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HEAVY METALS CONTENT IN CHEESE: A STUDY OF THEIR DIETARY INTAKE AND HEALTH RISK ASSESSMENT

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Abstract: Cheese represents an important source of essential amino acids, vitamins, and trace elements such as copper (Cu), and zinc (Zn). Cheese can be contaminated with toxic heavy metals due to the use of contaminated ingredients, or during the manufacture process, and distribution. This study was taken to estimate the residual contents of some toxic metals including lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg), and trace elements such as zinc (Zn) and copper (Cu) in five of the most consumed cheese types in Egypt including Domiati, Feta, Karish, Mish, and Rumi. Moreover, calculation of the dietary intakes, and health risk assessment among children and adults were conducted. The obtained results revealed that Mish cheese had the highest residual content of Pb, and As. Karish cheese had the highest content of Cd, and Zn. Mercury was not detected in Feta and Domiati cheese. Calculation of the estimated daily intakes, hazard ratio, and hazard index revealed that consumption of cheese among Egyptian adults and children would not pose health risks. However, such data should be handled carefully as consumption of cheese will provide considerable concentrations of the total buildup of heavy metals to the Egyptian consumers. In addition, consumption of cheese will provide part of the human needs of the essential trace elements including Zn, and Cu.

Key words: heavy metals; trace elements; cheese; health risk assessment; dietary intakes

Introduction

Cheese is one of the dairy products that is consumed the most in the world. This is possibly due to its great nutritional content, distinct aroma and flavor, and availability of several varieties. The essential amino acids, vitamins, and trace minerals including calcium magnesium, copper, and zinc are all abundant in cheese (1, 2). The cheese Domiati, Feta, Karish, Mish, and Rumi, and are only a few of the varieties created from raw or pasteurized milk in Egypt. These kinds go through various processing steps with the potential for being contaminated with large varieties of biological contaminants and chemical contaminants such as heavy metals, mycotoxins, and biogenic amines (2).

Living animals can be exposed to a vast array of xenobiotics including heavy metals and other environmental pollutants. Such metals can accumulate in the different tissues of the animals and can cross the milk barrier (3). Milk also can be contaminated with heavy metals during the milking process. Metals can be leached from the milking tanks to the milk. Such contaminated

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milk might lead to contamination of the resultant dairy products from the processing of such milk (4). Dairy products such as cheese can be contaminated with heavy metals during the manufacture process (5), storage in tanks made from tin, and during marketing as in the case of Karish cheese which is sold open to air with street vendors (6).

Heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) are of no physiological functions for living organisms. Such metals are characterized by their bioaccumulation, and biomagnification nature, and find their way to the human body via ingestion of contaminated food and water leading to several toxicological implications. For instances, Pb is implicated in many cases of deaths among children as reported in Nigeria, and Zambia (7, 8). Moreover, Pb is also associated with the reduction in cognitive development and intellectual performance (9). Cadmium is the prime cause of itai-itai disease that is characterized by softening of bone and renal failure (10). Moreover, Cd is classified as an endocrine disruptor and associated with the occurrence of breast cancer (11). Another poisonous metal, Hg, was once widely employed in dentistry, antiseptics, and gold mining (12). However, chronic exposure to mercury was linked to serious neurological conditions, also known as Minamata illness. Arsenic is associated with dermal, nervous, respiratory mutagenic and carcinogenic effects (13).

Zinc serves as a crucial trace element for the catalytic activity of more than 100 enzymes in the body. It is also required for the regulation of the gene expression of many different cell components. Zinc is also necessary for maintaining the cell wall. In many underdeveloped countries, Zn deficiency is a serious problem that can result in anemia, and decreased immunity (14). Copper is an essential element that has a profound impact on the biochemistry and physiology of living creatures as a co-factor for many enzymes. Furthermore, Cu is essential for cellular respiration. However, prolonged contact with Cu may result in oxidative damage to the cell's organelles (15).

In sight of the previous facts, this study was taken to estimate the residual content of Pb, Cd, As, Hg, Zn, and Cu in five cheese types including Domiati, Feta, Karish, Mish, and Rumi retailed in Egypt. In addition, dietary intakes and health risks associated with the consumption of such cheese types were calculated among Egyptian children and adults.

Material and methods

The used reagents in the present study were of analytical grades and purchased from Merck, Darmstadt, Germany, unless specified.

Collection of samples

A hundred cheese samples including Domiati, Feta, Karish, Mish, and Rumi (20 of each) were collected from the grocery stores and street vendors in Zagazig city, Egypt. Samples (50 g of each) were packed separately and transferred in plastic tubes to the laboratory for heavy metals' extraction and measurement.

Sample preparation and extraction

One gram from each cheese sample was digested in a 10 mL liquid mixture composed of 3 parts of HNO3 and 2 parts of HClO4. The sample mixture was left overnight for digestion at room temperature. Then the resultant mixture was heated in a water bath at 70°C with continuous swirling for 3 h (16). The digested mixture was filtered, and the concentrations of the heavy metals (Pb, Cd, Zn, and Cu) were directly measured using the atomic absorption spectrophotometer (Shimadzu AAS 6800, Shimadzu, Japan) using hollow cathode lamps with an air-acetylene flame. While hydride generation/cold vapor atomic absorption spectroscopy was used to evaluate Hg, and As concentrations (Shelton, CT, USA). Based on standard curves created for each of the analyzed metals, the concentrations of the detected heavy metals were calculated. A wet weight (ww) basis was used to record the results, which were presented as μg/g.

Quality assurance

The accuracy of the analysis was established by measuring the samples twice and using the recognized reference material IAEA-142/TM (muscle homogenate), Vienna, Austria. The average recoveries for the metals under investigation ranged from 95 to 105%. The recovered concentrations of the certified samples were within 3% to 5% of the certified limits. All of the tools and supplies used in the

study were cleaned with diluted nitric acid to avoid external contamination with heavy metals.

Dietary intakes of heavy metals

The estimated daily intake (EDI) (µg/kg/day) values for the tested metals were calculated using the equation suggested by US Environmental Protection Agency, US EPA) (17):

$$EDI = C*F_{IR}/BW$$

Where C is the concentration of the tested metal in the sample (µg/g wet weight); F_{IR} is the food ingestion rate in Egypt, according to the global per capita consumption of cheese by nation in 2016, the average daily ingestion rate of cheese in Egypt was calculated to be 11.51 g/day based on an annual consumption of 4.2 kg of cheese (18). BW is the body weight of Egyptian consumers, which was set at 70 kg for adults, and 30 kg for children.

Health risk assessment

The non-cancer risks among the Egyptian consumers that associated with the consumption of cheese contaminated with metals were calculated using the guidelines recommended by the US EPA (17). The EDI was compared with the recommended reference doses (RfD) (0.001 mg/kg/day for Cd, 0.004 mg/kg/day for Pb, 0.0004 for As, and 0.3 mg/kg/day for Zn) (17), to generate the hazard ratio (HR) as following:

$$HR = EDI/RfD*10^{-3}$$

The hazard ratios can be summed to calculate a hazard index (HI) for estimation of the health risks associated with the mixed heavy metals.

$$HI = \sum HRi$$

where *i* represents each metal

A potential risk to human health is indicated if the HR and/or HI value is greater than one, whereas a result of one or less indicates no risk.

Statistical analysis

For statistical comparisons, the Tukey-Kramer HSD difference test (JMP) (SAS Institute, Cary, NC, USA) was applied ($p < 0.05$).

Results

The obtained results in Table 1, and Fig. 1A showed that 70%, 40%, 90%, 100%, and 80% of Domiati, Feta, Karish, Mish, and Rumi cheese samples were contaminated with Pb. Mish cheese had significantly ($p < 0.05$) the highest Pb residues, followed by Karish, Rumi, Domiati, and Feta with average Pb residual contents (µg/g ww) of 0.25±0.008, 0.14±0.009, 0.13±0.02, 0.07±0.01, and 0.02±0.004, respectively. It is evident from the obtained results 90%, 40%, 80%, 75%, and 65% of Domiati, Feta, Karish, Mish, and Rumi cheese samples were contaminated with Cd. Feta cheese had significantly ($p< 0.05$) the lowest Cd residues, however, no significant difference was observed in Cd residues among the other cheese kinds. Cd residual concentrations (µg/g ww) in the examined cheese kinds were 0.06±0.01, 0.08±0.01, 0.05±0.01, 0.07±0.007, 0.01±0.006 in the examined Mish, Karish, Rumi, Domiati, and Feta cheese samples, respectively (Table 1, Fig. 1B).

Arsenic was also detected at 100%, 30%, 100%, 100%, and 100% of Domiati, Feta, Karish, Mish, and Rumi cheese samples. The recorded As contents (µg/g ww) in the examined cheese kinds were 0.34±0.02, 0.13±0.003, 0.11±0.005, 0.16±0.06, and 0.01±0.003 in the examined Mish, Karish, Rumi, Domiati, and Feta cheese samples, respectively (Table 1, Fig. 2A). Mercury was not detectable in Feta and Rumi cheese but detected at 60%, 75%, and 65% of Domiati, Karish, and Mish cheese samples. The recorded concentrations (µg/g ww) of Hg were 0.02±0.001, 0.03±0.004, and 0.002±0.001 in the examined Mish, Karish, and Domiati cheese samples, respectively (Table 1, Fig. 2B).

In the case of trace elements, both Zn and Cu were detectable in all examined cheese types (Table 1). The average concentrations of Zn were 1.55±0.07, 3.35±0.07, 2.15±0.04, 2.18±0.08, and 2.55±0.08 in the examined Mish, Karish, Rumi, Domiati, and Feta cheese samples, respectively (Fig. 3A). While the average Cu residual contents were 0.05±0.008, 0.06±0.006, 0.09±0.003, 0.06±0.004, and 0.04±0.004 in the examined Mish, Karish, Rumi, Domiati, and Feta cheese samples, respectively (Fig. 3B). However, the recorded concentrations of Zn and Cu did not exceed the established maximum permissible limits. The recorded results in Table 2 showed that consumption of cheese among the Egyptian population could contribute to the daily intake of the measured heavy metal and subsequently to their buildup in the human body. However, calculation of the hazard ratio and hazard index for the tested metals recorded values far below 1 in both Egyptian adults and children (Table 2).

Table 1: Percentage of cheese samples contaminated with the tested heavy metals and that exceeding maximum per-missible limits (MPL)

	Domiati		Feta		Karish		Mish		Rumi	
	Positive	**Exceeding MPL**	**Positive**	**Exceeding MPL**	**Positive**	**Exceeding MPL**	**Positive**	**Exceeding MPL**	**Positive**	**Exceeding MPL**
Pb	70	70	40	35	90	90	100	100	80	80
Cd	90	85	40	5	80	80	75	55	65	50
As	100	20	30	0	100	15	100	95	100	10
Hg	60	0	0	0	75	45	65	30	0	0
Zn	100	0	100	0	100	0	100	0	100	0
Cu	100	0	100	0	100	0	100	0	100	0

MPL of Pb is 0.02, Cd is 0.05, As is 0.1, Hg is 0.01, Zn is 50, and Cu is 5 µg/g according to EU (2006)

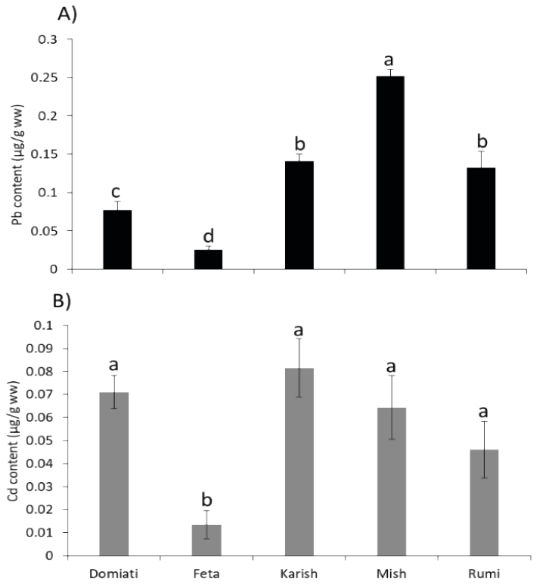

Figure 1: Toxic metals content A) lead (Pb), and B) cadmium (Cd) in the examined cheese samples. Values repre-sent mean ± SD (µg/g ww). Columns with different letter are significantly different at $p < 0.05$

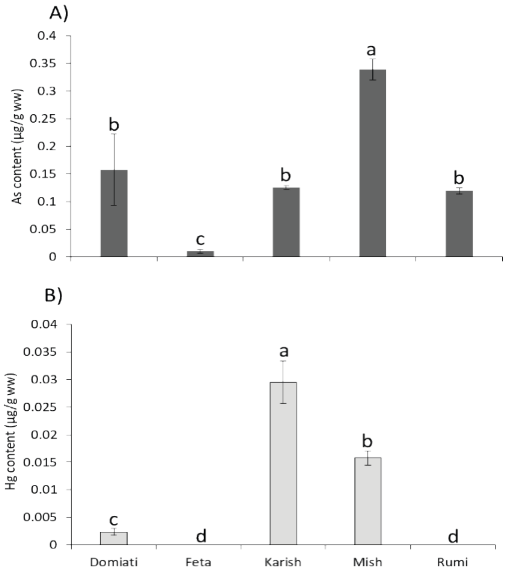

Figure 2: Toxic metals content A) arsenic (As), and B) mercury (Hg) in the examined cheese samples. Values repre-sent mean ± SD (µg/g ww). Columns with different letter are significantly different at $p < 0.05$

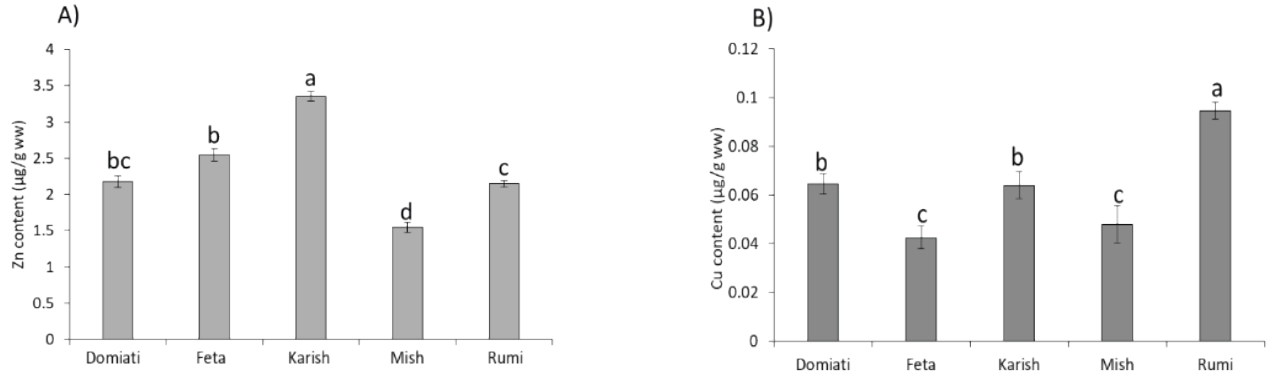

Figure 3: Trace elements content A) zinc (Zn), and B) copper (Cu) in the examined cheese samples. Values repre-sent mean ± SD (µg/g ww). Columns with differ-ent letter are significantly different at $p < 0.05$

Table 2: Estimated daily intake and health risk assessment of consumption of different cheese kinds

	EDI		HR	
	Adult	**Children**	**Adult**	**Children**
Domiati cheese				
Pb	0.013	0.029	0.003	0.007
Cd	0.012	0.027	0.017	0.027
As	0.026	0.061	0.086	0.201
Hg	0.001	0.001	0	0
Zn	0.361	0.835	0.001	0.003
Cu	0.011	0.025	0	0
HI			0.102	0.239
Feta cheese				
Pb	0.003	0.008	0.001	0.002
Cd	0.002	0.005	0.002	0.005
As	0.001	0.004	0.005	0.013
Hg	0	0	0	0
Zn	0.418	0.977	0.001	0.003
Cu	0.007	0.016	0	0
HI			0.010	0.023
Karish cheese				
Pb	0.023	0.054	0.006	0.013
Cd	0.013	0.031	0.013	0.031
As	0.021	0.048	0.069	0.161
Hg	0.005	0.011	0	0
Zn	0.551	1.286	0.002	0.004
Cu	0.011	0.024	0	0
HI			0.090	0.210
Mish cheese				
Pb	0.041	0.096	0.010	0.024
Cd	0.011	0.025	0.010	0.025
As	0.056	0.130	0.186	0.434
Hg	0.003	0.006	0	0
Zn	0.251	0.593	0.001	0.002
Cu	0.008	0.018	0	0
HI			0.208	0.484
Rumi cheese				
Pb	0.022	0.051	0.005	0.013
Cd	0.008	0.017	0.007	0.017
As	0.019	0.045	0.065	0.153
Hg	0	0	0	0
Zn	0.353	0.823	0.001	0.008
Cu	0.016	0.036	0	0
HI			0.079	0.186

Discussion

Heavy metals are characterized by their bioaccumulation and biomagnification nature. In addition, heavy metals are hardly affected by heat treatment. The obtained results of the present study revealed contamination of different cheese types retailed in Egypt with the toxic metals such as Pb, Cd, As, and Hg. Mish cheese had significantly the highest Pb residues followed by Karish, and Rumi cheese, while Feta cheese had the lowest Pb residues. Feta cheese also had the lowest Cd residues among the examined cheese types. Lead residues exceeded the established MPL (0.02 µg/g) in 100%, 90%, 80%, 70%, and 35% of Mish, Karish, Rumi, Domiati, and Feta cheese, respectively. Cadmium concentrations recorded in the current investigation exceeded the established MPL of Cd (0.05 µg/g) (19) in 85%, 80%, 55%, 50%, and 5% of Domiati, Karish, Mish, Rumi, and Feta cheese, respectively. Similarly, Pb and Cd were detected at higher levels that exceeded MPL in 48% & 44%, 64% & 68%, and 72% & 64% of soft, Ras, and processed cheese retailed in Ismailia governorate, Egypt (20). However, Pb and Cd were detected at lower levels that were within MPL in the soft cheese Sold in Ogbomoso, Southwestern Nigeria (21). Lead and Cd were detected at higher levels in cheese samples retailed in Arak, Iran (22), and in Karish cheese retailed in Egypt (6). Comparable levels of Pb were recorded in ranchero cheese in Mexico (23). Lower Cd residues (0.0001-0.007 ppb) were detected in milk, butter milk, cream, cheese, and yoghurt samples from different regions in Poland (24).

Arsenic was also detected in all examined cheese types in the present study, where 95%, 20%, 15%, 10%, and 0% of Mish, Domiati, Karish, Rumi, and Feta cheese exceeded the established MPL. While Hg was found at higher levels in Karish (45%), and Mish (30%), respectively. For both As, and Hg, Feta cheese had the lowest residual contents. Mercury was within the acceptable levels in the soft, Ras, and processed cheese retailed in Ismailia governorate, Egypt (20). While both As, and Hg exceeded the EU established MPL (19) in cheese samples retailed in Arak, Iran (22).

Detection of such toxicants in the different cheese kinds indicate contamination of the used milk in the processing of cheese with such toxic metals. Animals are exposed during their lifetime to a vast array ofxenobiotics including heavy metals which by turn can cross the milk barrier and find their way to the milk. Animals can be exposed to heavy metals via inhalation or ingestion of contaminated feed and water (4). Besides, As is commonly used in livestock farmsas a feed additive to increase the feed conversion ratio. This could explain the detected concentrations of As in the present study (25). Raw milk was found to be contaminated with heavy metals such as Pb, Cd, As, and Hg in several reports worldwide (6, 22, 24).

Zinc and Cu are trace elements that are routinely added for livestock and in dairy farms to avoid nutritional disorders. In the present study, Zn and Cu were detected in all examined samples but with no samples exceeded MPL of Zn and Cu. Relatively comparable Zn and Cu levels were recorded in dairy products including milk, and Karish cheese retailed in Beni-Suef, and Zagazig cities, Egypt (6, 26). Besides, Zn and Cu were recorded at higher levels in the marketed dairy products in Poland (24). Copper exceeded the MPL in 80%, 100% and 60% of soft, Ras, and processed cheese samples, respectively that sold in Ismailia, Egypt (20).

The EDI (µg/kg/day) values for Pb among the Egyptian consumers ranged between 0.003 (Feta cheese) and 0.09 (Mish cheese); for Cd ranged between 0.002 (Feta cheese) and 0.03 (Karish cheese); for As ranged between 0.001 (Feta cheese) and 0.130 (Mish cheese); for Hg ranged between 0.001 (Domiati cheese) and 0.011 (Karish cheese); for Zn ranged between 0.251 (Mish cheese) and 1.286 (Karish cheese); and for Cu ranged between 0.007 (Feta cheese) and 0.036 (Rumi cheese).

For both Egyptian adults and children, the computed non-carcinogenic hazard ratios and hazard indices were much below one, indicating that there were no potential hazards related to the consumption of the tested cheese varieties. The examined heavy metals' EDIs were comparable to those previously reported for consumption by Egyptian populations (6). Compared to Ghana (27), and Egypt (28), the reported HR and HI values were lower. The examined metals have been linked to several adverse health impacts, including cancer risk, neurological abnormalities, and toxic effects (4). As a result, even very low quantities of the tested hazardous metals will build up over time and produce a variety of toxicological effects.

It also notes worthy to mention that different cheese types tested in the present study were proven to afford at least part of the human needs of the essential trace elements such as Zn, and Cu.

Conclusion

The obtained results of the present study showed that different cheese kinds consumed by Egyptian population contained considerable concentrations of toxic and trace elements. Calculation of the HR and HI values for different cheese kinds demonstrated no potential non carcinogenic risks associated with the consumption of such cheese types. However, such results should be handled carefully as cheese also supports the total buildup of heavy metals with other food sources for the Egyptian population.

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