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Determination of Some Heavy Metals in Flavored Milk by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Their Public Health Importance

Karima G. Abdel Hameed and Mona A. El-Zamkan

Department of Food Hygiene and Control, Faculty of Veterinary Medicine,
South Valley University, Qena, 83523, Egypt

Abstract: Sixty flavored milk samples were analyzed for the presence of some heavy metals using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Aluminum (Al), Arsenic (As), Cadmium (Cd) and Lead (Pb) were detected in the analyzed samples. None of samples was contaminated with Mercury (Hg). The results were compared with Maximum permissible Limit (MPL). The maximum estimated dietary intake (MEDI) of detected heavy metals was calculated for children and adults. The concentration of Al and Cd in all samples exceed MPL (100%). The highest MEDI was calculated for Al (925 and 396%) and the lowest MEDI was reported for As (8 and 3%) from the provisional tolerable daily intake (PTDI) for children and adults in chocolate flavored milk, respectively. The results of this study exceed the permissible limits, suggesting health hazard. Therefore, strict regulations must be established during manufacture of such type of dairy products in developing countries.

Key words: Flavored milk · (ICP-OES) · Heavy metals · Health hazard

INTRODUCTION

Flavored milk is defined as UHT sweetened dairy drink made with milk, sugar, colorings and artificial or natural flavorings. It is has longer shelf-life than plain milk.It includes chocolate, banana and strawberry milk, etc. Although flavored milk has the same nutritional value as plain milk [1], it can be a source of heavy metals as plain milk when ingested over a long time period [2]. It is well established that heavy metals are toxic to humans and children may have an increased exposure to these metals compared with adults because of their lower body weight, especially if this product is marketed for daily use [3].

Toxicity of metal is closely related to age, route of exposure, daily intake, solubility, duration of exposure and frequency of intake [4]. Occurrence of heavy metals in dairy products may be attributed to contamination of the original cow's milk [5], or during its manufacture [6]. Moreover contamination may also occur from the use of aluminum utensils and cans in milk and dairy products [7].

Contamination of food by heavy metals as Aluminum (Al), arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) even in low concentrations, leads to metabolic disturbance and causing serious problems as it causes many health problems such as, heart failure, cancer, weakness and also affects the kidneys [8, 9]. Additionally, Al has neurotoxic effect and plays a factor in Alzheimer's disease, a very common cause of dementia [10,11]. Since, as far to our knowledge, reports regarding heavy metals in flavor milk are scarce, as well as their public health significance, the present work was designed to throw some light over the concentration of Al, As, Cd, Hg and Pb in flavored milk and compare them with the maximum permissible limits (MPL). In addition to calculation of maximum estimated daily intake (MEDI) of those metals for both children and adults.

MATERIALS AND METHODS

Samples Collection: A total of 60 flavored milk samples of commercially available (liquid cow's milk) were purchased

Corresponding Author: Karima G. Abdel Hameed, Department of Food Hygiene and Control, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt. E-mail: karima_galal2004@yahoo.com.

Table 1: The operating parameters of determination of metals by ICP-OES

Parameter	Setting	
Pump Tubing	Tygon Orang/White	
Pump speed	45rpm	
Nebulizer	Standard concentric	
Nebulizer Argon Flow	0.6L/min	
Spray Chamber	Standard cyclonic	
Center Tube	1.5mm	
RF Forward power	1150W	
Purge Gas	Argon	
Coolant gas flow	12L/min	
Auxiliary gas flow	0.5L/min	
Camera temperature	-45	
Element	Detection limit (µg L^{-1})	Wavelength (nm)
Al	0.12	167
As	1.43	189
Cd	0.07	214
Hg	0.14	185
Pb	1.06	220

from supermarkets in Qena City, Egypt. Samples were belonging to 3 different flavors (chocolate, strawberry and banana) consequently, 20 samples from each flavor were collected. Milk samples in their original containers (200 ml) were transported under refrigerated condition to the laboratory for analysis.

Samples Preparation: All glass tubes and bottles were washed before use with distilled water, soaked in nitric acid 30%, then rinsed in distilled water and air dried and kept clean to avoid contamination [12]. All milk samples were soaked in 1:1 nitric acid for 1 day and well rinsed with double distilled water (ddH2O).

Digestion of Samples and Estimation of Heavy Metals Concentration: A total of 25 mL of the milk samples were acid digested with 7 mL of HNO3 (Merck, Germany) and 7 mL of 30% hydrogen peroxide (H2O2; Merck) in a semiclosed glass digestion apparatus. After cooling, volume was adjusted to 50 mL with doubled distilled water [13]. The clear filtrate of each sample was kept in refrigerator to avoid evaporation. A blank (without sample) was prepared in the same way. Digested samples were being analyzed for Al, As, Cd, Hg and Pb using Inductivity Coupled Plasma Optical Emission Spectrometry (ICP-OES, thermo iCAP 6000 series). The analysis was carried out in the Central Laboratory of Faculty of Agriculture, Assiut University, Egypt. Three replicates were done for more accuracy.The ICP-OES operating conditions are listed in Table 1.

Calculation of the Maximum Estimated Daily Intake (MEDI): The acceptable daily intake of Al, As, Cd, Hg and Pb for an adult person (60 kg b.w.) and for children (25 kg b.w.) was calculated according to Nutrition Institute recommendations [14] using the following equation:

$$MEDI=\frac{\text{milk intake (ml/day) x maximum heavy metal concentration (mg/kg)}}{\text{average bory weight (kg)}}$$

The results obtained were compared with MEDI recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Codex Alimentarius Commission) [15] and with MPL recommended by IPCS [16], WHO [17] and Pennington [18] for Al, according to (Codex Alimentarius Commission) [15] for As, [19] for Cd and Pb.

Statistical Analysis: Data were analyzed using General Linear Model (GLM) procedure of SAS [20] according to the following model:

$$Yij = \mu + Ti + Eij$$

where: µ = Mean, Ti = Effect of treatment and Eij= Standard error

Duncan's multiple range test [21] was used to compare between means of groups.

RESULTS AND DISCUSSION

Contamination of milk by heavy metals constitutes serious hazardous impacts on human health because milk is mainly used for feeding infants and children [22]. Therefore, to increase health awareness of consumer, discarding contaminated food, concentration on regulations of international food commissions, as well as to pay consumers attention toward flavored milk what does really it contain, we construct this study to determine the concentration of several heavy metals in flavored milk sold in city of Qena Egypt.

Concentration of Heavy Metals in the Examined Flavored Milk: The results presented in Table 2 summarized the concentration of heavy metals in the examined flavored milk. Al, Cd and Pb were detected in all of the examined chocolate, strawberry and banana flavored milk samples.

Table 2: Metals concentration (mg/L) in flavored milk samples (N = 60)

METAL	Type of flavored milk		
Al	Chocolate, n=20	Strawberry, n=20	Banana, n=20
Min.	0.74	0.72	0.71
Max.	1.19	1.12	1.13
Mean ± SD	0.984 ± 0.1058	0.964 ± 0.1352	0.957 ± 0.1303
As			
Min.	ND	ND	ND
Max.	0.01	0.028	0.018
Mean ± SD	0.0038 ± 0.0028	0.0057 ± 0.0070	0.0047 ± 0.0052
Cd			
Min.	0.02	0.02	0.02
Max.	0.07	0.04	0.03
Mean ± SD	0.040 ± 0.0171 [a]	0.024 ± 0.0060 [b]	0.022 ± 0.0041[b]
Hg			
Min.	ND	ND	ND
Max.	ND	ND	ND
Mean ± SD	ND	ND	ND
Pb			
Min.	0.02	0.02	0.02
Max.	0.16	0.18	0.19
Mean ± SD	0.1074 ± 0.0343	0.1064 ± 0.0459	0.1067 ± 0.0549

N= total number of samples; n= number of samples /each type of flavored milk)

ND= non-detected

a and b: values with the different superscripts on the same row differ at P<0.05

Al was detected in 100% of the flavored milk samples at mean concentrations mg/L of 0.984 ± 0.1058 (chocolate), 0.964 ± 0.1352 (strawberry) and 0.957 ± 0.1303 (banana), respectively (Table 2). These values were higher than the MPL (<0.05 mg/L), reported by IPCS [16] and WHO [17] and were higher than the results obtained by Rezaei *et al.* [23], they stated that the mean concentration of Al in pasteurized milk was (0.135 ± 0.0857) mg/L. As well AI-Ashmawy [24], reported a mean Al concentration of 0.081 ± 0.010 mg/L in market milk in Dakahlia Governorate, Egypt. On the contrary, our results were lower than level measured in sterilized cow's milk in Dakahlia Governorate, Egypt by Salah *et al.* [25], who detected an average Al concentration of 1.324 ± 0.112 mg/kg.

In the present study, the mean concentrations of As (mg/kg) in flavored milk samples were 0.0038 ± 0.0028 for (chocolate), 0.0057 ± 0.0070 (strawberry) and 0.0047 ± 0.0052 (banana) (Table 2). The levels in our samples were higher than the permissible level (0.002 mg/kg) set by Pennington [18] and that estimated by Rezaei *et al.* [23] (0.0023 ± 0.0016) mg/L. However it was lower than results recorded by Algan *et al*. [26], who found that Arsenic concentration in raw milk was (0.0839 mg/kg) and values (0.0087, 0.0075 and 0.0094 mg/kg) were reported by Amponsah [27] in tin milk produced in Ghana.

Concerning Cd in the current study, the mean concentrations (mg/kg) (Table 2) were 0.040 ± 0.0171, 0.024 ± 0.0060 and 0.022 ± 0.0041 for chocolate, strawberry and banana flavored milk, respectively. These values were higher than the limit (0.002 mg/kg) established by EOSQC [19] and concentration of (0.00395 ± 0.0023) mg/L reported by Rezaei *et al.* [23]. Higher result (0.355 ± 0.039 mg/kg) was detected by Salah *et al.* [25], in sterilized cow's milk in Dakahlia Governorate, Egypt. Nearly similar results of 0.0228 and 0.0388 mg/L were reported by Licata *et al.* [9] and Algan *et al.* [26] in cow's milk respectively. Lower result was reported by Tripath *et al.* [22] who found 0.00007 mg/L of Cd in cow's milk. Significant differences were found in Cd level between chocolate, strawberry and banana flavored milk.

When findings of this study were compared to the literature, it was noted that Cd content was found to be negligible. However, it shouldn't be ignored that the level of cadmium, is likely to go up depending on the growing environmental pollution and it can be a source of danger for both human and animal health.

Regarding Pb in the examined chocolate, strawberry and banana flavored milk we found a mean of concentration of 0.1074 ± 0.0343, 0.1064 ± 0.0459 and 0.1067 ± 0.0549 mg/L, respectively (Table 2). The mean concentrations of lead were disclosed above the permitted one by EOSQC [19] which is 0.02 mg/L. Higher results of 0.230 ± 0.12 mg/L and 0.910 ± 0.051 were reported by Vanessa *et al.* [28] in pasteurized milk in Brazil and Salah *et al.* [25] in sterilized cow's milk in Dakahlia Governorate, Egypt, respectively. Lower value of 0.0125 ± 0.0076 mg/L was detected by Rezaei *et al.* [23] in pasteurized milk. Only six samples of the total studied flavored milk (10%) did not show that contaminant. One of the most important sources of Pb contamination in milk is water, especially in more contaminated areas (Codex Alimentarius Commission) [29].

Mercury, which is one of the most toxic elements, was not determined in the examined samples (Table 2). This may be due to being very low from detection limit (185 nm) of the apparatus of amounts present in milk and its products.

There was no significant difference ($p < 0.05$) between types of flavored milk in terms of heavy metals content (Table 2).

Comparison with MPLs: The levels of Al and Cd in all flavored milk samples exceeded the MRL values set by IPCS and [16] and WHO [17] for Al and EOSQC [19] for Cd. Additionally, the level of As exceeded the permissible

Table 3: Comparison of detected heavy metals with MPL in examined flavored milk

		Samples above MPL					
		Chocolate		Strawberry		Banana	
Metals	MPL mg/Kg	n=20	%	n=20	%	n=20	%
Al	< 0.05a	20	100	20	100	20	100
As	0.002b	11	55	10	50	10	50
Cd	0.002c	20	100	20	100	20	100
Pb	0.02c	19	95	17	85	18	90

a =<0.05 mg/L) reported byIPCS and WHO [16,17and 18], respectively.

b= (Codex Alimentarius Commission)[15]

c= EOSQC [19]

Table 4: The maximum estimated daily intake (MEDI) value of heavy metals for children (1 mg/kg b.w./d)[a] within the calculated intake from the examined flavored milk for children

		Calculated daily intake of heavy metals from consumption of flavored milk (mg/kg body weight/d) MEDI (mg/kg b.w./d)[b]					
		Chocolate		Strawberry		Banana	
Metals	ADI (mg/kg) (Codex (2014)	MEDI	%	MEDI	%	MEDI	%
Al	0.286	9.52	925	8.96	896	9.04	904
As	0.0021	0.08	8	0.224	22.4	0.144	14.4
Cd	0.001	0.56	56	0.32	32	0.24	24
Pb	0.0036	1.28	128	1.44	144	1.52	152

a= According to (Codex Alimentarius Commission),WHO and NHF [15, 17, 30].

b= Daily consumption for children (25 kg b.w.) according to Nutrition Institute, Egypt [14,31].

Table 5: The maximum estimated daily intake (MEDI) value of heavy metals for adults (1 mg/kg b.w./d)[a] within the calculated intake from the examined flavored milk for adults

		Calculated daily intake of heavy metals from consumption of flavored milk (mg/kg body weight/d) MEDI (mg/kg b.w./d)[b]					
		Chocolate		Strawberry		Banana	
Metals	ADI (mg/kg) (Codex (2014)	MEDI	%	MEDI	%	MEDI	%
Al	0.286	3.966	396	3.733	373.3	3.78	378
As	0.0021	0.033	3.33	0.0933	9.33%	0.06	6%
Cd	0.001	0.233	23%	0.1333	13	0.1	10
Pb	0.0036	0.533	53.3%	0.6	60	0.64	64%

a = According to (Codex Alimentarius Commission)WHO and NHF [15,17, 30].

b = Daily consumption for adult person (60 kg b.w.) according to Nutrition Institute, Egypt [14,31]

limit recommended byPennington [18] in 55, 50 and 50 % of chocolate, strawberry and banana flavored milk samples, respectively. Moreover, Pb levels exceeded MPLs indicated by EOSQC [19] in 95, 85 and 90% of the examined chocolate, strawberry and banana flavored milk samples, respectively (Table 3).

The Calculated Maximum Estimated Daily Intake in Children and Adults: The results presented in Table 4 revealed that the calculated MEDI of Al, As, Cd and Pb for children (25 kg) consuming 200 mL flavored milk daily were (9.52, 0.08, 0.56 and 1.28 mg/L b.w./d) for chocolate, (8.96, 0.224, 0.32 and 1.44 mg/L b.w./d) for strawberry and (9.04, 0.144, 0.24 and 1.52 mg/L b.w./d) for banana flavored milk and these contribute about (925, 8, 56 and 128%) of chocolate, (896, 22.4, 32 and 144%) of strawberry and (904, 14.4, 24 and 152%) of banana flavored milk of the provisional tolerable daily intake (PTDI) of Al, As, Cd and Pb recommended by Codex Alimentarius Commission [15].

However the calculated MEDI of Al, As, Cd and Pb for an adult person (60 kg) consuming 200 mL flavored milk daily were approximately (396, 3.33, 23 and 53.3%) of chocolate, (373.3, 9.33, 13 and 60%) of strawberry and (378, 6, 10 and 64%) mg/L b.w./d of banana flavored milk of the PTDI, respectively (Table 5).

According to the maximum estimated daily intake (MEDI) for children and adult person, we found that the MEDI of Al, As, Cd and Pb from consumption of 200 ml flavored milk were relatively high and represented high proportion as compared to acceptable daily intake (ADI) recommended by Codex Alimentarius Commission [15]. MEDI can be exceeded to a large extent by some population groups, particularly children who regularly consume foods that include heavy metals containing additives.

CONCLUSION

From the above mentioned data, it can be concluded that the examined flavored milk samples were contaminated with heavy metals especially Al, Pb and Cd in various levels, exhibiting a wide array of hazardous impacts on human health. This may be attributed to manufacturing processes that play a key role in distribution of heavy metals, greater pollution of the environment which have resulted in an increased concentration of heavy metals in air, water and soil subsequently; these metals are taken by plants and animals to take their way into milk. Also, heavy metals may be added to milk during production, processing and storage as well as by contamination from containers.

REFERENCES

1. USDA National Nutrient, 2011. U.S. Department of Agriculture, USDA National Nutrient Database for Standard Reference, Release 24, Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl.
2. Unak, P., F.Y. Lambrecht, F.Z. Biber and S. Darcan, 2007. Iodine measurements by isotopedilution analysis in drinking water in Western Turkey. J. Radioanaly. Nucl. Chem., 273: 649-651.
3. Khaniki, G.R.J., 2007. Chemical contaminants in milk and public health concerns: a review. Int. J. Dairy Sci., 2: 104-115.
4. Mertz, W.E.D., 1986. Trace elements in human and animal nutrition. Vol. I and II, 5 th ed. Academic Press, New York.pp: 209.
5. Okada, I.A., A.M. Sakuma, F.D. Maio, S. Dovidauskas and O. Zenebon, 1997. Evaluation of lead and cadmium levels in milk due to environmental contamination in the Paraiba Valley region of Southeastern Brazil. J. Public Health, 31(2): 140-143.
6. Ukhun, M.E., J. Nwazota and F.O. Nkwocha, 1990. Level of toxic mineral elements in selected foods marketed in Nigeria. Bull. Environ. Contam. Toxicol., 44: 325-330.
7. Masten, S., 2000. Aluminum compounds, Review of Toxicological Literature Abridged Final Report. Integr. Lab. Sys., 27709, North Carolina.
8. McCally, M., 2002. Human health and heavy metals exposure. The Environment and Human Health (Chapter 4).In: McCally M, editor. Life Support. Cambridge MIT Press, pp: 1.
9. Licata, P., D. Trombetta, M. Cristani, F. Giofre, D. Martino and M. Calo, 2004. Levels of ‘‘toxic” and ‘‘essential” metals in samples of bovine milk from various dairy farms in Calabria, Italy. Environ. Int., 30: 1-6.
10. Gupta, V.B., S. Anitha and M.L. Hegde, 2005. Aluminum in Alzheimer’s disease: are we still at a crossroad? Cell Mol. Life Sci., 26(2): 143-58.
11. Edwards, M.R., 2006. Toxicity, aluminum. E. Medicine from WebMD. http://www. Emedicine. Com/MED/topic113.htm.
12. Valiukenaite, R., M. Stankeviciene, H. Stankevicius and K.A. Skibniewska., 2006. Polish J. Food Nut. Sci., 15/56(1): 243-246.
13. Goudarzi, A., P. Parsaei, F. Nayebpour and E. Rahimi, 2013. Determination of mercury, cadmium and lead in human milk in Iran. Toxicol. Indus. Health, 29: 820-823.
14. Nutrition Institute, Cairo, 2007. Daily consumption of adult person in Egypt. No. 43906. http://www. elharm.com/9134/INVES.HTM.
15. Codex Alimentarius Commission., 2014. Joint FAO/WHO food standards programme contaminants in foods, Eighth Session, The Hague, The Netherlands, 31 March - 4 April.
16. International Programme on Chemical Safety “IPCS”. 2009. Inter-Organization Programme for the Sound Management of Chemicals, A Cooperative Agreement Among UNEP, ILO, FAO, WHO, UNIDO, UNITAR and OECD, 657. Aluminum (WHO Food Additives Series 24).
17. World Health Organization (WHO), 2007. Evaluation of certain food additives and contaminants. Sixty-Seventh Report of the Joint FAO.WHO Expert Committee on Food additives. Geneva. WHO Techn. Rep. Ser. No. 940.
18. Pennington, J.A.T., 1987. Aluminum content of food and diets. Food Addit. Contam., 5: 161-232.

19. Egyptian Organization for Standardization and Quality Control (EOSQC). 1993. Maximum residue limits for heavy metals in food. Ministry of Industry Cairo, Egypt. No. 2360/1993, pp: 5.
20. SAS, 1998. SAS User's guide: Statistics. SAS Inst. Inc., Cary, NC, Releigh.
21. Duncan, D.B., 1955. Multiple Range and Multiple F Tests. Biometrics, 11: 11-42.
22. Tripathi, R.M., R. Raghunath, V.N. Sastry and M. Krishnamoorthy, 1999. Daily intake of heavy metals by infants through milk and milk products. Sci. Total Environ., 227: 229-235.
23. Rezaei, M., H.D. Akbari, H. Jafari, A. Farahi, A. Shahabi, H. Javdani, H. Teimoory, M. Yahyaei and A.A. Malekirad, 2014. Assessment of dairy products consumed on the Arakmarket as determined by heavy metal residues. Health, 6(5): 323-327. http://dx.doi.org/10.4236/health.2014.65047.
24. Al Ashmawy, M.A., 2011. Prevalence and public health significance of aluminum residues in milk and some dairy products. J. Food Sci., 76(3): 73-76.
25. Salah, F.A., E.I. Awad and R.M. Kamal, 2012. Prevalence of some trace and toxic elements in raw and sterilized cow's milk. J. Amer. Sci., 8(9): 753-761.
26. Algan, G., C.O. Tekinşen and V. Gök, 2012. Investigation of some heavy metal presence in the milk of cattle. Eurasian J. Vet. Sci., 28(3): 159-163.
27. Amponsah, D., 2014. Determination of levels of heavy metals (arsenic, lead, cadmium and mercury) in tin milk produced in Ghana. Inter. J. Adv. Res. Tech., 3(6): 129-131.
28. Vanessa, A.S., M.M. Mahyara, A.L. Kus, S.C. Juliana, F.S. Rodrigo and J.I. Hélcio, 2010. Determination of nutritional and toxic elements in pasteurized bovine milk from Vale do Paraiba region (Brazil). J. Food Contam., 21: 45-49.
29. Codex Alimentarius Commission. 2004. Joint FAO/WHO Food Standards Programme, CX/FL 0415 – ADD.2, Agenda Item No. 5.
30. National Health Federation (NHF), 2008. Beijing- The Codex Food Additives Committee Meeting, Codex: Aspartame, Sucralose & Aluminum are Still Dangers. http://www.Thenhf.Com/pressreleases/pr28apr 2008.html.
31. Nutrition Institute, Cairo, 1996. Guide of healthy food for Egyptian family. 2nd ed. Nutrition Institute, Cairo, A.R.E.