Korean Journal for Food Science of Animal Resources

pISSN 1225-8563
eISSN 2234-246X

Korean J. Food Sci. An. 37(1): 79~86 (2017)
DOI https://doi.org/10.5851/kosfa.2017.37.1.79

ARTICLE

Determination of Aflatoxin M_1 and Heavy Metals in Infant Formula Milk Brands Available in Pakistani Markets

Saeed Akhtar[1], Muhammad Arif Shahzad[1], Sang-Ho Yoo[2], Amir Ismail[1], Aneela Hameed[1], Tariq Ismail[1], and Muhammad Riaz[1,2]*

[1]Institute of Food Science and Nutrition, Bahauddin Zakariya University, Multan-Pakistan
[2]Department of Food Science and Biotechnology, College of Life Sciences, Sejong University, Seoul 143-747, Korea

Abstract

Aflatoxin M_1 (AFM_1) after its bioconversion from aflatoxin B_1 in animal liver becomes the part of milk while heavy metals get entry into milk and milk products during handling in the supply chain. Aflatoxin M_1 and heavy metals being toxic compounds are needed to be monitored continuously to avoid any ailments among consumers of foods contaminated with such toxicants. Thirteen commercially available infant formula milk (IFM) brands available in Pakistani markets were analyzed for the quantitative determination of AFM_1 and heavy metals through ELISA and atomic absorption spectrophotometer, respectively. AFM_1 was found positive in 53.84% samples while 30.76% samples were found exceeding the maximum EU limit i.e. 0.025 µg/kg for AFM_1 in IFM. Heavy metals lead (Pb) and cadmium (Cd) were found below the detection limits in any of the sample, whereas the concentrations of iron (Fe), zinc (Zn) and nickel (Ni) ranged between 45.40-97.10, 29.72-113.50 and <0.001-50.90 µg/kg, respectively. The concentration of Fe in all the tested brands was found in normal ranges while the concentrations of Zn and Ni were found exceeding the standard norms. Elevated levels of AFM_1, Zn and Ni in some of the tested IFM brands indicated that a diet completely based on these IFM brands might pose sever health implications in the most vulnerable community i.e., infants.

Keywords aflatoxin M_1, heavy metals, permissible, milk

Received November 23, 2016
Revised December 13, 2016
Accepted December 25, 2016

Corresponding author
Muhammad Riaz
Institute of Food Science and Nutrition, Bahauddin Zakariya University, Multan-Pakistan
Tel: +82-10-4584-5564
+92-3067905770
E-mail: muhammad.riaz@sejong.ac.cr; riaz@bzu.edu.pk

Introduction

Mother milk being a rich source of all the basic nutrients is the only food for infants during the early few months of their lives. Infants up to 6 mon of age undergo some vulnerable changes in growth and development. Infants require mother milk for at least 2 years as it helps establish healthy immune system, nervous system, digestive system, reproductive system and strong physical structure of body (Kazi *et al.,* 2010). World Health Organization (WHO) recommends breast-feeding as best and sole source of infant feeding (WHO, 2009). However, rapid urbanization and advanced life style of recent days has led to concentrate on ready-made foods for infants in the form of infant formula milk (IFM). IFM has become popular all around the globe. If mother faces health issues or an infant denies mother's milk, the usage of infant formula becomes right choice as an alternative to breast feeding.

A number of food commodities available in the markets is specifically designed and manufactured for infants. Care is always taken to protect infant foods from any of the harmful compounds due to the fact that these food items are consumed by one of the most vulnerable age groups of people i.e., infants. Mycotoxin contamination in food chain is considered as one of the brazen food safety issues worldwide (Bouaziz *et al.,* 2013; Gao *et al.,* 2016; Li *et al.,* 2014; Pattono *et al.,* 2011; Tatay *et al.,* 2014). However, it is still reported that these infant formulas include a number of toxic compounds, such as aflatoxins (Torović, 2015), heavy metals (Fernandes *et al.*, 2015), melamine (Meng *et al.,* 2015; Yang *et al.,* 2016), residues of hormones (Barreiro *et al.,* 2015), nitrates (Chamandust *et al.,* 2016), antibiotics (Díaz-Bao *et al.,* 2015) and pesticide residues (Bessaire *et al.,* 2015; Sharma *et al.,* 2016).

Aflatoxins are the secondary metabolites of fungus including *Aspergillus niger, A. flavus, A. paraciticus and A. nominous.* Aflatoxins are reported to cause 0.025-0.15 million cases of hepatocellular carcinoma each year (Liu and Wu, 2010). There are different types of aflatoxins and their toxicities ranked in decreasing order as follows; aflatoxin B1 (AFB1) > aflatoxin B2 (AFB2) > aflatoxin G1 (AFG1) > aflatoxin G2 (AFG2), respectively. AFB1 if ingested by the animals is converted into aflatoxin M1 (AFM1) inside the liver it appears in the milk of dairy animals and is also reported in human milk. AFM1, although ten times less carcinogenic as compared to AFB1, it is still categorized as probable human carcinogen (group 2B) by the International Agency for Research on Cancer (IARC, 2002). The permissible limit for AFM_1 in animal milk (0.05 μg/L) set by European Commission (2006) is 40 times stricter than the total aflatoxins limit in food and feed items (20 μg/L) while more stringent permissible limits are adopted for AFM_1 in IFM i.e., 0.025 μg/L. Infants health may also be adversely affected due to the prolonged feeding on aflatoxin M_1 contaminated food i.e., milk, infant formulas and related products (Ismail *et al.,* 2016; Kunter *et al.,* 2016). The elevated levels of aflatoxin M_1 may also result in hepatocellular carcinoma, immunosuppression as well as teratogenic and mutagenic effects including impairment of kidney and liver (Afum *et al.,* 2016; Clarke *et al.,* 2015; Giolo *et al.,* 2012; Zheng *et al.,* 2013).

From last few decades, heavy metals appeared in different food commodities as a matter of serious concern and threat for human health. The penetration of heavy metals in food chain is a result of escalating industrialization and agricultural processes throughout the world. Heavy metals are the elements having densities higher than 5 g/cm^3 and among the most toxic ones are Arsenic (As), Pb, Mercury (Hg) and Cd. Although, Zinc (Zn) and Iron (Fe) are among the essential micronutrients having wide range of biochemical functions in human body, these elements may cause toxicity when consumed in higher concentrations. Heavy metals may enter human body by inhalation and ingestion (Tripathi *et al.,* 1999). The intake of metals in infants mainly depends on the bioavailability of metal content in milk and milk based foods (Kazi *et al.,* 2009). A number of studies have reported the prevalence of heavy metals in milk and milk products (Ismail *et al.,* 2015; Najarnezhad *et al.,* 2015; Pilarczyk *et al.,* 2013; Ping *et al.,* 2012; Suturović *et al.,* 2014). The induction of heavy metal in food chain is due to increased industrialization or municipal waste water; the elements may also find route in food commodities through processing, packaging and other unit operations in food industry (Ljung *et al.,* 2011; Muchuweti *et al.,* 2006; Saracoglu *et al.,* 2007). Several health disorders including cells, tissues and skeletal damage, failure of lungs and kidneys, cancer of lungs and blood, and osteoporosis and anemia are associated with heavy metals intake (Ashraf and Mian, 2008; Ikem and Egiebor, 2005; Ismail *et al.,* 2014; Rebelo and Coldas, 2016).

The envisaged project was designed to investigate the concentrations of two major chemical toxicants i.e., AFM_1 and metals (Pb, Cd, Fe, Zn and Ni) in thirteen IFM brands available in Pakistani markets.

Materials and Method

The analysis for AFM_1 and heavy metals in IFM samples were performed in the Food Analysis Laboratory of Institute of Food Science & Nutrition, Bahauddin Zakariya University, Multan-Pakistan.

Collection of samples

IFM of thirteen different brands used for infants aged 1-6 mon were purchased from pharmaceutical stores and local markets. Ten samples of each IFM brand were collected and the analyses were performed in triplicate. The samples were stored in refrigerator until analyzed.

Analysis of AFM_1 in IFM

Sample preparation

Powdered IFM (1 g) was taken and mixed with 10 mL of distilled or deionized water. Samples were defatted through centrifugation (Germany) for 10 min at 2000×*g*. The upper layer of fat was removed carefully by using a spatula. Defatted milk samples were used for the determination of AFM_1 through ELISA kits.

ELISA assay protocol

ELISA kits were purchased from Euro Proxima, (catalogue No. 5121AFM_1, Netherlands) and the analysis for AFM_1 was performed according to the guidelines provided in the kit manual. Defatted samples, standard solutions and blanks (100 µL each) were added to their respective wells and were incubated in dark for a period of 1 hr. Specific antibody binding sites in each well bounded free AFM_1 available in samples or standards. After incubation the remaining solution in each well was discarded and the wells were washed three times with rinsing buffer. Now, conjugate solution (AFM_1-horseradish peroxidase) was added to each well in the quantity of 100 µL. The plates were again incubated in dark at room temperature for a period of 30 min. The remaining solution was discarded and the microtiter plate wells were again washed three times with rinsing buffer. After drying, 100 µL of substrate solution was added in each well and the plate was incubated at room temperature. After 30 min, 100 µL of stop solution was added to each well. The absorbance of each well at 450 nm was read through ELISA reader (Bio-Tek ELx800, Indonesia). The limit of detection (LOD) for AFM_1 was 0.006 ng/mL. The concentration of AFM_1 in each sample was calculated through standard calibration curve.

Quality control

For validation of results, a standard solution of AFM_1 was purchased from Sigma Aldrich Chemicals (A6428). AFM_1 free milk samples were spiked with standard solution of AFM_1 at the concentrations of 0.01, 0.05, 0.1 and 0.2 µg/L. AFM_1 recovery percentages were recorded in the range of 96.3-98.4% (Table 1).

Heavy Metals Assessment in IFM Brands

Apparatus and chemicals

All the chemicals and reagents used were of analytical grade purchased from Merck Chemicals, USA. Hot plate (Lab Tec; EH 35A plus) was used for digestion of the samples. Flame atomic absorption spectrophotometer (Thermo Scientific; iCE-3000 series) was used for the analysis of heavy metals. The standards for heavy metals were purchased from CPA chemicals limited. Samples and standards were diluted with double distilled water to make final volumes.

Quantification of heavy metals

Metals included in current study were Pb, Cd, Fe, Zn and Ni. For the estimation of heavy metals in IFM samples, wet digestion method of Weldegebriel *et al*. (2012) was adopted. Briefly, 0.5 g sample was weighed by using Digital Weighing Balance (Precisa XB 120A) followed by addition of 10 mL of nitric acid (HNO_3) and 5 mL perchloric acid ($HClO_4$), and kept at room temperature for one night. Next day, the sample was heated on a hot plate until the volume of solution dropped down to 2-3 mL and the color became transparent. The sample was diluted with double distilled water up to convenient volumes and filtered through Whatman's filter paper No. 42. Finally, the samples and blanks were loaded on flame atomic absorp-

Table 1. ELISA method efficiency verification by spiking various levels of AFM_1 in milk

Spiked AFM_1 (pg/L)	Observed value (pg/L)	Recovery (%)	Coefficient of variance (%)
10	9.63	96.3	0.24
50	48.4	96.8	0.53
100	98.1	98.1	0.94
200	196.8	98.4	0.64

Table 2. Operating parameters for flame atomic absorption spectrophotometer for determination of heavy metals in infant formula milk brands

Metal	Wavelength (nm)	Flow rate (l/min)	Band pass (nm)
Lead	217	0.8-1.1	0.45
Cadmium	227.8	0.9-1.4	0.48
Iron	248	0.9-1.1	0.2
Zinc	214	1-1.3	0.2
Nikel	231.8	0.9-1.1	0.2

tion spectrophotometer for quantification of the metals. A mixture of air and analytical grade acetylene was used for burning of flame. The limits of detection (LOD) for various elements were calculated according to the method of Ismail *et al.* (2015). The LODs for Fe, Zn, Ni, Pb and Cd were 0.01, 0.03, 0.001, 0.004 and 0.002 mg/kg, respectively. The operating parameters of flame atomic absorption spectrophotometer are presented in Table 2.

Statistical analysis

Statistical evaluation of data obtained from each parameter was done through Statistix 8.1 software (Statistix Inc., USA). For comparison purpose, the data were subjected to one-way analysis of variance (ANOVA) followed by LSD (least significant difference) test. The differences were considered statistically significant at the probability level of $p<0.05$. Mean values and the measurement uncertainty (standard deviations) were computed through Microsoft Excel 2013.

Results and Discussion

Aflatoxin M_1 in different IFM brands

The results of mean AFM_1 level in different brands of IFM (n=13) are presented in Table 3. Statistical analysis revealed significant differences in the concentration of AFM_1 in various brands of IFM ($p<0.05$). AFM_1 was found positive in seven brands (53.84%) while the range of AFM_1 in all the tested brands was <0.006-0.108 μg/kg. European Union permissible limit for AFM_1 in IFM samples is 0.025 μg/kg (European Commission, 2010). The current study revealed that 30.76% of the samples of IFM brands were found exceeding the maximum permissible limits set by European Commission.

The presence of AFM_1 beyond permissible limit in such a high percentage of IFM samples is a serious health issue particularly for infants who rely on this food during very early stage of their life with developing immunity. Higher levels of AFM_1 in animal milk samples from Pakistan are also reported by Ismail *et al.* (2016), according to which 93% out of a total of 520 milk samples were found positive for $AFM_{1,}$ while 53% samples were reported exceeding the EU maximum limit for AFM_1 in milk (0.05 μg/L). Kanungo and Bhand (2015) reported the level of AFM_1 in IFM samples from India and found that all samples had AFM_1 above the maximum EU limits. The range of AFM_1 in IFM samples was 0.501-0.713 μg/kg. The level of AFM_1 in IFM samples from India is much higher as compared to our findings. In a study conducted in Spain by Beltran *et al*. (2011), AFM_1 level was measured in 14 baby food samples, 7% of which were found positive for AFM_1 while the reported mean concentration for AFM_1 was 0.006 μg/kg, however none of the samples was found exceeding the maximum EU limit. Meucci *et al*. (2010) measured the level of AFM_1 in 185 infant milk samples in Italy and found only 1% samples positive for AFM_1 with a range of 0.0118-0.0153 μg/kg. Alvito *et al*. (2010) analyzed 27 baby food samples for the quantification of AFM_1 in Portugal, 7.4% of which were found positive for AFM_1 having a range of 0.017-0.041 μg/kg. The reported values of AFM_1 from Spain, Italy and Portugal showed less level of AFM_1 as compared to our results indicating a better control of aflatoxins in these countries as compared to Pakistan. The availability of IFM brands contaminated with AFM_1 beyond set standards might be associated with favorable environment for the growth of fungus responsible for the production of aflatoxins, lack of surveillance system and lack of control of law enforcement agencies on manufactures and suppliers.

Table 3. Concentration of AFM_1 (μg/kg) in infant formula milk samples of various brands

Sr. No.	Brand Codes	AFM_1 Concentration
1	Brand-A	0.04 ±0.002 [c]
2	Brand-B	0.108 ±0.006 [a]
3	Brand-C	0.056 ±0.003 [b]
4	Brand-D	<0.006 [e]
5	Brand-E	<0.006 [e]
6	Brand-F	0.032±0.002 [d]
7	Brand-G	0.0062±0.002 [e]
8	Brand-H	<0.006 [e]
9	Brand-I	0.0093±0.001[e]
10	Brand-J	0.0092±0.001 [e]
11	Brand-K	<0.006 [e]
12	Brand-L	<0.006 [e]
13	Brand-M	<0.006 [e]

Determination of heavy metals in IFM brands

The results of minerals elements in IFM samples of various brands are presented in Table 4. Statistical analysis showed significant differences in the concentration of mineral elements among various brands however the differences were non-significant for Pb and Cd ($p<0.05$). Mineral elements concentrations in various brands were found in the order of Fe > Zn > Ni. Heavy metals like Pb and Cd were found below detection limits in all of the tested brands, indicating the adoption of good manufac-

Table 4. Concentration of heavy metals and mineral elements (mg/kg) in IFM samples of different brands

Brand codes	Fe	Zn	Ni	Pb	Cd
Brand-A	51.39±0.2^{i}	41.05±0.3^{h}	50.90±0.4^{a}	<0.0004	<0.0002
Brand-B	56.45±0.3^{e}	37.22±0.2^{k}	17.58±0.1^{d}	<0.0004	<0.0002
Brand-C	92.07±0.5^{b}	35.23 ±0.2^{l}	19.35±0.2^{b}	<0.0004	<0.0002
Brand-D	50.65±0.3^{j}	52.33 ±0.4^{c}	18.29±0.2^{c}	<0.0004	<0.0002
Brand-E	62.08±0.4^{c}	50.31±0.4^{d}	<0.001 ^{f}	<0.0004	<0.0002
Brand-F	46.85±0.2^{k}	48.19±0.4^{e}	0.12±0.01 ^{f}	<0.0004	<0.0002
Brand-G	51.59±0.2^{i}	40.17±0.3^{i}	<0.001 ^{f}	<0.0004	<0.0002
Brand-H	97.11±0.6^{a}	62.44 ±0.5^{b}	17.55±0.1^{d}	<0.0004	<0.0002
Brand-I	54.50±0.3^{g}	37.93±0.2^{j}	<0.001 ^{f}	<0.0004	<0.0002
Brand-J	54.09±0.3^{h}	113.50±0.7^{a}	0.18±0.1^{f}	<0.0004	<0.0002
Brand-K	55.37±0.3^{f}	29.72±0.1^{m}	<0.001^{f}	<0.0004^{NS}	<0.0002^{NS}
Brand-L	57.21±0.4^{d}	47.07 ±0.3^{f}	17.20±0.1^{e}	<0.0004	<0.0002
Brand-M	45.40±0.2^{l}	44.14±0.3^{g}	<0.001 ^{f}	<0.0004	<0.0002

turing practices by the IFM manufacturers to control these highly toxic heavy metals.

Fe is an essential element for the normal growth and development of human body. The results of current study showed the presence of iron in all the IFM brands ranged from 45.40-97.10 mg/kg. The statistical analysis showed significant differences in the level of iron among various brands of IFM (Table 4). The level of iron found in our study showed 5-10% (±) variations as compared to the labeled values. Lesniewicz *et al*. (2010) quantified the concentration of Fe in 12 different types of IFM brands available in the markets of Poland The mean level of Fe in different brands ranged 35-74 mg/kg which is almost in agreement with our findings. Pandelova *et al*. (2012) measured the level of Fe in baby milk samples available in the markets of Germany and found mean Fe value as 47.7 mg/kg that is almost in line with our study. These results showed that the concentration of Fe found in current study was in normal range.

Zn is a minor inorganic element necessary for the growth and development of infants. It is believed to be involved in cellular metabolism. Zn is also needed for the synthesis of DNA, division of cells and for catalytic activity of more than 100 enzymes (Beigi and Maverakis, 2015; Tariba *et al.,* 2016). The results of present study revealed that the concentration of Zn in different infant formula samples ranged between 29.72-113.50 mg/kg (Table 4). A difference of about 2-3% was observed among the calculated and labeled values. According to Polish standards the Zn content in IFM samples must not exceed 55 mg/kg (PN-A-94015). Comparing this limit with our results the IFM of two brands were found exceeding the permissible limit. Level of Zn reported from Poland in 12 different brands of IFM samples ranged between 16-56 mg/kg and these results are lower as compared to our findings. The level of Zn reported by Melø *et al*. (2008) in IFM samples available in Norway markets was in the range of 35-39 mg/kg and these results are in line with our findings.

Ni is one of the metals which pose severe complications especially in the new born babies and infants, if consumed in higher concentrations. The results of present study showed the presence of Ni in some of the IFM samples. The levels of Ni in various infant brands showed huge variations and the mean values ranged between <0.001-50.903 mg/kg. Some of the IFM samples showed Ni level below the detection limit (<0.001 mg/kg). The statistical analysis showed significant differences in the level of Ni among tested IFM brands. Concentration of Ni in IFM samples is reported by a few researchers. Pandelova *et al*. (2012) reported Ni level below detection limit in all the tested IFM samples available in the markets of Germany while Odhiambo *et al*. (2015) reported 0.022-0.032 mg/kg Ni in the IFM samples available in the markets of Nigeria. The results of these studies are although in line with our study but the higher levels of Ni in some of our tested samples indicated the chances of Ni toxicity in infants.

Conclusion

The results of current study indicated that the concentrations of toxic metals Pb and Cd in IFM samples were detected within safe limits. The level of Fe was also found in normal ranges but the levels of Zn and Ni in some of the IFM brands were found above the normal ranges. The analysis of AFM_1 in IFM samples revealed that 30.76% infant formula samples exceeded the EU maximum permissible limit which might result in severe toxicity in infants being immunity compromised group of age. The elevated levels of AFM_1, Zn and Ni in some of the IFM brands demand surveillance and implementation of regulations to avoid any severe and irrecoverable health implications as a result of bioaccumulation of these toxic compounds in infants.

Acknowledgements

Financial supports from The Higher Education Commission Islamabad-Pakistan under the project No. 20-1932 titled “safety status of street vended raw milk in Southern Punjab” and Sejong University Seoul-Korea under the project titled, “Microbial Decontamination of Aflatoxin M_1 in Bovine Milk” are highly recognized and appreciated.

References

1. Afum, C., Cudjoe, L., Hills, J., Hunt, R., Padilla, L. A., Elmore, S., Afriyie, A., Opare-Sem, O., Phillips, T., and Jolly, P. E. (2016) Association between aflatoxin M1 and liver disease in HBV/HCV infected persons in Ghana. *Int. J. Environ. Res. Publ. Health*. **13**, 377.
2. Alvito, P. C., Sizoo, E. A., Almeida, C. M. M., and Egmond, H. P. V. (2010) Occurrence of aflatoxins and ochratoxin A in baby foods in Portugal. *Food Anal. Methods* **3**, 22-30.
3. Ashraf, W. and Mian, A. A. (2008) Levels of selected heavy metals in black tea varieties consumed in Saudi Arabia. *Bull. Environ. Conta. Toxicol*. **81**, 101-104.
4. Barreiro, R., Regal, P., Díaz-Bao, M., Fente, C. A., and Cepeda, A. (2015) Analysis of naturally occurring steroid hormones in infant formulas by HPLC-MS/MS and contribution to dietary intake. *Foods* **4,** 605-621.
5. Beigi, P. K. M. and Maverakis, E. (2015) Role of Zinc in different body systems. In Acrodermatitis Enteropathica. *Springer*, 61-75.
6. Beltran, E., Ibanez, M., Sancho, J. V., Cortes, M. A., Yusa, V., and Hernandez, F. (2011) UHPLC-MS/MS highly sensitive determination of aflatoxins, the aflatoxin metabolite M1 and ochratoxin A in baby food and milk. *Food Chem*. **126**, 737-744.
7. Bessaire, T., Tarres, A., Goyon, A., Mottier, P., Dubois, M., Tan, W. P., and Delatour, T. (2015) Quantitative determination of sodium monofluoroacetate “1080” in infant formulas and dairy products by isotope dilution LC-MS/MS. *Food Addit. Contam. A*. **32**, 1885-1892.
8. Bouaziz, C., Bouslimi, A., Kadri, R., Zaied, C., Bacha, H., and Abid-Essefi, S. (2013) The *in vitro* effects of zearalenone and T-2 toxins on Vero cells. *Exp. Toxicol. Pathol*. **65**, 497-501.
9. Chamandust, S., Mehrasebi, M. R., Kamali, K., Solgi, R., Taran, J., Nazari, F., and Hosseini, M. J. (2016) Simultaneous determination of nitrite and nitrate in milk samples by ion chromatography method and estimation of dietary intake. *Int. J. Food Prop*. **9**, 1-11.
10. Clarke, R., Connolly, L., Frizzell, C., and Elliott, C. T. (2015) Challenging conventional risk assessment with respect to human exposure to multiple food contaminants in food: A case study using maize. *Toxicol. Lett*. **238**, 54-64.
11. Díaz-Bao, M., Barreiro, R., Miranda, J. M., Cepeda, A., and Regal, P. (2015) Fast HPLC-MS/MS method for determining penicillin antibiotics in infant formulas using molecularly imprinted solid-phase extraction. *J. Anal. Methods Chem*. **2015**, 1-8.
12. European Commission. (2006) Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur Union*. **364**, 5-24.
13. European Commission. (2010) Commission regulation EC No. 165/2010 of 26 February 2010 amending regulation (EC) No 1881/2006 setting the maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Off. J. Eur. Union.* 8-11.
14. Fernandes, T. A., Brito, J. A., and Gonçalves, L. M. (2015) Analysis of micronutrients and heavy metals in Portuguese infant milk powders by wavelength dispersive X-ray fluorescence spectrometry (WDXRF). *Food Anal. Methods* **8**, 52-57.
15. Gao, Y. N., Wang, J. Q., Li, S. L., Zhang, Y. D., and Zheng, N. (2016) Aflatoxin M1 cytotoxicity against human intestinal Caco-2 cells is enhanced in the presence of other mycotoxins. *Food Chem. Toxicol*. **96**, 79-89.
16. Giolo, M. P., Oliveira, C. M. D., Bertolini, D. A., Lonardoni, M. V. C., Gouveia, M. S., Netto, D. P., Nixdorf, S. L., and Junior, M. M. (2012) Aflatoxin M1 in the urine of non-carriers and chronic carriers of hepatitis B virus in Maringa, Brazil. *Braz. J. Pharm. Sci*. **48**, 447-452.
17. Ikem, A. and Egiebor, N. O. (2005) Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) marketed in Georgia and Alabama (United States of America). *J. Food Comp. Anal*. **18**, 771-787.
18. Ismail, A., Akhtar, S., Levin, R. E., Ismail, T., Riaz, M., and Amir, M. (2016) Aflatoxin M1: Prevalence and decontamination strategies in milk and milk products. *Crit. Rev. Micro*. **42**, 418-427.
19. Ismail, A., Riaz, M., Akhtar, S., Ismail, T., Ahmad, Z., and Hashmi, M. S. (2015) Estimated daily intake and health risk of heavy metals by consumption of milk. *Food Addit. Contam. B*. **8**, 260-265.
20. Ismail, A., Riaz, M., Akhtar, S., Ismail, T., Amir, M., and Zafar-ul-Hye, M. (2014) Heavy metals in vegetables and respective soils irrigated by canal, municipal waste and tube well waters. *Food Addit. Contam B*. **7**, 213-219.
21. International Agency for Research on Cancer. (2002) Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. In: IARC monograph on the evaluation of car-

cinogenic risks to humans. Lyon. *World Health Organization* **82**, 1-556.

22. Kanungo, L. and Bhand, S. (2015) A survey of Aflatoxin M1 in some commercial milk samples and IFM samples in Goa, India. *Food Agri. Immuno*. **25**, 467-476.
23. Kazi, T. G., Jalbani, N., Baig, J. A., Afridi, H. I., Kandhro, G. A., Arain, M. B., Jamali, M. K., and Shah, A. Q. (2009) Determination of toxic elements in infant formula by using electrothermal atomic absorption spectrometer. *Food Chem. Toxicol*. **47**, 1425-1429.
24. Kazi, T. G., Jalbani, N., Baig, J. A., Arain, M. B., Afridi, H. I., Jamali, M. K., Shah, A. Q., and Memon, A. N. (2010) Evaluation of toxic elements in baby foods commercially available in Pakistan. *Food Chem*. **119**, 1313-1317.
25. Kunter, İ., Hürer, N., Gülcan, H. O., Öztürk, B., Doğan, İ., and Şahin, G. (2016) Assessment of aflatoxin M1 and heavy metal levels in mothers breast milk in Famagusta, Cyprus. *Biol. Trace Elem. Res*. **175**, 42-49.
26. Lesniewicz, A., Wroz, A., Wojcik, A., and Zyrnicki, W. (2010) Mineral and nutritional analysis of Polish infant formulas. *J. Food Comp. Anal.* **23**, 424-431.
27. Li, Y., Zhang, B., He, X., Cheng, WH., Xu, W., Luo, Y., Liang, R., Luo, H., and Huang, K. (2014) Analysis of individual and combined effects of ochratoxin A and zearalenone on HepG2 and KK-1 cells with mathematical models. *Toxins*. **6**, 1177-1192.
28. Liu, Y. and Wu, F. (2010) Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. *Environ. Health Persp*. **118**, 818.
29. Melø, R., Gellein, K., Evjea, L., and Syversen, T. (2008) Minerals and trace elements in commercial infant food. *Food Chem. Toxicol.* **46**, 3339-3342.
30. Meng, Z., Shi, Z., Liang, S., Dong, X., Lv, Y., and Sun, H. (2015) Rapid screening and quantification of cyromazine, melamine, ammelide, ammeline, cyanuric acid, and dicyandiamide in infant formula by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry and triple quadrupole mass spectrometry. *Food Control* **55**, 158-165.
31. Meucci, V., Razzuoli, E., Soldani, G., and Massart, F. (2010) Mycotoxin detection in IFMs in Italy. *Food Addit. Contam*. **27**, 64-71.
32. Muchuweti, M., Birkett, J. W., Chinyanga, E., Zvauya, R., Scrimshaw, M. D., and Lester, J. N. (2006) Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: Implications for human health. *Agri. Ecosystems Environ.* **112**, 41-48.
33. Najarnezhad, V., Jalilzadeh-Amin, G., Anassori, E., and Zeinali, V. (2015) Lead and cadmium in raw buffalo, cow and ewe milk from west Azerbaijan, Iran. *Food Addit. Contam. B.* **8**, 123-127.
34. Ljung, K., Palma, B., Grander, M., and Vahter, M. (2011) High concentrations of essential and toxic elements in infant formula foods- a matter of concern. *Food Chem*. **40**, 1-9.
35. Odhiambo, V. O., Wanjau, R., Odundo, J. O., and Nawiri, M. P. (2015) Toxic trace elements in different brands of milk infant formula in Nairobi market, Kenya. *Afr. J. Food Sci.* **9**, 437-440.
36. Pandelova, M., Lopez, W. L., Michalke, B., and Schramm, K. W. (2012) Ca, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn contents in baby foods from the EU market: Comparison of assessed infant intakes with the present safety limits for minerals and trace elements. *J. Food Comp. Anal*. **27**, 120-127.
37. Pattono, D., Gallo, P. F., and Civera, T. (2011) Detection and quantification of Ochratoxin A in milk produced in organic farms. *Food Chem*. **127**, 374-377.
38. Pilarczyk, R., Wójcik, J., Czerniak, P., Sablik, P., Pilarczyk, B., and Tomza-Marciniak, A. (2013) Concentrations of toxic heavy metals and trace elements in raw milk of Simmental and Holstein-Friesian cows from organic farm. *Environ. Monit. Asses*. **185**, 8383-8392.
39. Ping, J., Wu, J., and Ying, Y. (2012) Determination of trace heavy metals in milk using an ionic liquid and bismuth oxide nanoparticles modified carbon paste electrode. *Chin. Sci. Bull*. **57**, 1781-1787.
40. Polish standard: PN-A-94015. Infant formula and follow-on formula.
41. Saracoglu, S., Saygi, K., Ozgur, O., Uluozlu, D., Tuzen, M., and Soylak, M. (2007) Determination of trace element contents of baby foods from Turkey. *Food Chem*. **105**, 280-285.
42. Sharma, N. D., Sharma, I. D., Chandel, R. S., and Wise, J. C. (2016) Presence of pesticides in breast milk and infants' formula in Himachal Pradesh, India. *Int. J. Environ. Anal. Chem.* **96**, 225-236.
43. Suturović, Z., Kravić, S., Milanović, S., Đurović, A., and Brezo, T. (2014) Determination of heavy metals in milk and fermented milk products by potentiometric stripping analysis with constant inverse current in the analytical step. *Food Chem*. **155**, 120-125.
44. Rebelo, F. M. and Caldas, E. D. (2016) Arsenic, lead, mercury and cadmium: Toxicity, levels in breast milk and the risks for breastfed infants. *Environ. Res*. **151**, 671-688.
45. Tariba, B., Živković, T., Gajski, G., Gerić, M., Gluščić, V., Garaj-Vrhovac, V., and Pizent, A. (2016) *In vitro* effects of simultaneous exposure to platinum and cadmium on the activity of antioxidant enzymes and DNA damage and potential protective effects of selenium and zinc. *Drug Chem. Toxicol*. DOI: 10.1080/01480545.2016.1199564
46. Tatay, E., Meca, G., Font, G., and Ruiz, M. J. (2014) Interactive effects of zearalenone and its metabolites on cytotoxicity and metabolization in ovarian CHO-K1 cells. *Toxicol. Vitro.* **28**, 95-103.
47. Torović, L. (2015) Aflatoxin M1 in processed milk and infant formula and corresponding exposure of adult population in Serbia in 2013-2014. *Food Addit. Contam. B*. **8**, 235-244.
48. Tripathi, R. M., Raghunath, R., Sastry, V. N., and Krishnamoorthy, T. M. (1999) Daily intake of heavy metals by infants through milk and milk products. *Sci. Total Environ*. **227**, 229-235.
49. World Health Organisation. (2009) Infant and young child fee-

ding. Model chapter for textbooks for medical students and allied health professionals. Geneva, Switzerland: WHO Press, World Health Organisation.

50. Yang, X., Jia, Z., Tan, Z., Xu, H., Luo, N., and Liao, X. (2016) Determination of melamine in infant formulas by fluorescence quenching based on the functionalized Au nanoclusters. *Food Control* **70**, 286-292.

51. Zheng, N., Sun, P., Wang, J. Q., Zhen, Y. P., Han, R. W., and Xu, X. M. (2013) Occurrence of aflatoxin M1 in UHT milk and pasteurized milk in China market. *Food Control* **29**, 198-201.