Food Chemistry 119 (2010) 1313–1317

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Evaluation of toxic elements in baby foods commercially available in Pakistan

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ARTICLE INFO

Article history:
Received 4 June 2009
Received in revised form 11 August 2009
Accepted 1 September 2009

Keywords:
Toxic element
Ultrasound energy
Baby food extraction
Atomic absorption spectrometry

ABSTRACT

In present work, the concentrations of toxic elements, aluminium (Al), cadmium (Cd), nickel (Ni) and lead (Pb) were measured in different solid baby foods (BFs), primarily to evaluate whether the intakes comply within permissible levels of these toxic elements (TEs). The BFs were evaluated for total contents of TEs, using a simple and fast ultrasound-assisted extraction (UAE) method. The accuracy of the proposed UAE method was ensured by using certified reference materials and results obtained by conventional wet acid digestion method on same CRM, at 95% confidence level. The range of the investigated TEs in different BFs were 4770–35,200, 25.6–88.3, 124–332 and 52.5–90.6 μg/kg for Al, Cd, Ni and Pb, respectively. The results indicated that BFs including rice cereals have high level of all four TEs. The daily intakes of TEs for children through BFs have also been estimated, and are well below the recommended tolerable levels.

1. Introduction

Feeding patterns during infancy are important for their growth and development, which may affect preferences and eating habits later in childhood (Briefel, Reidy, Karwe, & Devaney, 2004). Milk is the fundamental food for infants. The most natural and best source is from breast feeding and this is greatly encouraged for the first 6 months of life and should be continued for as long as 2 years. In certain situations, special diets are required due to different metabolic reasons or in view of increasing nutritional requirements, which are no longer met by breast milk alone. Accordingly, pediatric guidelines advocate the gradual replacement of exclusive milk feeding by complementary foods from the fifth month of life onwards (Kersting, Kaiser, & Schoch, 1995).

Infant feeding deserves top priority in any plan aimed at sound child healthcare, irrespective of ethnic, cultural and religious considerations. It is anticipated that appropriate infants and children feeding can prevent millions of deaths occurring from childish gastroenteritis and malnutrition in developing and developed countries (Oskarsson, Hallen, Sundberg, & Graw, 1998). Multi-element surveys of baby foods (BFs) and the need to establish limits for infant exposure to such elements from the diet have extensively studied (Saracoglu, Saygi, Uluozlu, Tuzen, & Soylak, 2007). The toxic elements (TEs) found throughout the environment and are present in virtually all food, and these contaminants are more likely to affect children (Ghaedi, Fathi, Marahel, & Ahmadi, 2005; Mendil, Tuzen, Yazici, & Soylak, 2005).

Many developmental problems in infants and children have been directly linked with exposure of TEs (American Academy of Pediatrics, 1996). The high levels of TEs in BFs may avoid the intestinal barrier, while due to renal immaturity of neonates and children (less than 12 months) impairs their elimination (Bougle, Bureau, Morello, Guillois, & Sabatier, 1997). The early postnatal period of infants is characterised by rapid growth and development, their energy requirements and hence their food consumption is on average much higher in relative to their body weight than that of older children and they are more vulnerable to TEs present in their atmosphere and food (Campbell, 2006). An excess of aluminium can inhibit several important enzymes activities and contribute to impairment of mental development and bone mineralisation (Fernandez-Lorenzo, Cocho, Rey Goldar, Couce, & Fraga, 1999). Lead in milk products and cereals is of particular concern because they are considered as first solid food for infants and children (Kazi et al., 2009), and the determination of lead level in baby feed is particularly monitored by international organisations (Codex Alimentarius Commission, 2003) The body of children absorbs a larger percentage of lead that they ingest, and exhibit lead toxicity even at lower levels of exposure (Ikeda et al., 2000). Cadmium intake via respiration of atmospheric air is minimal for

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0308-8146/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2009.09.003

the general population, and exposure is almost exclusively through ingestion of daily foods (Tsukahara et al., 2003). It was also made clear that, even in recent years, Cd in rice is the most influential factor of daily dietary Cd intake among the general population in world (Matusiewicz, 2003, chap. 6).

Determination of TEs in different environmental and biological samples requires the use of sensitive and selective techniques such as electrothermal atomic absorption Spectrophotometry, inductively coupled plasma-optical emission and mass spectrometry (Ghaedi, Ahmadi, & Shokrollahi, 2007; Shokrollahi, Ghaedi, Niband, & Rajabi, 2008).

These techniques require the destruction of sample matrix to render a solution of the analyte ready for analysis (Lamble & Hill, 1998). The determination of elements in food samples traditionally been performed by digestion with acid (or acid mixtures), which is time consuming and involves some potential drawbacks such as analyte losses and sample contamination. The microwave-assisted digestion is also an adequate determinative technique, but it requires intensive sample handling, a long time to cool the reactor before opening and has a high operational cost (Ashley, Andrews, Cavazos, & Demange, 2001). The use of ultrasonic energy can be considered as another alternative for sample pre-treatment, since ultrasound facilitates an auxiliary energy and accelerates some steps, such as dissolution, fusion and leaching, among others (Bendicho & de Loos-Vollebregt, 1991). Partial extraction of the analytes into the liquid phase, promoted by acids and ultrasonic agitation is particularly effective in promoting the extraction of elements (Jalbani et al., 2006).

The objective of this study was to determine the toxic elements (Al, Cd, Ni and Pb) in different baby solid foods, to evaluate the changes in the intake pattern of TEs when the children switches over from milk to a solid cereal-based diet. The TEs were determined by electrothermal atomic absorption spectrometry, after an efficient and rapid ultrasonic assisted extraction procedure. The different variables such as sonication time, acid concentration and centrifugation were optimised for proposed method, to achieve the complete extraction of understudy TEs. The analytical results obtained by proposed extraction method were compared with those obtained by conventional wet acid digestion method on same certified and real samples. The daily intake of TEs by infants and children through different baby foods was also calculated for possible health risks.

2. Materials and methods

2.1. Reagents

Ultrapure water obtained from ELGA labwater system (Bucks, England), was used throughout the work. All the reagents and chemicals used were of analytical grade Merck (Darmstadt, Germany). Concentrated 65% HNO_3 and 30% H_2O_2 were spectroscopic grades (Merck). Standard solutions of Al, Cd, Ni and Pb were prepared by dilution of certified standard solutions (1000 μg ml^{-1}) obtained from Fluka (Bushs, Switzerland) of corresponding metal ions. Stock standard solution of chemical modifiers, $Mg(NO_3)_2$ (2.00 g L^{-1}), was prepared from $Mg(NO_3)_2$ (Merck) and Pd stock standard solution (3.00 g L^{-1}), was prepared from Pd 99.999%. Sigma (St. Loius, MO, USA). Accuracy was evaluated by certified reference material for trace metals, DORM-2 (Dogfish muscle), was purchased from the National Research Council Canada (Ottawa, Ontario, Canada). All materials were dried in an oven at 80 °C for 4 h before use. All glass wares and plastic materials used were previously treated for 24 h in 5 M suprapur nitric acid and rinsed with double distilled water then with ultrapure water (Jalbani et al., 2007).

2.2. Instrumentation

A Perkin–Elmer (Norwalk, CT, USA) atomic absorption spectrophotometer model AA-700, equipped with a graphite furnace (HGA-400) and an auto-sampler plus high intensity deuterium lamp for background correction was used. Pyrolytic graphite coated graphite tubes were used. The electrothermal parameters and thermal program are listed in (Table 1). The ultrasonic assisted extraction (UAE) was carried out with a Model No. SC-121TH (Sonicor, Copiague, NY). The following technical specifications were used for UAE: timer 0–30 min, volts 220, cycles 50/60 Hz, programmable for temperature ranging from 0 to 90 °C with intensification frequency of 35 kHz and a total volume of 4 L. Centrifugation was carried out using a WIROWKA Laboratoryjna type WE-1, nr-6933 centrifuge; speeds range 0–6000 rpm, timer 0–60 min (Mechanika Phecyzyjna, Poland). A Retsch (Haan, Germany) mixer mill MM 2000, equipped with 10 ml capacity agate cups and agate balls.

2.3. Sampling

On the basis of results of questionnaire from population of different cities of Pakistan (n = 500), BFs having different gradients, cereals, rice, wheat, fruit, vegetables, honey and powdered milk, were selected. A total of eight different brands of commercially available solid BFs, retailed in the Pakistan market over the period of (2005–2006) were purchased. Thirty-two samples of each baby solid food packed on different dates (four batches) were collected randomly. Four composites samples of each baby food (packed on different dates) were made. The all BFs were dried for 48 h in an oven at 60 °C to constant weight. The dried BFs were ground with agate ball mill and powder samples were passed through nylon sieve mesh size of <50 μm.

2.4. Ultrasonic assisted extraction method

Weighed 0.2–0.5 g of replicate eight samples of CRMs (DORM-2), while four samples of each collected BFs were weighed accurately in centrifuge tubes (25 ml in capacity) separately. The half of CRM and real samples were treated with 1 ml concentrated nitric acid (65%), kept for 5 min at room temperature and then added 9.0 ml of deionised water. whilst, other set of CRM and real samples were treated with 10 ml of 10% HNO_3 (v/v) directly. All the tubes were shaken energetically and the mixtures were subjected to an ultrasound water bath for different time intervals (1–10 min), in order to obtain homogeneous dispersions. The temperature of ultrasonic bath kept constant at 60 °C. The half tubes of each acid treated contents were subjected to centrifugation at 3000 rpm (3–15 min), the supernatant aliquots and other half sub-samples as suspension (without centrifugation) of each certified and real samples were transferred to auto-sampler cups, then subjected to electrothermal atomic absorption spectrometer with modifiers. For comparative purposes conventional wet acid digestion method was applied on same CRMs (Arain et al., 2008).

Table 1
Measurement conditions for electrothermal atomic absorption spectrometry.

Parameter	Al	Cd	Pb	Ni
Lamp current (mA)	10	7.5	7.5	7.5
Wave length (nm)	309	228.8	283.3	232.0
Slit-width (nm)	0.7	0.7	0.7	0.2
Cuvette	Tube	Cup	Cup	Cup
Common parameters				
Sample volume = 10 μL analyte + 10 μL corresponding modifiers				
Background correction: D2 lamp				
Carrier gas argon = 200 (mL/min)				

The $Pd(NO_3)_2$ was used as a chemical modifier for Pb determination, $Mg(NO_3)_2$ for Al and Ni, while a solution containing $Pd(NO_3)_2 + Mg(NO_3)_2$ was used for Cd determinations. Ten microlitres of extracted and digested solution of samples and 10 μL modifiers were simultaneously injected into the pyrocoated graphite tube of furnace, using auto-sampler AS-800. Aqueous calibration was a real possibility for Cd, Cr, Ni and Pb. Blank extractions were carried out in the same way.

2.5. Quality control

The linear range of the calibration curve reached from the detection limit up to 250, 10, 50 and 100 μg/L, for Al, Cd Pb, and Ni, respectively. The detection limit (LOD) was defined as $3s/m$, where s is the standard deviation corresponding to 10 blank injections and m is the slope of the calibration graph. The LODs of 1.2, 0.05, 0.2 and 0.55 μg/L were calculated for Al, Cd, Ni and Pb, respectively.

The accuracy of results was verified by analysing the concentration of Al, Cd, Ni and Pb in certified reference material, DORM-2 and with those obtained from wet acid digestion method on same CRM. The significance of the numerical deviations seen in Table 2, estimated by the statistical paired t-test, was also applied to compare the results of both methods. A $t_{experimental}$ values was calculated at degrees of freedom $n - 1 = 5$, the experimental values are lower than the $t_{critical}$ (2.57), at a confidence interval of 95% ($P = 0.05$), indicate non-significant difference in obtained values of TES by wet acid digestion method and UAE method, which may be applicable for the extraction of metals from different food samples.

3. Results and discussion

3.1. Optimisation of method

The proposed UEA procedure had three main variables (concentration of nitric acid, sonication time and centrifugation) which may potentially affect on % recoveries of TEs. The UAE method is effective for determining the toxic elements, Al, Cd, Ni and Pb in different solid BFs and has sufficient sensitivity, accuracy and reproducibility as shown in Table 2.

3.1.1. Effects of sonication intervals

The UAE rate curves with different sonication time intervals for recovery of TEs were compared in Fig. 1. Each value in line graph represents the mean of generally six replicates with standard deviation. For some elements in certified sample, the results were multiplied by a factor (indicated on the bar and line) in order to be plotted in the same graph. The integrated absorbance signal for each element was enhanced with increasing ultrasonic treatment time, up to 10 min. The optimum recoveries of Cd, Ni, Pb and Al were achieved at ultrasonic exposure intervals of 4, 6, 7 and 8 min, respectively. For longer sonication times, no significant

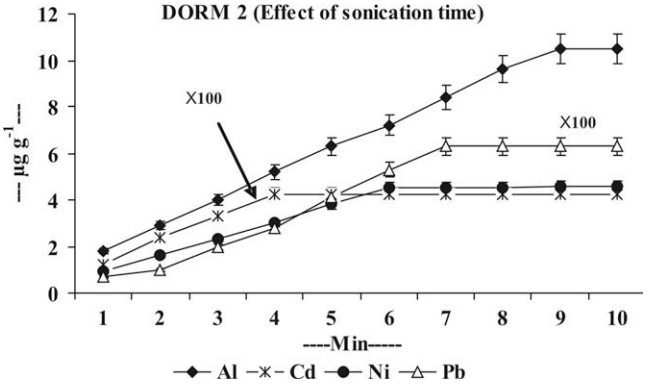

Fig. 1. Effects of sonication time on toxic elements recovery from certified samples (DORM-2).

effect on recoveries of understudy TEs was observed at 95% confidence limit ($P > 0.05$). A treatment time of 10 min in the ultrasonic bath was used for further experiments.

3.1.2. Effect of acid concentration

The concentrated HNO_3 (65%) and 10% HNO_3 (v/v) treatment of BFs may influence the precision and the extraction of the analyte to the aqueous phase. The pre-treatment of samples with concentrated HNO_3 prior to dilution enhance the % recoveries of TEs, ranging from 15% to 20% at optimum level of other variable (sonication and centrifugation), as compared to extracting directly with 10% acid (Fig. 2). The effects of different variables on the recoveries of TEs are consistent with literature reported studies (Jalbani et al., 2006).

3.1.3. Effect of centrifugation

The effect of the centrifugation time on the extraction efficiency was also studied within a range of 3–15 min. The advantages of centrifugation in relation to slurry sampling are the minimisation to build up of carbonaceous residues inside the graphite tube and lower background values because significantly less matrix is introduced in the former case (Davis & Grant, 1992), as well as increase life of graphite tube. It was observed that recovery of all TEs were enhanced 10–15% on centrifugation (Fig. 3).

The results obtained for all BFs having different composition are given in Table 3. The data revealed that in some baby food samples, the concentrations of Al, Cd, Ni and Pb were found to be in lower range (341–6520), (26.6–57.5), (124–213) and (52.5–64.8 μg/kg), while others showed higher levels of these elements, ranged as (13,500–17,800), (72.7–88.3), (246–332) and (78.94.8) μg/kg, respectively. This high quantity of TES in some BFs may due to the food additives or other ingredients, especially rice and vegetables. Children are more sensitive to toxic effects of Al, Cd, Ni and Pb because there are significant differences in absorption, distribu-

Table 2
Validation of ultrasound-assisted extraction (UAE) and conventional wet acid digestion (CAD) methods against certified reference materials ($n = 6$) (μg/g).

	Certified values	CAD, $\bar{x} \pm ts/\sqrt{n}$[a]	UAE, $\bar{x} \pm ts/\sqrt{n}$[a]	% Recovery[b]	$t_{experimental}$ $t_{critical} = 2.57$[c]
DORM-2 (Dogfish muscles)					
Al	10.9 ± 1.7	10.8 ± 1.3	10.5 ± 0.85	96.0	0.543
Cd	0.043 ± 0.008	0.044 ± 0.004	0.0425 ± 0.002	98.8	0.401
Ni	4.64 ± 0.260	4.72 ± 0.26	4.50 ± 0.14	97.1	0.213
Pb	0.065 ± 0.007	0.065 ± 0.004	0.063 ± 0.004	96.9	0.250

[a] Average value ± confidence interval ($P = 0.05$).
[b] Recovery % = [TEs found with UAE/certified values of CRM] × 100.
[c] Paired t-test between UAE vs. CAD, at 95% confidence limit at degree of freedom ($n - 1$) = 5.

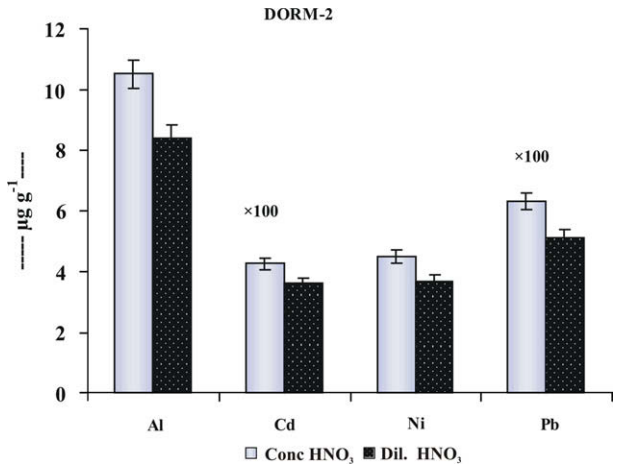

Fig. 2. Effects of acid concentration on toxic elements recovery from certified samples (DORM-2).

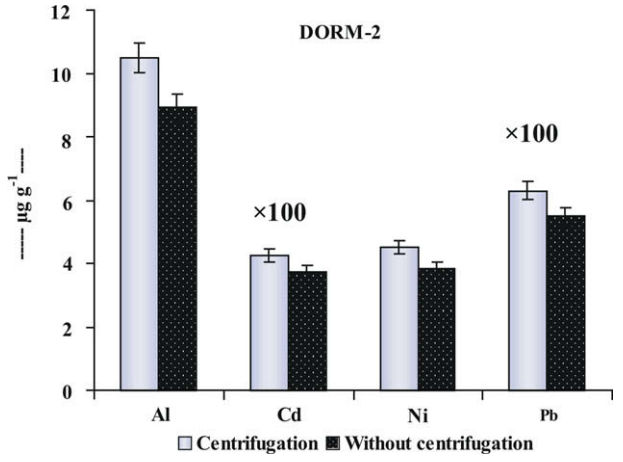

Fig. 3. Effects of centrifugation on toxic elements recovery from certified samples (DORM-2).

tion, metabolism and excretion of these TEs in neonates and adults (Navaro & Alvarez, 2003).

The great variability found in the aluminium contents in different BFs, suggests that contamination occurs during the manufacturing or storage processes of baby solid food, particularly in glass containers. A previous report also determined high Al concentrations in some processed formulas, such as low birth weight infants and soy formulas (Plessi, Bertelli, & Monzani, 1997). The high level of Al intake in children, avoids the intestinal barrier and the renal immaturity of newborns impairs its elimination (Sipahi, Eken, Aydin, Sahin, & Baydar, 2006). Infants, especially preterm neonates, display a narrow tolerance to aluminium (Martino, Sanchez, & Medel, 2000).

Cadmium and lead are toxic and can be cumulative. The high level of TEs in air, water and food are detrimental to human health and children are more sensitive to these metals than adults (Divrikli, Horzum, Soylak, & Elci, 2006). Childhood exposure to lead may induce suppression of mental capacity or retardation, aggressive behaviours and there is a high negative association between lead exposure and children's intelligence quotient (Bogden, Oleske, & Louria, 1997). It is therefore important to monitor the levels of TEs in solid baby food, which forms a source of nutrition in childhood other than milk (Divrikli et al., 2006). The proportion of Pb absorbed from gastrointestinal tract is about 10% in adults, where as 40–50% of ingested lead have been reported for infants (Health Canada, 2004).

The daily intake of TEs by infants and children (>6 months) through BFs was calculated on the basis of feeding tables provided by the manufacturers of different branded baby food. On average, a 6–12-month-old infant would require approximately 5 kg of milk powder every month. On the other hand, the requirement of baby food containing cereals along with milk works out to be only 3 kg/month. The daily intake of understudy TEs through different types of BFs are computed for infants older than 6–12 months, assuming the daily consumption rates of 100 g/day with average body weight of 7.0 kg. The daily intakes of TEs by the infants and children up to 12 months through different BFs were calculated on the basis of consumption of 3 kg BFs/month/kg body weight (≈7 kg) were found in the range of 74.3–254, 0.386–1.26, 0.743–1.34 and 1.77–4.03 μg/kg/day for Al, Cd, Pb and Ni, respectively.

The estimated intake of Cd from understudy BFs was high in some rice-based solid baby food as compared to other cereal-based BFs, the high level of Cd in rice-based BFs is consistent with other study [18]. In some cases, the intake of Cd was higher than Joint FAO/WHO, Expert Committee on Food Additives (JECFA) for Provisional Tolerable Weekly Intake (PTWI) for cadmium which is equivalent to 1 μg/kg bw/day (WHO, 2001). The FDA is established 100 μg Al/kg/day for infants, while 350 μg/kg/day for 2-year-old children.

A tolerable daily intake (TDI) for aluminium of 1000 μg/kg bw/day has been established by an international committee of experts under the auspices of the World Health Organization (WHO) and the Food and Agricultural Organization of the United Nations (WHO, 2001). The estimated intake of Al from different BFs was lower than the TDI, approximately 3.94–14.5% of the permissible tolerance intake. Even at the highest level of Al in all BFs, intake of aluminium is well below the TDI. The estimated intake range of Pb in different BFs understudy was 2.69–4.84% lower than the PTWI of lead, which is equivalent to 3.6 μg/kg bw/day. The estimated intake range of Ni from different BFs understudy was 1.24–2.82% lower than the recommended WHO total daily intake of 5 μg/kg bw/day (WHO, 2001).

Table 3
Concentration of Al, Cd, Ni and Pb indifferent baby food samples (BFs) (dried basis (μg/kg)).

Code no.	Composition of BFs	Al	Cd	Ni	Pb
BF1	Cerelac (cereal, honey and milk)	6540 ± 288	25.6 ± 1.2	213 ± 17.2	52.5 ± 3.8
BBF2	Cerelac (wheat and milk)	5200 ± 256	32.6 ± 1.4	127 ± 10.2	54.6 ± 4.8
BF3	Cerelac (rice and milk)	13,500 ± 340	74.8 ± 4.4	246 ± 21.4	94.5 ± 6.54
BF4	Cerelac (rice and three fruits)	4770 ± 427	88.3 ± 4.25	281 ± 23.2	81.5 ± 3.0
BF5	Cerelac (oat cereal and fruit)	4560 ± 245	57.5 ± 2.85	124 ± 12.2	90.6 ± 4.2
BF6	Cerelac (milk and wheat)	3410 ± 234	64.8 ± 3.25	178 ± 10.6	87.7 ± 4.85
BF7	Farex (cereal and milk)	7950 ± 249	47.9 ± 2.78	184 ± 13.2	64.8 ± 5.24
BF8	Nestum (rice and vegetable)	17,800 ± 423	72.7 ± 3.8	332 ± 27.5	78.5 ± 4.45

4. Conclusion

The method described offers a rapid, easy and efficient sample preparation for the determination of Al, Cd, Ni and Pb in solid baby foods. A simple procedure based on the use of a low cost and easily available routine ultrasonic bath, employed for determination of TEs in BFs, to avoid the time-consuming acid digestion procedure. The nitric acid concentration strongly influences the extraction efficiency. The advantages in comparison with and without centrifugation of extracted sample suspensions can be anticipated such as lower background signals, decreased build up of carbon residues inside the graphite tube and improved accuracy and precision, since both sedimentation and volumetric errors inherent to the slurry technique are removed. The levels of the toxic elements in analysed baby food samples were found to be under legal limits except in some cases (rice-based BFs contain high level of Cd). It is essential to take special care throughout the entire process of manufacturing foods for infants and young children, as the fragility of them requires an increased safety. We suggest that contaminations with metals, especially aluminium should be routinely monitored in foods for babies in order to reduce food-borne hazards in infants and young children.

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