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Element fractionation analysis for infant formula and food additives by inductively coupled plasma optical emission spectrometry

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Summary Fractionation analyses are essential to investigate the effects of dietary constituents on bioavailability of elements. A sequential separation procedure has been applied for elemental fractionation patterns in infant formula, coffee cream, milk powder, whey powder and rice flour. The protein, lipid and serum fractions were separated successfully, and fractions were analysed for various essential (Fe, Cu, Zn, Mn, Mg, Ca, Cr and Co) and nonessential (Ni, V, Al, Mo, Pb, Sn, Ba and Cd) elements by ICP-OES. Basically, three main fractions namely protein, lipid and serum were separated. Organically bounded fraction was calculated from the sum of the element contents in protein and lipid fractions. The organically bounded fraction can be retained longer in the body than the other fractions, and its percentages of whole elements are between 3.8% and 92.2% in the samples. Additionally, the distribution tendency of each studied metal was variable, which is based on the sample characteristics and complexation reactivity of the metal. The organically bounded fraction for Fe, Cu, Zn and Mn is higher than the other elements in whole samples except whey powder. Investigated elements are basically included in ionic forms in whey powder. Additionally, Mg and Ca are usually observed as uncomplexed structures in the samples.

Keywords Infant formula, food additives, fractionation, metals, sequential extraction.

Introduction

Breast milk is the first food for the human kind, and it is the source for all the nutrients including trace elements required for the growth, development and long-term health of infants. Many studies in the literature recognise the importance of milk and milk products for the newborns and infants. Particularly the 6–12 month age groups of infants are disposed to infection due to their immature immune system, and this is the time they are weaned from breast milk, started to feed on additional foods. Infant formula is one of the primary foods that infants ingest during this period. Inadequate and inaccurate nutrient intakes can affect infant growth and can have long-term consequences on organ development and function, which may result in unfavourable health effects later in life (Ljung *et al.*, 2011). Infant formula may have toxic elements as a result of either their natural presence in raw materials or contamination during food processing.

The substances added into the food to preserve flavour or enhance its taste and appearance are called as food additives. As a food additive, whey powder behaves as binder and extender for plenty of food products. It is considerably used in bakery products, dry mixes, frozen desserts, meat emulsions, sauces, salad dressing, processed cheese products and spreads, confections, gravies, snack foods and beverages. Whey powder consisting primarily of carbohydrates (lactose), protein (mainly lactalbumins), various minerals and vitamins is characterised by high nutritional and protein values. Nutritional and functional properties of whey powder are associated with the biological functions of its proteins. Powdered milk includes such items as whey powder. Powdered milk is a manufactured dairy product made by evaporating milk to dryness for preserving it. Coffee cream is defined as whitener and used as a hot drink additive in common. Some part of the world requires the alternate term nondairy whiteners so as not to imply the presence of real cream.

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Flame atomic absorption spectrometry (FAAS) (Zamir & Hussain, 2001; Yaman & Çokol, 2004; Yebra *et al*., 2004), graphite furnace atomic absorption spectrometry (GFAAS) (Plessi *et al*., 1997; Campillo *et al*., 1998; Saracoğlu *et al*., 2007; Kazi *et al*., 2010), inductively coupled plasma optical emission spectrometry (ICP-OES) (Ikem *et al*., 2002; Navarro-Blasco & Alvarez-Galindo, 2004; Zand *et al*., 2011) and inductively coupled plasma mass spectrometry (ICP MS) (Ho & Jiang, 2002; Martino *et al*., 2002; Zand *et al*., 2011) are different analytical techniques applied to analyze element contents of milk products such as infant formula and milk powder.

Researches on this field mostly deal with the total amount of elements, but the biological behaviour of a given element strongly depends on the chemical form in which this element occurs in the biological sample (Martino *et al*., 2002). Considering food analysis, fractionation studies are more elucidative than total element determinations to comprehend bioavailability and toxicity of elements. The process of classification of analyte or a group of analytes from a certain sample according to physical (e.g. size, solubility) or chemical (e.g. bonding, reactivity) properties was defined as fractionation (Templeton *et al*., 2000). To understand and predict the availability and absorption of micronutrient in food, fractionation analysis may be beneficial. Distribution and binding patterns of minerals in fractions are of great importance for their intestinal absorption (Goes *et al*., 2002). Decreased bioavailability because of the milk processing may be critical for minerals that are required for human beings, especially infants.

Metals are found in different forms and distributed in different fractions of the food samples. Fractionation studies are helpful for more comprehensive understanding of the partitioning of metals with regard to prediction of metal bioavailability and toxicity to human. In the present work, a sequential analytical fractionation scheme, suggested in our previous work (Bağdat Yaşar *et al*., 2013), has been applied to infant formulas and some food additives. Additionally, the detailed investigation was performed for a better understanding of nutritional quality of these samples with respect to trace element absorption.

Materials and methods

Chemicals and instrumentation

All the reagents were analytical grade, and water purified by reverse osmosis system was used to prepare all the solutions. Protein precipitation and washing of the precipitated protein were made by 50% (w/w) trichloroacetic acid (TCA) (Merck 1.00810) (Darmstadt, Germany), and cold acetone (Merck 1.00020) was used for precipitation and washing of proteins, respectively. The extraction of lipids was succeeded by n-hexane (Merck 1.04368). Digestion of samples was performed by HNO_3 (Merck 1.00456) and H_2O_2 (Merck 1.08600).

The precision and accuracy of the method were ensured using a wheat flour certified reference material (CRM), NIST SRM 1567a (Gaithersburg, MD, USA). The investigated samples were collected from Turkish market. Infant formula, coffee cream, milk powder, whey powder and rice flour were chosen for the application of the procedure. Samples were stored below +4 °C in polyethylene containers.

Perkin Elmer Optima 3100 XL (Waltham, MA, USA) axial simultaneous inductively coupled plasma optical emission spectrometer (ICP-OES), equipped with a CCD detector was used for the determination of all metals. The optical system was purged with argon, and the operating conditions are listed in Table 1. Sigma Laborzentrifugen (Harz, Germany) 3K15 ultracentrifuge was used for precipitation of some fractions. Thermo Scientific (Beverly, USA) Orion 5 Star model pH metre, Heidolph (Schwabach, Germany) MR 3001K model magnetic stirrer, Medline (Oxfordshire, UK) heating mantle, Biohit Proline (Helsinki, Finland) and Eppendorf (Hamburg, Germany) Research micropipetts were used for the present work.

Fractionation studies

A previously designed sequential separation procedure was used to make element fractionation in the sam-

Table 1 ICP-OES operating parameters

Polychromator	Echelle-based polychromator, UV region (167–403 nm)
Torch viewing	Axial
Recalibration system	Hg lamp
Detector	Segmented array charge coupled device detector
RF generator	40 MHz, free running, 750–1000 W
Nebuliser	Cross-flow
Plasma gas flow	15 L min^{-1}
Auxiliary gas flow	0.5 L min^{-1}
Nebulisation gas flow	0.5 L min^{-1}
View height	15 mm
Sample flow rate	1.5 mL min^{-1}
Sample flush time	4 s
Sample flush rate	4.0 mL min^{-1}
Delay time	60 s
Wash rate	1.5 mL min^{-1}
Wash time	20 s
Wavelengths (nm)	238.204 (Fe); 327.393 (Cu); 206.200 (Zn); 257.610 (Mn); 285.213 (Mg); 317.933 (Ca); 267.716 (Cr); 231.604 (Ni); 202.031 (Mo); 308.215 (Al); 220.349 (Pb); 228.616 (Co); 233.527 (Ba); 228.802 (Cd); 290.880 (V); 189.927 (Sn)

ples. The fractionation scheme was based on separation of three main fraction and element determinations by ICP-OES in these fractions.

Lipid fraction
The mixture of 2.0 g sample and 10.0 mL n-hexane was shaken in falcon tube for 5 min; the mixture was centrifuged at 1914 ***g*** for 15 min for the separation of the phases. After solvent evaporation, the residue was treated with 5.0 mL HNO_3 for mineralisation in open vessels on hot plate and diluted to 10.0 mL. The elements included in n-hexane extract were called as lipid bounded elements.

Protein fraction
The obtained supernatant removed from lipid phase was solubilised in 15 mL distilled water; the pH was adjusted to 4.8 and supplemented with 1.0 mL of 50% TCA; the mixture was incubated 10 min at 4 °C; the emulsion was centrifuged at 23447 ***g*** for 5 min at the same temperature. Proteins were coagulated, and *precipitate* was decanted, so *protein bounded* fraction was obtained. The precipitate was washed with cold acetone and mineralised by addition of HNO_3 similar to lipid phase digestion.

Serum fraction
The final solution after removing lipid and protein fractions was called serum fraction. It was diluted to 50 mL. Low molecular weight (LMW) compounds and ions were included in serum fraction. 4.0 mL concentrated HNO_3 was added to 5.0 mL serum fraction before ICP-OES determination.

Total element determination

For determination of total metal contents, 0.5 g of samples was weighed and digested with 10 mL concentrated HNO_3 under reflux for 1 h; 5 mL 30% H_2O_2 was added and continued to digestion under reflux for another 1 h. After cooling the mixture, the solution was diluted to 25.0 mL with pure water.

Results and discussion

The sequential fractionation procedure was improved and suggested for the fractionation of Fe, Cu, Zn, Mn, Mg, Ca, Cr and Ni in our previous work (Bağdat Yaşar *et al.*, 2013). The proposed analytical scheme given in Fig. 1 consists of three fractions: (i) protein, (ii) lipid and (iii) serum (ionic and LMW compounds). The distribution of mentioned elements between the fractionations was observed and evaluated for whole samples. The limit of detection (LOD) was calculated as the concentration equivalent to the three times the standard deviation (3σ) of ten replicate measurements of the calibration blank solution. The LOD values of the investigated elements for ICP-OES were found to be 59.8 μg kg^{-1} for Fe, 34.3 μg kg^{-1} for Cu, 134.9 μg kg^{-1} for Zn, 3.4 μg kg^{-1} for Mn, 64.5 μg kg^{-1} for Mg, 144.5 μg kg^{-1} for Ca, 3.6 μg kg^{-1} for Cr, 5.5 μg kg^{-1} for Ni, 36.0 μg kg^{-1} for Ba, 9.4 μg kg^{-1} for V, 13.0 μg kg^{-1} for Co, 13.9 μg kg^{-1} for Cd, 51.1 μg kg^{-1} for Al, 26.8 μg kg^{-1} for Mo, 14.5 μg kg^{-1} for Pb and 17.7 μg kg^{-1} for Sn.

CRM analysis

The accuracy of multielement determination and efficiency of the separation were checked using wheat flour CRM (NIST SRM 1567a). The metal concentrations of each separated fractions were determined, and the total values calculated from the sum of the fractions were called as *theoretical total values*. Additionally, the samples were digested under reflux, and the total element contents were determined directly. These amounts were called as *experimental total values*. The element values mentioned in certificate of CRM were also called as *certified values*. All of these values were compared with each other, and all results presented in Table 2 are in good agreement. Certified and experimental results were evaluated statistically. According to the Student's *t*-test, *t* values (t_{calc}) were calculated as 4.2, 14.1, 7.8, 28.3, 14.0 and 10.8 for Fe, Cu, Zn, Mn, Mg and Ca, respectively. At the 99.9% confidence, level t_{crit} is 31.6 and t_{calc} values are lower than t_{crit}; consequently, there was no significant difference between the certified and experimental results. As it is understood from the CRM analysis, it is possible to say that the method was applied successfully.

Evaluation of metal distributions between fractions

The previously suggested analytical scheme was applied to infant formula, coffee cream, milk powder, whey powder and rice flour samples for fractionation

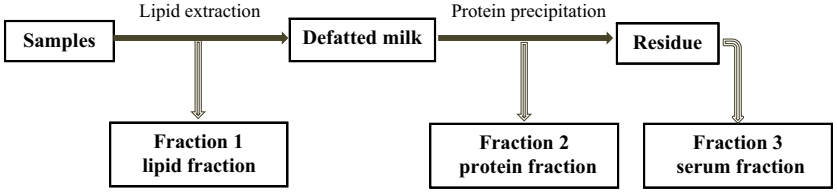

Figure 1 Analytical scheme for element fractionation in infant formula and food additives.

Table 2 The distribution of elements between fractions and total concentration of each element in NIST SRM 1567a

	Concentrations (in mg·kg^{-1};[a] in μg·kg^{-1})						
Element	**Protein (A)**	**Lipid (B)**	**Serum (C)**	**Theoretical Total (A + B + C)**	**Experimental Total (D)**	**Certified Value (E)**	**Rec.% (D/E) × 100**
Fe	12.0 ± 0.1	1.2 ± 0.006	1.5 ± 0.1	14.7 ± 0.1	14.4 ± 0.1	14.1 ± 0.5	102.1
Cu	0.8 ± 0.01	0.1 ± 0.009	1.2 ± 0.1	2.1 ± 0.1	2.3 ± 0.02	2.1 ± 0.2	109.5
Zn	3.0 ± 0.03	0.3 ± 0.004	9.0 ± 0.3	12.3 ± 0.3	12.7 ± 0.2	11.6 ± 0.4	109.5
Mn	1.2 ± 0.02	0.1 ± 0.001	8.0 ± 0.06	9.2 ± 0.1	8.4 ± 0.05	9.4 ± 0.9	89.4
Mg	56.3 ± 0.6	2.4 ± 0.02	334.4 ± 2.2	393.1 ± 2.3	417.8 ± 1.8	400 ± 20	104.4
Ca	38.4 ± 0.6	1.4 ± 0.02	147.6 ± 1.7	187.4 ± 1.8	184.1 ± 0.9	191 ± 4	96.4
Cr[a]	52.3 ± 1.6	33.0 ± 1.0	336.4 ± 2.2	421.7 ± 2.9	446.2 ± 9.6	–	–
Ni[a]	60.6 ± 0.6	15.9 ± 1.0	202.1 ± 7.6	483.6 ± 7.7	419.4 ± 7.0	–	–

[a] mean was explained as "microgram/gram" in the table.

Table 3 The distribution of elements between fractions in different types of samples

		Elements (in mg·kg^{-1};[a] in μg·kg^{-1})							
Sample	**Fractions**	**Fe**	**Cu[a]**	**Zn**	**Mn[a]**	**Mg**	**Ca**	**Cr[a]**	**Ni[a]**
Infant	A	57.50 ± 5.9	2551.7 ± 247.1	19.6 ± 0.6	403.0 ± 33.4	57.0 ± 4.3	1236.4 ± 8.9	90.8 ± 4.8	86.3 ± 3.0
Formula	B	1.4 ± 0.1	51.2 ± 7.1	0.4 ± 0.05	20.2 ± 2.8	0.4 ± 0.05	3.6 ± 0.2	Nd	14.5 ± 2.8
(6–9 months)	C	10.8 ± 0.2	548.3 ± 64.7	18.2 ± 1.4	1105.3 ± 120.4	216.3 ± 16.0	2660.6 ± 252.8	319.6 ± 41.4	221.3 ± 22.2
Coffee	A	4.6 ± 0.2	146.8 ± 12.0	6.9 ± 1.3	43.0 ± 0.7	0.6 ± 0.1	9.0 ± 0.7	65.8 ± 2.1	65.5 ± 13.3
Cream	B	0.5 ± 0.06	53.0 ± 1.3	0.2 ± 0.01	9.0 ± 0.5	0.2 ± 0.01	0.8 ± 0.2	Nd	13.0 ± 1.4
	C	8.0 ± 0.5	37.0 ± 4.9	20.2 ± 3.4	195.5 ± 12.5	2.7 ± 0.3	13.2 ± 3.0	749.3 ± 88.3	53.4 ± 10.3
Milk	A	4.2 ± 0.7	373.2 ± 2.8	36.0 ± 0.8	350.0 ± 2.8	135.1 ± 3.2	2392.4 ± 98.1	61.5 ± 8.1	17.6 ± 1.2
Powder	B	0.8 ± 0.01	82.2 ± 13.4	0.5 ± 0.02	7.0 ± 0.9	9.8 ± 0.3	222.4 ± 1.3	25.0 ± 0.9	11.9 ± 1.1
	C	0.7 ± 0.1	263.1 ± 19.7	13.6 ± 0.4	69.8 ± 11.0	864.2 ± 24.9	7552.6 ± 325.9	431.0 ± 21.0	152.4 ± 11.1
Whey	A	2.8 ± 0.02	180.2 ± 3.7	4.4 ± 1.3	50.2 ± 6.8	29.6 ± 0.3	249.6 ± 3.8	28.0 ± 1.3	32.5 ± 1.2
Powder	B	0.8 ± 0.02	53.8 ± 9.7	0.5 ± 0.05	17.6 ± 3.0	4.5 ± 0.2	20.2 ± 1.6	21.1 ± 3.9	26.5 ± 0.9
	C	8.7 ± 0.7	409.5 ± 84.0	15.4 ± 2.4	706.6 ± 12.4	859.4 ± 43.9	4194.9 ± 360.3	411.9 ± 20.0	328.8 ± 27.9
Rice	A	52.2 ± 1.1	324.7 ± 47.6	28.1 ± 0.4	1163.0 ± 78.6	377.7 ± 9.4	4339.4 ± 414.6	84.4 ± 8.5	58.0 ± 2.2
Flour	B	0.9 ± 0.1	43.6 ± 4.3	0.2 ± 0.01	16.4 ± 2.5	5.1 ± 0.9	41.8 ± 9.2	31.2 ± 5.3	21.8 ± 2.2
	C	4.5 ± 0.5	256.7 ± 31.5	17.8 ± 0.8	1256.7 ± 67.5	277.3 ± 0.1	1713.8 ± 221.1	371.1 ± 50.1	197.4 ± 22.8

[a] means was explained as "microgram/gram" in the table.
A, Protein fraction; B, Lipid Fraction; C, Serum Fraction.

analysis of them all. The analytical results of the concentrations from elements, as an average of three determinations, can be seen in Table 3, as well as the values of standard deviation.

The highest contents of iron and zinc were found mainly in protein fraction for infant formula, milk powder and rice flour, it ranges from over 73.7% to 90.6% and 51.3% to 71.9% of the total amount for iron and zinc, respectively. In our previous work, it is observed that the majority of iron in cow, goat and human milk was associated with the protein fraction (Bağdat Yaşar *et al.*, 2013). It should be mentioned here that de La Flor St Remy *et al.* (2004) observed that iron in human milk whey appears mainly associated with high molecular weight proteins. Al-Awadi & Srikumar (2001) were reported that cow and camel milk contained a higher concentration of zinc. Nevertheless, the iron and zinc concentrations of serum fraction were the highest for coffee cream and whey powder. Iron and zinc percentages of serum fraction are 61.1% and 74.0% in coffee cream; 70.7% and 75.9% in whey powder. The present data on concentration of iron and zinc in serum fraction of investigated samples were in accordance with the literature (Al-Awadi & Srikumar, 2001; Bermejo *et al.*, 2001). In the case of whey powder, the serum fraction includes the highest level of all elements. Similarly, the serum fraction is reported to have included the most of the copper concentration in human milk (Al-Awadi & Srikumar, 2001; Bağdat Yaşar *et al.*, 2013). However, copper is mainly distributed in protein fraction for the other samples, ranging from 51.9% to 81.0% of the total concentration. The main amount of manganese has been found in serum fraction except milk powder.

In previous works, manganese had been predominantly found in the serum fractions in cow and goat milks, whereas for the human milk, the copper distribution was changeable according to the mature (Al-Awadi & Srikumar, 2001; Bağdat Yaşar *et al.*, 2013). In a similar way, magnesium and calcium exist in serum fraction as the highest proportion aside from rice flour. Mg and Ca are in the range of 77.1–96.2% and 57.4–94.0%, respectively. Furthermore, the main fraction of chromium and nickel is serum for whole samples. Although Ni is mainly distributed in serum fraction, it is distributed almost equally between serum and protein fractions in coffee cream. Considering all the results, lipid bounded fraction does not contain a great amount of metal.

In addition to the metal contents in fractions, total metal contents in five different samples determined by ICP-OES are given in Table 4. The results were obtained by repeating in triplicate. Table 4 contains not only the direct determination results but also the sum of the fractions. The harmony between direct determination and sum of the fractions supports the achievement of the analytical scheme. The recoveries of most of the elements from the fractions given in Table 4 indicated that no contamination and/or lose of elements occurred. The contents of iron were found in the range of 6.7–68.3 mg kg^{-1}. Iron concentrations in the present study are similar to the range of 1.02–67.5 μg g^{-1} reported for baby foods in the literature (Saracoğlu *et al.*, 2007). The copper level varied from 254.2 μg kg^{-1} in coffee cream to 3485.0 μg kg^{-1} in infant formula. Copper level in some baby food samples has been reported in the range of 0.52–4.38 μg g^{-1} (Saracoğlu *et al.*, 2007). As can be seen, the results obtained in the present study are in good agreement with the ones in the literature. The lowest and the highest contents of zinc were found as 20.5 mg kg^{-1} for whey powder and 51.8 mg kg^{-1} for milk powder. The zinc concentration found in this study is consistent with the literature data (Plessi *et al.*, 1997; Saracoğlu *et al.*, 2007). The concentration of manganese was determined in the range of 250.0–2333.5 μg·kg^{-1} in this work. Saracoğlu *et al.* (2007) obtained some results supporting our study and have reported that manganese level varies from 0.22 to 7.20 μg g^{-1} in baby foods in Turkey. Additionally, Yaman & Çokol (2004) have investigated some baby formulas and determined Mn concentration in the range of 0.29–10.50 mg kg^{-1}. Some other elements such as V, Al, Mo, Co, Pb, Sn, Ba and Cd were also determined in whole samples, but their concentrations were measured below the LOD.

The lowest magnesium and calcium levels were found as 3.6 and 22.3 mg·kg^{-1} for coffee cream, whereas the highest Mg and Ca levels were 1036.7 and 10 583.8 mg kg^{-1} in milk powder, respectively. The lowest and highest contents of chromium were 406.8 μg·kg^{-1} in infant formula and 745.2 μg kg^{-1} in coffee cream, respectively. The concentrations of nickel in infant formula and food additive samples were found in the range of 126.3–457.9 μg kg^{-1}. In a research in Turkey, nickel content of baby foods has been reported in the range of 0.05–10.3 μg g^{-1}, and the concentrations of nickel in the present work were, therefore, similar to those found in the literature (Saracoğlu *et al.*, 2007).

Table 4 The comparison of theoretical and experimental total concentration of each element in different types of samples

Sample	Total Concentration	Elements (in mg·kg^{-1};[a] in μg·kg^{-1})							
		Fe	Cu[a]	Zn	Mn[a]	Mg	Ca	Cr[a]	Ni[a]
Infant Formula (6–9 months)	Theoretical	69.7 ± 5.9	3151.2 ± 255.5	38.2 ± 1.5	1528.5 ± 125.0	273.7 ± 16.6	3900.6 ± 253.0	410.4 ± 41.7	322.1 ± 22.6
	Experimental	68.3 ± 3.3	3485.0 ± 648.2	43.8 ± 1.8	1295.3 ± 8.5	309.5 ± 2.23	4019.6 ± 3.8	406.8 ± 31.3	363.3 ± 33.7
	Rec.%	102.0	90.4	87.2	118.0	88.4	97.0	100.9	88.7
Coffee Cream	Theoretical	13.1 ± 0.5	236.8 ± 13.0	27.3 ± 3.6	247.5 ± 21.8	3.5 ± 0.3	23.0 ± 3.1	815.1 ± 88.3	131.8 ± 16.9
	Experimental	14.7 ± 0.1	254.2 ± 60.1	28.8 ± 0.4	250.0 ± 1.0	3.6 ± 0.6	22.3 ± 0.4	745.2 ± 90.8	126.3 ± 48.6
	Rec.%	89.1	93.2	94.8	99.0	97.2	103.1	109.4	104.4
Milk Powder	Theoretical	5.7 ± 0.7	718.5 ± 24.0	50.1 ± 0.9	426.8 ± 11.4	1009.1 ± 25.1	10167.4 ± 340.3	517.5 ± 22.5	181.9 ± 11.2
	Experimental	6.7 ± 1.1	690.5 ± 7.4	51.8 ± 1.2	437.0 ± 82.7	1036.7 ± 85.4	10583.8 ± 37.6	480.0 ± 15.6	199.5 ± 16.3
	Rec.%	85.1	104.0	96.7	97.7	97.3	96.1	107.8	91.2
Whey Powder	Theoretical	12.3 ± 0.7	643.5 ± 84.6	20.3 ± 2.7	774.4 ± 14.5	893.5 ± 43.9	4464.7 ± 360.3	461.0 ± 20.4	387.8 ± 27.9
	Experimental	13.5 ± 4.6	702.5 ± 20.3	20.5 ± 2.4	694.8 ± 28.0	971.9 ± 3.3	4551.6 ± 64.7	464.4 ± 28.0	457.9 ± 50.9
	Rec.%	91.1	91.6	99.0	111.5	91.9	98.1	99.3	84.7
Rice Flour	Theoretical	57.6 ± 1.2	625.0 ± 57.2	46.1 ± 0.9	2436.1 ± 103.6	660.1 ± 9.4	6095.0 ± 470.0	486.7 ± 51.1	277.2 ± 23.0
	Experimental	61.7 ± 1.1	541.2 ± 2.3	40.7 ± 4.1	2333.5 ± 8.4	769.7 ± 17.7	5772.1 ± 21.8	446.3 ± 21.0	266.1 ± 4.0
	Rec.%	93.4	115.5	113.3	104.4	85.8	105.6	109.0	104.2

[a] means was explained as "microgram/gram" in the table.

Conclusion

An application of the previously developed fractionation scheme on infant formula and food additives was introduced in this article. The distribution of Fe, Cu, Zn, Mn, Mg, Ca, Cr, Co, Ni, V, Al, Mo, Pb, Sn, Ba and Cd between the fractions – protein, lipid and serum – was investigated. The distribution ratio of elements between fractions has been found variable up to the elements. The metal distribution between the fractions shows a similarity according to their chemical properties. The lipid and protein bounded fractions can be seen as organically bounded fractions. It is known that ions and low molecular weighed compounds are soluble in water phase, so uncomplexed and ionic forms of elements are usually included in the serum fraction. Fe, Cu and Zn exist mainly in protein fraction, yet others were included in serum fraction for infant formula. On the other hand, all elements are mainly distributed in serum fraction for coffee cream apart from Cu. The main fraction of Fe, Cu, Zn and Mn is protein for milk powder, whereas serum is the main fraction for Mg, Ca, Cr and Ni. Additionally, when whey powder is considered, serum is the main fraction for all elements. Almost all elements are mainly distributed in protein fraction for rice flour except for toxic elements, Cr and Ni. The theoretical total values and the experimental total values are also compatible with each other for all elements. This means the separation procedure is successful, loss of elements and the contamination of samples did not occur. It is known that the ionic ones remain shorter in the body than the metals bound to organic and/or biomolecules (Hocquellet & L'Hotellier, 1997). The sum of protein and lipid fractions is considered as metals bounded to organic molecules. According to the results in the present work, the ranges of metal concentrations included in organics are 21.0–84.5%, 8.1–84.4%, 14.4–87.7%, 6.0–36.4% and 23.8–92.2% for infant formula, coffee cream, milk powder, whey powder and rice flour, respectively. The minimum percentage of the organic fractions of elements was observed in whey powder. That is because the sample is supernatant of cheese production process, so organic fraction substantially was retained in cheese phase. The toxic elements, Cr and Ni, are not usually included in and/or bounded to organic structure. Additionally, these elements do not have a tendency for complexation. Mg and Ca have low organically bounded percentages for whole samples. Generally, the highly soluble samples (coffee cream and whey powder) in water show low organically bounded fractions for whole elements. It is noteworthy that the organic fraction of iron in infant formula and rice flour is higher than other elements.

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