



Short communication

Prevalence of Melamine and Cyanuric Acid in Powdered Dairy Products in Egypt



Aml S. Ibrahim, Saad, M.F., Nagah M. Hafiz

Cairo University, Faculty of Veterinary Medicine, Department of Food Hygiene and Control, Giza, 12211 Egypt.

Abstract

This study was conducted to screen melamine and cyanuric acid in powdered dairy products, which becomes a high safety concern, as considered one of the scanty surveys in Egypt. A total of 140 samples (fifty whole milk powder, thirty samples of each; skim milk powder, powdered infant milk formula, and dried milk-cereal-based infant formula) were collected from Egyptian markets and pharmacies. Samples were analyzed for the presence of melamine and cyanuric acid using Liquid Chromatography-Mass Spectrometry (LC-MS/MS). Melamine was detected in 15/50 of whole milk powder, 22/30 of skim milk powder, 20/30 of powdered infant milk formula, and 25/30 milk-cereal-based infant formula samples. Meanwhile, cyanuric acid couldn't be detected in any examined samples, as its concentration was < 0.05 mg/kg. This investigation initially proceeded in Egypt for melamine and cyanuric acid detection in milk-cereal-based infant formula and skim milk powder. It is targeted for re-attention from concerned authorities to these toxic chemicals.

Keywords: Powdered milk; Milk-cereal-based infant formula; Melamine; Cyanuric acid; Mass spectrometry.

1. Introduction

Milk powder recently becomes a communal dairy product as it is facilely in handling, preparation, conveyance, and formulation (used in the production of infant formula), in addition, it is characterized by high protein content [1, 2].

In some cases, producers to compensate for this high protein content may use melamine. Since 2008, melamine (2, 4, 6-triamino-1, 3, 5-triazine) was considered one of the adulterants that have become a troubleshoot to food safety, as recalls for contamination of infant food with melamine are still happened. It is known to have a high nitrogen% (66% by weight) and is used as a melamine-formaldehyde resin in the food to resist heat and maintain its stability [3, 4].

Cyanuric acid is an oxytriazine analog of melamine, which is considered the output of melamine synthesis. It is applied significantly in the processing

of feed additives for animals or in sanitizing substances. The occurrence of cyanuric acid in food is related to the use of dichloroisocyanurates as a disinfectant for water and product contact items [5, 6].

The consumption of melamine was studied to cause stones in the kidney; subsequently, renal failure and even death may happen. Like, what emerged in China (2008); death of six children and more than fifty-one thousand babies suffered from kidney dysfunction due to consumption of dried infant formula that was adulterated by adding melamine [7].

FDA [8]; has proposed the use of interim Mass Spectrometry (MS) method LC-MS/MS in the analysis of melamine and cyanuric acid in powdered milk and dried infant formula at a level of 0.25 mg/kg. This method is characterized by being highly specific and sensitive to contaminants [9]. In this study, we planned to detect melamine and cyanuric acid in dried milk products and elucidate on still utilized

*Corresponding author e-mail: mena.saad@vet.cu.edu.eg; (Mena Saad).

Receive Date: 15 July 2021, Revise Date: 24 July 2021, Accept Date: 01 August 2021

DOI: [10.21608/EJCHEM.2021.86412.4186](https://doi.org/10.21608/EJCHEM.2021.86412.4186)

©2022 National Information and Documentation Center (NIDOC)

them as a source of nitrogen.

2. Materials and methods

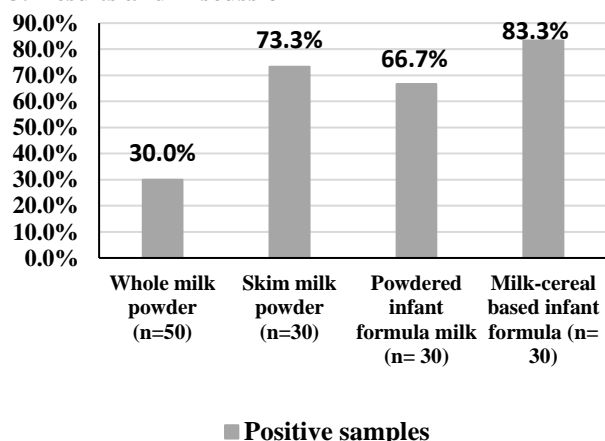
2.1. Collection of samples

Fifty whole milk powder and thirty samples of each following products: skim milk powder, powdered infant milk formula (0-6 months), and dried milk-cereal-based infant formula that applied for infants from six-month age, were gathered from pharmacies and markets in Cairo and Giza Governorate, Egypt. All samples were kept at room temperature (25°C) until the time of analysis.

2.2. Detection of melamine and cyanuric acid according to [10, 11]

After samples preparation by extraction, 2g of each powder milk sample using 14 ml of formic acid 2.5% (Sigma-Aldrich, Germany) and diluting by acetonitrile (ACN, Sigma-Aldrich, Germany). The extracted were tested via a Liquid Chromatography triple quadruple tandem Mass Spectrometry (LC-MS/MS) 4000 QTRAP® (Applied Bioscience, Canada). The separation of melamine and cyanuric acid were preceded by a Hydrophilic Interaction Chromatography (HILIC) column. As detection of melamine was measure in positive ion mode but negative ion mode for cyanuric acid. This method exhibited a Limit of Detection (LOD) of 0.25 mg/kg for melamine and 0.05 mg/kg for cyanuric acid. The analysis was done at Regional Center for Food, Giza, Egypt.

3. Results and Discussion



n = number of the examined samples

Fig. 1. Percentages of melamine adulteration in the examined powdered milk samples.

Melamine was detected in a percentage of 30,

73.3, 66.7, and 83.3 in whole, skim milk powders, powdered infant milk formula, and milk-cereal-based infant formula, respectively (Fig.1). These confirmed that all positive samples were polluted with melamine at the level of 0.25 mg/kg, which was above the limit of detection of LC-MS/MS. Several authorities set that melamine and cyanuric acid shouldn't exceed a maximum limit of 2.5 mg/kg in all products based on milk and 1 mg/kg for infant formula [5, 12]. All the examined samples were free from cyanuric acid, similar to results were obtained by Hassani [13] and nearly identical to data reported by Wu [14]; who detected melamine in 87 of 111 infants formula samples, but low prevalence of cyanuric acid. WHO [5]; European Food Safety Authority (EFSA) [15], reported that cyanuric acid was found to migrate and contaminate food at low levels. In addition, less evidence of using it as an adulterant with proving it alone has weak toxicity for infants than melamine, this due to its fast absorption in GIT and excretion through urine.

Various investigations were applied to elucidate that the existence of melamine in these milk products. It may result from the contamination by decomposition of cyromazine pesticides [16] or packaging materials as an origin of melamine immigration to such food [17]. Liu et al. [18] recorded that milk may be contaminated with melamine through animal feeds, especially when melamine-containing fertilizers were used. Elkhawaga et al. [19] detected melamine in 48.0% and 60.0% of analyzed milk powder samples, respectively. The highest findings were obtained by Deabas [20] as 100.0% of milk powder and powdered infant formula samples were positive for melamine. WHO [5] declared a Tolerable Daily Intake (TDI) of 0.2 mg/kg body weight for melamine. Li et al. [21] announced that; when TDI increased, it was observed to cause nephrolithiasis and Tolerable Daily Intake (TDI) of 1.3 mg/kg body weight for cyanuric acid.

As reported by EFSA [15] some producers have used melamine as a source of nitrogen in wheat gluten and rice to enhance protein content. This explained the high percentage of melamine in milk-cereal-based infant formula samples, in which skim milk powder was included in its ingredients. Also, this was proved by Zhu [22], who revealed melamine in 100% of the examined cereal products.

A recent study achieved by Tawfik [23]; reported that most Egyptian mothers were dependent on powdered infant formula feeding instead of exclusive breastfeeding with early introduction of milk-cereal-

based baby formula before infants reach six-month age. These infants were fed on powdered infant milk, and milk-cereal-based baby formulae may be exposed to melamine. So the application of the Hazard Analysis and Critical Control Points system is a precondition for the uppermost safe such products and regular monitoring of these products plus increasing awareness of Egyptian mothers will help safeguard the health of their infants [24, 25, 26, 27].

Conclusion

This investigation for melamine and cyanuric acid in milk-cereal-based infant formula and skim milk powder is the initial study in Egypt. So, implementation of Good Manufacturing Practices (GMPs) becomes necessary for control melamine levels to prevent its adverse health effect on human beings, especially infants. More limitations by Egyptian and international authorities should be applied to prevent melamine using as a nitrogenous compound in animal feeds or preparation of pesticides or fertilizers by application of Good Agricultural Practices (GAPs). As well as dried milk and powdered infant milk formula should be checked and monitored recurrently for melamine by employing fast and sensitive methods. More research needs to explain the relation between fat percentages in dairy products and the melamine detected, as in our study, low-fat powders were the highest contaminated with melamine.

Conflicts of interest

“There are no conflicts to declare”.

Bibliography

- [1] A.M.M. Deeb, I.I. Al-Hawary, I.M. Aman, D. M. H. A. Shahine, Bacteriological investigation on milk powder in the Egyptian market with emphasis on its safety, *Global Veterinaria*. 4, 24-433 (2010). [http://www.idosi.org/gv/gv4\(5\)10/1.pdf](http://www.idosi.org/gv/gv4(5)10/1.pdf)
- [2] O. Oussaief, Z. Jrad, M. Dbara, T. Khorchani, H. El Hatmi, Physicochemical and antioxidant properties of freeze-dried dromedary skim colostrum and milk powder, *Mljekarstvo*. 71, 69-78 (2021). DOI: 10.15567/mljekarstvo.2021.0107
- [3] M. Huang, M.S. Kim, S.R. Delwiche, K. Chao, J. Qin, C. Mo, C. Esquerre, Q. Zhu, Quantitative analysis of melamine in milk powders using near-infrared hyperspectral imaging and band ratio, *Journal of Food Engineering*. 181, 10-19 (2016). DOI: 10.1016/j.jfoodeng.2016.02.017.
- [4] S.A. Abo-El-Enein, I.A. Aiad, M.A. Heikel, S.M.A. El-Gamal, M. Mahmoud, Effect of some superplasticizers on the physico-chemical properties of the hardened cement pastes, *Egyptian Journal of Chemistry*. 59, 195–208 (2016). DOI: [10.21608/ejchem.2016.1367](https://doi.org/10.21608/ejchem.2016.1367)
- [5] WHO, World Health Organization. Expert meeting to review toxicological aspects of melamine and cyanuric acid. 1–4 December 2008, Geneva: WHO Press.
- [6] K. Rovina, S. Siddiquee, A review of recent advances in melamine detection techniques, *J. Food Compos. Anal* 43, 25-38 (2015). <http://dx.doi.org/10.1016/j.jfca.2015.04.008>
- [7] F. Sun, W. Ma, L. Xu, Y. Zhu, L. Liu, C. Peng, L. Wang, H. Kuang, C. Xu, Analytical methods and recent developments in the detection of melamine, *Trends in Analytical Chemistry*. 29, 1239-1249 (2010). <https://doi.org/10.1016/j.trac.2010.06.011>
- [8] FDA, Food and Drug Administration. Analytical Methods for Melamine and Triazine Analogs, (2009). Available from: <http://www.fda.gov/AnimalVeterinary/ScienceResearch/ToolsResources/ucm135002.htm>.
- [9] P. W.S. Chu, K.M. Chan, S.T. C. Cheung, Y.C. Wong, Review of analytical techniques used in proficiency-testing programs for melamine in animal feed and milk, *Trends Anal. Chem*. 29, 1014–1026 (2010). <https://doi.org/10.1016/j.trac.2010.06.007>
- [10] S. Turnipseed, C. Casey, C. Nocketto, D.N. Heller, Determination of melamine and cyanuric acid residues in infant formula using LC–MS/MS, *United States Food and Drug Administration*. 1–14 (2008). Corpus ID: 36062519
- [11] E.R. Attala, T.M. Khedr, M.M. Abo-Aly, Development and validation of analytical method for determination of melamine in baby milk and liquid milk using LC-MS/MS, *Res J. Chem. Environ. Sci* 4, 24-30 (2016). http://www.aelsindia.com/oct_2016.htm
- [12] European Commission, Commission Decision 2008/798/EC, Imposing special conditions governing the import of products containing milk or milk products originating in or consigned from China, and repealing Commission Decision 2008/757/EC. *Official Journal of the European Union L 273 / 18, 15. 10.* (<http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:273:0018:0020:EN:PDF>).
- [13] S. Hassani, F. Tavakoli, M. Amini, F. Kobarfard, A. Nili-Ahmadabadi, O. Sabzevari, Occurrence of melamine contamination in powder and liquid milk in market of Iran, *Food Additives & Contaminants: Part A*. 30, 413-420 (2013). DOI: 10.1080/19440049.2012.761730
- [14] Y. N. Wu, Y.F. Zhao, J. G. Li, Melamine Analysis Group, A survey on occurrence of melamine

- and its analogues in tainted infant formula in China, *Biomedical and Environmental Sciences*. 22, 95–99 (2009). [https://doi.org/10.1016/S0895-3988\(09\)60028-3](https://doi.org/10.1016/S0895-3988(09)60028-3).
- [15] EFSA, European Food Safety Authority. Panel on contaminants in the food chain (CONTAM) and EFSA panel on food contact materials, enzymes, flavourings and processing aids (CEF). Scientific opinion on melamine in food and feed, *EFSA Journal*. 8(4), 1573(2010).
- [16] G. Patakioutas, D. Savvas, C. Matakoulis, T. Sakellarides, T. Albanis, Application and fate of cyromazine in a closed-cycle hydroponic cultivation of bean (*Phaseolus vulgaris L.*), *Journal of Agricultural and Food Chemistry*. 55, 9928-9935(2007). DOI: [10.1021/jf071726i](https://doi.org/10.1021/jf071726i)
- [17] Commission Directive 2002/72/EC, Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of the European Communities (L220)*, 18-58 (2002).
- [18] Y. Liu, J. Deng, L. An, J. Liang, F. Chen, H. Wang, Spectrophotometric determination of melamine in milk by rank annihilation factor analysis based on pH gradual change-UV spectral data, *Food Chem*. 126 (2), 745-750 (2011). <https://doi.org/10.1016/j.foodchem.2010.11.057>
- [19] E. Elkhawaga, E. Shukry, A.H. Nassar, O. Abdelaziem, Z.A. Kheder, Detection of melamine in milk powder and some dairy products, *Animal Health Research Journal*. 5, 348-357 (2017).
- [20] M.M. Deabas, R. El-Habib, Determination of melamine in infant milk formula, milk powder and Basaa fish samples by HPLC/DAD, *J Environ Anal Toxicol*. 2, 137-140 (2012). DOI:10.4172/2161-0525.1000137.
- [21] G. Li, S. Jiao, X. Yin, Y. Deng, X. Pang, Y. Wang, The risk of melamine-induced nephrolothiasis in young children starts at a lower intake level than recommended by the WHO, *Pediatric Nephrology*. 25, 135-141 (2010). DOI: [10.1007/s00467-009-1298-3](https://doi.org/10.1007/s00467-009-1298-3)
- [22] H. Zhu, K. Kannan, Melamine and cyanuric acid in foodstuffs from the United States and their implications for human exposure. *Environment International* 130, 104950 (2019). <https://doi.org/10.1016/j.envint.2019.104950>
- [23] S. Tawfik, D. Saied, O. Mostafa, M. Salem, E. Habib, Formula feeding and associated factors among a group of Egyptian mothers, *Open Access Macedonian Journal of Medical Sciences*. 7, 1854-1859 (2019). DOI: [10.3889/oamjms.2019.462](https://doi.org/10.3889/oamjms.2019.462)
- [24] N.M. Hafiz, M.F. Saad, M.H. Hanafy, E.F. Abdel-Latif, Detection of *Mycobacterium avium subsp. paratuberculosis* in raw buffalo's milk, *International Journal of ChemTech Research*. 9, 123- 128 (2016).
- [25] A.S. Ibrahim, M.F. Saad, N.M. Hafiz, Toxic elements in dried milk and evaluation of their dietary intake in infant formula, *Int J Vet Sci*. 9, 563-567 (2020). DOI: 10.37422/IJVS/20.070.
- [26] N.H. Youssif, N.M. Hafiz, M.A. Halawa, M.F. Saad, Association of selected risk factors with bovine subclinical mastitis, *Acta Veterinaria Brasilica*. 15, 153-160 (2021). <https://doi.org/10.21708/avb.2021.15.2.9785>
- [27] A.S. Ibrahim, M.F. Saad, N.M. Hafiz, Safety and quality aspects of whole and skimmed milk powders, *Acta Scientiarum Polonorum Technologia Alimentaria*. 20, 165–177 (2021). <https://doi.org/10.17306/J.AFS.2021.0874>