اللهب لتقدير كمية الحديد، النحاس والزنك. وقد أظهرت النتائج أن تركيز عنصر الرصاص السام فى عينات الجبن المختبرة يتراوح من 19, 0 ± 0,03 –44, 0 ± 0,04 مجم/كجم وكان أعلى تركيز فى الجبن الرومى والجبن الجودة بينما أقل تركيز كان فى جبن فيتا، بينما كان تركيز عنصر الكادميوم السام يتراوح من 16, 0 ± 0,02 –0,54 ± 02, 0 مجم/كجم فى الجبن الشيدر والجبن الفلمنك على التوالى0 أما بالنسبة للعناصر الضرورية لجسم الإنسان فوجد أن تركيز عنصر الحديد يتراوح من 2,9 ± 85, 0 مجم/كجم فى الجبن الفلمنك الى 24,4 ± 12,73مجم/كجم فى الجبن الدمياطى وعنصر النحاس يتراوح من 1,45 ± 14, 0 مجم/كجم فى الجبن المطبوخ الى 4,18 ± 0,71 مجم/كجم فى الجبن الشيدر، بينما يتراوح تركيز عنصر الزنك من 21,72 ± 1,19 مجم/كجم فى جبن فيتا الى 43,18 ± 0,53 مجم/كجم فى الجبن الفلمنك0وقد أظهرت الدراسة أن تركيز العناصر الثقيلة مثل الرصاص والكادميوم فى عينات الجبن المختبرة أعلى من الحدود العليا المسموح بها فى الجبن مما يؤثر على صحة المستهلك لذلك يجب مراعاة الشروط الصحية أثناء تصنيع ، تسوية، تخزين وتداول هذه الأنواع من الجبن0 أما بالنسبة للعناصر الضرورية فوجد أنها فى حدود المعدلات اليومية المطلوبة0

SUMMARY

One hundred samples of cheese [10 Cheddar cheese; 10 Edam cheese; 10 Gouda cheese; 10 Ras cheese; 10 Roquefort cheese; 15 Processed cheese; 10 Feta cheese; 10 Damietta cheese and 15 Kareish cheese] were randomly collected from different supermarkets in Kafr El- Sheikh Governorate, Egypt. All samples were analyzed before their expiry date. The collected samples were homogenized and packed in polyethylene bags and stored below -20 ^{o}C prior to analysis. Samples were digested within a microwave digestion system. A Perkin–Elmer Analyst 3110 model atomic absorption spectrometer (AAS) was used in this work. Pb and Cd in samples were determined by graphite furnace, using argon as the inert gas. Other measurements were carried out in an air/acetylene flame. The results revealed that the Lead concentrations of investigated cheese samples were in the range of 0.19±0.03– 0.44±0.04 mg/kg (the highes wast in Ras & Gouda cheese and the lowest was in Feta cheese). Cd levels in examined cheese samples varied from 0.16±0.02 to 0.54±0.02 mg/kg in Cheddar and Edam cheese. The lowest and highest iron concentrations were found as 2.90±0.85 mg/kg in Edam cheese samples and 24.4±12.73 mg/kg in Damietta cheese samples. Highest copper level of examined cheese samples was found as 4.18±0.71 mg/kg in Cheddar cheese, while lowest in processed cheese as 1.45±0.14 mg/kg. Mean zinc concentrations ranged from 21.72±1.19 to 43.18±0.53 mg/kg. The minimum and maximum zinc levels were found in Feta cheese and Edam cheese respectively. In conclusion, in all types of examined cheese, extremely toxic heavy metals (Pb and Cd) were detected above the toxicity levels but, the levels of Fe, Cu and Zn were within the recommended daily intake.

***Key words**: Trace metals, cheese, atomic absorption spectrometer.*

INTRODUCTION

Nowadays, studies on dietary adequacy assessment are focused more on the qualitative aspects of the diet composition than on the quantitative adequacy of daily dietary intake. Milk and milk products are important components of the human diet. Moreover cheese, being one of the basic dairy products, rich in protein, fat, calcium, riboflavin and some other vitamins (Yüzbasi *et al*., 2003). More than 1000 varieties of cheese are produced around the world. In Egypt, 40–50 cheese varieties are known, but only two of them are national: Damietta cheese (full cream soft cheese) and Kareish cheese (skimmed milk soft cheese).

From nutritional point of view, metal elements in the composition of cheese can be grouped in essential metals (Fe, Cu, Zn, Mn, Co, etc.) and non essential or toxic metals (represented mainly by Pb, Cd, Hg, etc.) (Orak *et al.*, 2005). The toxic metal content of milk and dairy products is due to several factors in particular environmental conditions, the manufacturing process and the possible contamination during several steps of the manufacturing processes. The presence of the latter, even in low concentrations, leads to metabolic disorders with extremely serious consequences. Likewise it is very important to mention that for both trace metal categories the increase of their concentration above admitted limits considered optimal has toxic effects on consumers of cheese. It is well established that Pb and Cd are toxic and the children are more sensitive to these metals than adults. The metals, namely Fe, Cu and Zn, are essential micro nutrients and have a variety of biochemical functions in all living organisms. While Fe, Cu and Zn are essential, they can be toxic when taken in excess; both toxicity and necessity vary from element- to- element (Tripathi *et al*., 1999).

In this study, the levels of trace metals in some cheese samples collected from Kafr –El-Sheikh Governorate, Egypt, were determined by flame and graphite furnace AAS, after a microwave digestion method.

MATERIALS and METHODS

1. Collection of samples

One hundred cheese samples [10 Cheddar cheese; 10 Edam cheese; 10 Gouda cheese; 10 Ras cheese; 10 Roquefort cheese; 15 Processed cheese; 10 Feta cheese; 10 Damietta cheese and 15 Kareish cheese] were randomly collected from different supermarkets in Kafr El-Sheikh Governorate, Egypt. All samples were analyzed before their expiry date. The collected samples were homogenized and packed in polyethylene bags and stored below -20 ^{o}C prior to analysis.

2. Samples and standards preparation (AOAC, 2005)**.**

Commercial standard solutions for AAS, BDH chemicals Ltd., Poole, UK, were used for all metal standard solutions. All reagents were of analytical reagent grade. Double-deionized water (18.2 MΩ· cm.) was used for all dilutions. HNO_3 and H_2O_2 were of supra-pure quality (Merck, Darmstadt, Germany). The glasses used were thoroughly cleaned with water and detergent, rinsed with tap water followed by deionized water then with 10% v/v nitric acid and finally rinsed 4-5 times with deionized water. Teflon digestion vessels were rinsed with acetone, washed with deionized water, covered with 0.1 *M* HNO_3 for 30 min, rinsed with

deionized water. Samples (2.0 g) were digested with 5 ml of HNO_3 (65%) and 2 ml of H_2O_2 (30%) in a microwave digestion system (Speed-wave four, Berghof, GmbH, Germany) for 31 min and diluted to 25 ml with deionized water. A blank digest was carried out in the same way (digestion conditions for microwave system were: 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, vent: 8 min, respectively).

3. Analytical parameters

A Perkin–Elmer Analyst 3110 model atomic absorption spectrometer with deuterium background corrector was used in this work. Pb and Cd in samples were determined by graphite furnace, using argon as the inert gas. Other measurements were carried out in an air/acetylene flame.

RESULTS

Table 1: Lead and Cadmium concentrations in the examined cheese samples (mg/kg).

Types of cheese	No. of examined samples	*Pb concentration (ppm)			*Cd concentration (ppm)		
		Min.	Max.	Mean ± SEM	Min.	Max.	Mean ± SEM
Cheddar cheese	10	0.18	0.55	0.39±0.04	0.11	0.25	0.16±0.02
Edam cheese	10	0.09	0.46	0.26±0.05	0.43	0.63	0.54±0.02
Gouda cheese	10	0.28	0.55	0.44±0.03	0.15	0.42	0.32±0.03
Ras cheese	10	0.28	0.64	0.44±0.04	0.24	0.38	0.28±0.01
Roquefort cheese	10	0.09	0.46	0.22±0.04	0.14	0.65	0.38±0.06
Processed cheese	15	0.09	0.46	0.36±0.05	0.09	0.55	0.31±0.04
Feta cheese	10	0.09	0.30	0.19±0.03	0.29	0.56	0.36±0.04
Damietta cheese	10	0.18	0.37	0.30±0.02	0.30	0.79	0.48±0.06
Kareish cheese	15	0.09	1.83	0.36±0.11	0.26	0.68	0.48±0.03

* 100% of examined samples were positive for Pb and Cd

Table 2: Iron, Copper and Zinc concentrations in the examined cheese samples (mg/kg).

Types of cheese	No. of examined samples	Fe concentration (ppm)					
		+ve samples		Min.	Max.	Mean ± SEM	+ve
		No.	%				No.
Cheddar cheese	10	6	60	2.1	6.0	4.37±0.68	10
Edam cheese	10	10	100	0.4	7.3	2.90±0.85	10
Gouda cheese	10	8	80	1.9	6.9	5.23±0.70	10
Ras cheese	10	10	100	10	14.7	11.56±0.81	10
Roquefort cheese	10	10	100	1.5	101.3	13.17±9.31	10
Processed cheese	15	13	86.7	5.5	50.8	15.03±4.30	15
Feta cheese	10	4	40	2.0	5.5	3.78±0.86	10
Damietta cheese	10	8	80	0.5	118	24.4±12.73	10
Kareish cheese	15	10	66.7	0.25	27.8	7.47±2.79	13

* 100% of examined samples were positive for Zn

DISCUSSION

In view of the worldwide interest concerning contamination of the environment with heavy metals, cheese samples have been examined to determine the trace elements concentrations (heavy and essential).

The results of trace metal contents of different types of cheese samples collected from Kafr El-Sheikh Governorate were given in Tables 1&2. Lead concentrations of investigated cheese samples were in the range of 0.19±0.03– 0.44±0.04 mg/kg (the highest was in Ras & Gouda cheese and the lowest was in Feta cheese) (Table 1). Mean lead level of Herby cheese was reported by Mendil (2006) as 0.32 µg/g. While, Orak *et al*. (2005) reported lead levels of Turkish white cheese as 0.415 µg/g. Lead concentration of cheese samples from Southern Italy was reported by Caggiano *et al*. (2005) as in the range of 0.35–0.58 µg/g. Lead concentrations, exceeding the maximum limit of 0.02 mg/kg wet weight (EC Regulation, 2001) were found in all of examined cheese samples. FAO/WHO (1999) has set a limit for heavy metal intakes based on body weight. For an average adult (60 kg body weight), the provisional tolerable daily intake (PTDI) for lead is 0.214 mg. According to USDA (2005) and HC (2007), the amount of milk group that are recommended each day 2-4 (for children and teens) and 2-3 cups (for adults), the cup equal 50-60 gm cheese. The examined cheese samples constitute about 19-44% of the daily dietary intake of Pb.

The sources of contamination with lead are lead piping and lead-lined tanks in water supplies. Lead, due to its wide use in industrial processes, ranks as the metal of largest diffusion through the atmosphere, one could speculate that this could in part explain the high concentrations found. This rise could be a consequence of contamination during the cheese making process, since only metals initially present are bound to milk proteins (Coni *et al.*, 1996), likewise cheese may be contaminated with lead from the contaminated salt and in case of Damietta cheese may picked up metals from tin containers. The further increase of lead levels in ripened cheese cannot be explained by water loss but was probably caused by environmental conditions at the ripening sites. The Pb contamination in cheese also could result from feeding cows with fodder collected from road sides and can be controlled by choosing the sources of the fodder without Pb contamination. Lead may accumulate in bone and lie dormant for years, and then pose a threat later in life during events such as pregnancy, lactation, osteoporosis, and hyperthyroidism and hyperparathyroidism, which mobilizes stores of lead in bones (HMRC, 2003).

Cadmium is found in low amounts in soil. It is transported via air and water from intensive industrial regions to the soil and water. It is considered to be the most important contaminant in modern times. Cd levels in examined cheese samples varied from 0.16±0.02 to 0.54±0.02 mg/kg in Cheddar and Edam cheese (Table 1). Nearly similar results (0.1-0.6 mg/kg) were reported by Vural *et al*. (2008). While, Orak *et al*. (2005) found that Cd level of white cheese samples from Turkey was 0.127 µg/g. Mean Cd content of cheese samples from Italy was 0.11 µg/g as recorded by Caggiano *et al*. (2005). The average Cd content of Otlu Lor cheese samples was reported by Kiliçel *et al*. (2004) as 0.20 mg/kg in Turkey. The Cd contents of cheese samples from several regions of Romania were noticed to be in the range of 0.003– 0.24 mg/kg (Hura, 2002).The results obtained by several researchers also show that the Cd contents of cheese vary over a wide range. A similar diversity in the Cd content of cheese was observed in this study. There is no maximum limit for Cd in milk or cheese, but the maximum limit in sweetened condensed milk and evaporated milk is 0.02 mg/kg and for milk powder is 0.03 mg/kg (Codex Alimentarius Commission, 2004).The high Cd content (0.09-0.7mg/kg) of examined cheese samples may be attributed to uncontrolled production and the use of contaminated water in the production process.

The tolerable daily intake of Cd established by the World Health Organization (WHO, 1996) is 0.060 mg/day for adult women and 0.070 mg/day for adult men. The contents of Cd in examined Cheddar cheese samples constitute about 53.3% (for women) and 45.7% (for men) of dietary daily intake, while Ras cheese samples constitute about 93.3% (for women) and 80% (for men) of dietary daily intake of Cd, except that, all examined cheese samples exceeded the permissible maximum daily intake of Cd recommended in the health criteria by WHO. Cadmium has a very long half-life in the body (10 to 30 years) and can build up over a long time. Over 80% of the body burden resides in the kidneys. Toxic contents of this element lead to renal insufficiency and metabolic abnormalities via enzyme inhibition (Hura, 2002).

The heavy metal contents of cheese varies due to the factors such as differences between species, characteristics of the manufacturing practices and possible contamination coming from the equipments during the process (Yüzbasi *et al*., 2003 and Caggiano *et al*., 2005). Oxidation of containers and equipments were affected by some parameters such as pH, quality of raw materials of containers and equipments. Enhancement of oxidation increases the metal contents of samples. Metal contents of used milk affect the metal levels in cheese. In addition, storage containers influence to metal levels. Therefore, heavy metal content of cheese varies in a big range.

Iron is an essential element for life and for our diets. Low iron levels can cause anemia. 40, 20, 13.3, 60, 20 and 33.3% of examined Cheddar, Gouda, Processed, Feta, Damietta and Kareish cheese samples respectively, were below the detection limit of Fe (0.01 mg/l)(DL= 3 × standard deviation of the mean of the blank determinations). The lowest iron concentration was found as 2.90±0.85 mg/kg in Edam cheese samples and the highest was 24.4±12.73 mg/kg in Damietta cheese samples (Table 2). Iron value for Herby cheese samples from Van-Turkey have been reported by Mendil (2006) as 12.5 µg/g and for Turkish white cheese was 3.610 µg/g as estimated by Orak *et al*. (2005).

As it can be seen in Table 2, Copper could be detected in all of examined samples except in Kareish cheese the Cu could be detected in 86.7% of the examined samples. Highest Cu level of examined cheese samples was found as 4.18±0.71 mg/kg in Cheddar cheese, while the lowest in processed cheese as 1.45±0.14 mg/kg. Mendil recorded Cu levels of Herby cheese from Van- Turkey as 0.13 µg/g (Mendil, 2006). Mean level of Cu in white cheese samples was 0.629 µg/g previously reported by Orak *et al*. (2005). Cu contents of various cheese samples were found in the range of 0.20–0.50 µg/g by Ferreira *et al*. (2005). On the other hand, Garcia *et al*. (2006) noted copper levels of fresh cheese and semi-hard cheese from Tenerife-Canary Islands as 0.80 and 0.86 µg/g, respectively.

Mean Zinc concentrations ranged from 21.72±1.19 to 43.18±0.53 mg/kg. The minimum and maximum Zn levels were found in Feta and Edam cheese samples, respectively (Table 2). Zn values are reported to be 0.35–4.50 mg/100 g (Gambelli *et al*., 1999).

If we take into consideration the recommended dose for daily diet (Recommended Daily Allowance- RDA) according to IMNAS (2000) and IMNAS, FNB (2001) of 8 mg/day Fe for men and 18 mg/day for women; 1.5-3 mg/day Cu and 12-15 mg/day Zn, from Table 2, it can be observed, that by normal consumption of 200 g cheese/day, the examined cheese samples constitute about 7.25-60% for men and 3.2-26.7% for women of Fe; 9.3-27.3% of Cu and 28-57.3% of Zn daily intake. The supply of Fe, Cu and Zn was within the recommended daily intake.

In conclusion, in all types of examined cheese samples, extremely toxic heavy metals Pb and Cd were detected above the toxicity levels but, the levels of Fe, Cu and Zn were within the recommended daily intake.

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