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Assessing the Bioaccessibilities of Some Elements in Fruit Based Complementary Baby Foods

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Abstract

In this study, total concentrations and bioaccessibility of some essential and toxic elements (As, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn) were determined in commercial fruit purees intended for baby consumption as complementary foods. Enzymatic *in vitro* digestions were performed to simulate the stomach and the intestine conditions. A five-level, three factor central composite design, was applied to optimize the open-wet digestion methodology as well as enzyme amounts used *in vitro* methods to achieve maximum elemental levels. Rice flour and baby food composite certified reference materials analysis were applied to optimize the digestion parameters and evaluate the accuracy of the optimized method. Water fractions of samples were analyzed and evaluated in terms of their suitability for risk assessment studies by inductively coupled plasma mass spectrometry. Recommended dietary allowance level of 1.3% for Zn, adequate intake levels of 15.3%, 0.04%, 4.4% and 68% for Cu, Mg, Mn and Cr, respectively were achieved as well as 20%, 6% level and 2% tolerable intakes of Cd, Ni and As, respectively. Thus, contribution of the consumption of one jar of fruit based complementary baby food to diet of infants aged up to 12 months were assessed.

Keywords: Baby complementary foods; Fruit puree; Essential element; Toxic element; Bioaccessibility; Inductively coupled plasma mass spectrometry

Introduction

The first year of life is a sensitive period for infants depending on rapid physical, biological, immunological and mental growth [1,2]. Any retardation in terms of proper development in this early life stage, can possibly be influential for life onward [3,4]. Thus, providing an appropriate diet with adequate levels of macro and micro nutrients to growing infant is critical and essential in terms of healthy growth, development and wellbeing of the baby [1,3,5].

After the first 4-6 months of life, only breast or formula feeding may not be sufficient to supply the increased nutrient requirements of infants. Thus, complementary baby food products may become an important part of the diet in addition to milk to ensure required energy and nutrient intake [1,4,6].

Baby food is any food given to infants, with a soft or liquid texture from the age of 6 months to 2 years [7] and also assessed as complementary foods as they seem additional rather than mother's or formula milk [1]. They could be commercially found in markets within various forms such as instant milk flour, salty foods, purees made from fruits, vegetables or processed mixed products [7,8]. These ready-to-feed food forms are intended to use for consumption of infants and toddlers to include minerals and vitamins at sufficient levels [1]. Especially, fruit-based products are important in this context as they are one of the first complementary solid foods and widely used for infants.

Food is the primary source of essential and toxic elements which necessitate to determination of their levels for consumers [9]. Elements like selenium, iron, nickel, copper, zinc and manganese are evaluated as essential depending on their important roles for biological systems, whereas aluminum, lead and cadmium are non-essential and evaluated as toxic ones even in trace amounts. Furthermore, even elements that seem necessary may be toxic when excessively intake [10]. As infants exposed to higher levels of food chemicals, than adults possibly as a result of high consumption levels of food relative to their body weight [2], it is expected that foods as a nutrient source will become even more important for early childhood. Thus, composition and consumption patterns of baby foods as well as right proportions of trace elements are highlighted in earlier works [2,3]. In this context, a variety of studies have been carried out on the levels of trace elements in baby foods by different analytical techniques [1-3,5,8,11-14]. Although atomic absorption spectrometry, inductively coupled plasma optical emission (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are leading examples of quantification of elements, ICP-MS come to the forefront as a multi elemental analytical technique for such analytes present in food at trace and ultra-trace levels that provides accurate and sensitive determinations [9,12].

The primary objectives of this study were to determine the total elemental content of baby foods together with the amount of the bioaccessibility of selected elements (As, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn) by ICP-MS after using a response surface methodology and *in vitro* methods. Despite the importance of these complementary foods for baby feeding, limited data could be achieved from the literature in terms of essential and non-essential elements with corresponding elemental bioaccessibilities. In this context, commercially fruit based baby foods consumed for baby feeding from the age of 4th month as complementary foods were selected for risk assessment studies originating from the matrix and screening the place of Turkish markets for any of the contribution of these foods for baby feeding. Central composite design

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(CCD) methodology was used in accordance with this purpose to improve the sample preparation step used in *in vitro* methods, since it would help to provide sufficient solubility of elements from matrix by obtaining maximum elemental levels as well as minimum interfered results.

Materials and Methods

Chemicals and reagents

A multi-element standard solution containing 100 µg mL^{-1} As, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn (instrument calibration standard 2) and initial calibration verification standard (N9301721 and N9303825, respectively) were purchased from Perkin Elmer (Lakewood, NJ, USA). HNO_3 (65%, v/v), H_2O_2 (30%, v/v) and HCl (30%, v/v) were of suprapure quality obtained from a local supplier of Merck KGaA (Darmstadt, Germany). Sodium hydrogen carbonate was a Carlo Erba Reagent (Rodano, Milan, Italy). Ultrapure water (18.3 MΩ.cm^{-1}) was produced using Zeneer Power I purification system (Human Corporation, Seoul, Korea). Enzyme salts, i.e. pepsin (P7000), pancreatin (P1750) and bile extract (B8631) were obtained from Sigma-Aldrich (St. Louis, MO, USA). NIST 2383a baby food composite was obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Additionally, NCS ZC73028 rice flour certified reference material was purchased from the National Analysis Center for Iron and Steel (Beijing, China). Argon gas (99.999% purity) was obtained from Asalgaz firm (Bursa, Turkey). 50-mL polypropylene centrifuge tubes manufactured from ISOLAB (Wertheim, Germany) obtained from local suppliers and used for sample preparation for *in vitro* methods. Hydrophilic polyvinylidene fluoride (PVDF) syringe filters (0.45 µm) that were used filtration were purchased from Millipore Corp. (Bedford, MA, USA).

Sampling

Four different brands and seven commercial products of fruit based complementary baby foods were purchased from local markets in Bursa (Turkey). Commercial baby foods were selected from conventional or organic production labeled samples suitable from the beginning of 4 months above baby feeding. Two of the total samples were apple and pear, three of them were apple and carrot and three of were apple and banana purees. As the weight of each commercial jars were 125 g, total sample amounts of 750 g for each brand and products were prepared mixing the 6 jars from the same production lot to give one representative and homogenized sample. Samples were coded from A to G and stored in closed polypropylene centrifuge tubes at 4°C or -22°C until further use.

Sample preparation

All equipment was cleaned using 10% (v/v) nitric acid. Calibration curves were constructed from eight-points using standard solutions from 0.1 to 250 µg L^{-1} for all the elements.

Water fractions were prepared by adding 10 mL of ultrapure water to 0.5 g of samples. Ultrasonic extraction was applied to samples for 1 h followed by filtration through PVDF filters.

For total elemental determinations, some of the samples (approximately 2 g) were subjected to microwave digestion with HNO_3 (6 mL) and H_2O_2 (1 mL) according to DS/EN 14084 (15) using a microwave apparatus with the following programmed digestion steps: 250 W (2 min), 0 W (2 min), 250 W (6 min), 400 W (5 min) and finally 600 W (5 min).

Open-wet digestion procedure was applied to samples after optimization process. The selected variables for optimization were amounts of sample (g), volumes of nitric acid (mL) and hydrogen peroxide (mL) as used in microwave apparatus. Baby food composite certified reference material and CCD was used to optimize the selected factors for maximum recovery of elements. Samples were then open-wet digested according to achieved results of parameters.

0.2 g of pepsin was dissolved in 5 mL of 0.1 M HCl to prepare enzyme solution of pepsin. In addition, 0.45 g of bile extract and 0.075 g of pancreatin were dissolved in 37.5 mL of 0.1 M $NaHCO_3$ to prepare pancreatin/bile enzyme solution. These solutions were prepared and used freshly. Samples were subjected to *in vitro* digestion method after CCD. The selected variables for optimization were amounts of sample (g), volumes of water (mL) and volumes of enzymes (either pepsin or pancreatine, seperately) (mL). Depending on the achieved results, 10 mL of water was added to 0.2 g of samples to initiate the *in vitro* method. The pH values of the solutions were adjusted to 1.9 with 0.1 M HCl. 4 mL of pepsin-HCl solution was then added and samples were incubated at 37°C for 1 h with 120 strikes/min in a stirred water bath. pH of the samples was adjusted to 6.9 with 0.1 M $NaHCO_3$ and 5 mL of pancreatin-bile solution was added. Samples were incubated at 37°C for 2 h. Agitation speed was kept at 120 strikes/min again. Finally, samples were centrifuged at 5000 rpm for 5 min and filtrated through PVDF syringe filters to separate remaining enzyme residues [15]. Three independent replicates were prepared for each of the microwave or open-wet digested samples, *in vitro* digested samples, reagent blanks, water fractions and certified reference materials.

Apparatus and instrumentation

Microwave apparatus (MLS 1200 Mega Microwave Lab Station, Milestone, Bergamo, Italy) was used digestion of some samples. A multi-hotplate stirrer (Wise Stir SMHS-3, DAIHAN Scientific Co., Seoul, Korea) with digital display was used for open-wet digestion of samples under temperature control (from 150°C to 190°C). An Elma LC-30H ultrasonic bath (Singen, Germany) operated at an ultrasonic frequency of 35 kHz and a power of 240 W was used for preparing water fractions. A pH meter dedicated to speciation studies (N8122257, PerkinElmer Sciex) were obtained from Perkin Elmer and used for pH adjustments in *in vitro* studies. MSE Mistral 2000 centrifuge (MSE Scientific Instruments, Crawley, UK) were used for centrifugation purposes. A Nuve ST 30 (Ankara, Turkey) thermostated water bath operated at 120 strikes/min at 37°C used for *in vitro* studies. ICP-MS analyses were performed using an Elan 9000 (PerkinElmer Sciex, Shelton, CT, USA) instrument equipped with Ryton cross-flow nebulizer, Scott-type double-pass spray chamber, glass torch, sampler and skimmer cones (nickel, i.d.: 1.1 mm and 0.9 mm, respectively). Optimized instrumental conditions detailed in Table 1.

Statistical analysis

The data obtained from the CCD were analyzed using the Design

ICP-MS conditions	
Radio frequency power	1000 W
Plasma flow rate	17.0 L min^{-1}
Auxiliary flow rate	1.2 L min^{-1}
Nebulizer flow rate	0.87 L min^{-1}
Sample uptake rate	1.5 mL min^{-1}
Scanning mode	Peak hopping
Detector mode	Dual

Table 1: Optimized ICP-MS conditions.

Expert (version 7.0.0, STAT-EASE Inc., Minneapolis, MN, USA) software package. Differences among the results were assessed using SPSS Statistics for Windows (Version 23.0, IBM Corp., Armonk, NY, USA) [16,17].

Results and Discussion

Baby food composite certified reference material was used for method selection for digestion and optimization purposes. Microwave assisted and open-wet digestion methods using the same chemicals and amounts were compared for obtained elemental levels within this scope and the results of microwave digestions were compared with the obtained results of open-wet digestion. A statistical *t*-test between the obtained levels indicated that there was a good agreement between the methods for this matrix and between the results of most of the analyzed elements at the 95% confidence level. Therefore, it was concluded that elemental volatilities from matrix could be ignored unless otherwise specified when all of the samples were open-wet digested instead of using microwave apparatus. Furthermore, optimization of open-wet digestion was performed using a baby food composite certified reference material by CCD to propose a sufficient digestion procedure with minimum consumption of chemicals and samples. In this context, the volumes of nitric acid and hydrogen peroxide (mL) as well as the amounts of sample (g) were optimized for elemental recoveries from certified material using a three factor and five-level central composite design. The low, middle and high levels of variables are shown in Table 2.

Detailed design matrix for some of the studied elements with experimental responses of 20 runs and six replicates of the central points were shown in Table 3. Achieved results were evaluated for the determination of maximum recovery values of elements from the certified reference material.

Variable	Unit	Symbol	Coded and actual values				
			(-α)	-1	0	+1	(+α)
Sample	g	X_1	0.06	0.2	0.4	0.6	0.74
Nitric acid	mL	X_2	2.64	4.0	6.0	8.0	9.36
Hydrogen peroxide	mL	X_3	0.32	1.0	2.0	3.0	3.68

Table 2: Variables, coded and actual values in optimization of open-wet digestion.

The data obtained from the CCD were analyzed using Design Expert software and all runs were analyzed in triplicate by ICP-MS. Detailed information was explained elsewhere [16]. Only the behaviors of Zn, Cu and Cr were fitted to a second-degree (quadratic) polynomial model with a recovery response function, thus the optimization procedure performed to increase their recovery from certified material. Analysis of the quadratic model by software offered the optimum digestion conditions of 0.2 g of sample amount, 4 mL of nitric acid and 1.55 mL of hydrogen peroxide. These optimum conditions were then used for digestion of fruit based complementary baby foods to determine the total levels of selected elements (As, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn) by ICP-MS. Achieved results were calculated using the instrumental signal, dilution factor, as well as the amount of sample and shown in Table 4.

Accuracy of the total elemental determination was assessed by analyzing the digested certified reference material with the obtained optimum conditions. A *t*-test indicated that there was a reasonable agreement between the obtained and certified values at the 95% confidence level. Certified values of 0.2; 0.758 ± 0.082; 4.42 ± 0.51; 212.2 ± 4.0 were compatible with the measured levels of 0.2; 0.580 ± 0.060; 3.66 ± 0.37; 246.7 ± 28.2 for Cr, Cu, Fe and Mg from baby food composite reference material. Unless the certified reference material has no certified or appropriate value nor without uncertainty, the results of rice flour certified reference material were used for comparison. In this context, certified values of 0.018 ± 0.002; 0.09 ± 0.03; 0.21 ± 0.06; 11.5 ± 0.6; 0.12 ± 0.03; 2.6 ± 0.1; and 14.6 ± 0.6 were in reasonable agreement with measured levels of 0.018 ± 0.001; 0.07 ± 0.01; 0.19 ± 0.06; 9.2 ± 1.3; 0.07 ± 0.01; 2.4 ± 0.4; and 10.0 ± 1.0 for Cd, Pb, Ni, Mn, As, Cu and Zn values under studied conditions. Limit of Detection (LOD) values for the mentioned elements were 0.03 μg L^{-1} for Cr and Mg, 0.003 μg L^{-1} for Cd, Pb, Ni, Co, Mn, As, Cu and Mo, 0.87 μg L^{-1} for Zn and 0.99 μg L^{-1} for Fe. These levels were corresponding in the range of 2.25E-05 to 0.007 as mg kg^{-1}. Concentrations below these range as well as the results with higher standard deviations when compared to the mean values were indicated as under detection limit (<LOD) in Table 4. Although similar levels were found for As and Cd

Run	X_1	X_2	X_3	Cr	As	Zn	Cu	Mg
14	0.2	4	1	36.8	106.1	84.7	167.9	110.8
5	0.6	4	1	43.6	118.5	73.2	91.4	114.7
20	0.2	8	1	44.3	42.3	139.6	98.1	119
3	0.6	8	1	47.5	102.8	62.2	98	119.6
19	0.2	4	3	65.7	77.6	123.6	112.1	108.6
17	0.6	4	3	51.4	136.8	99.4	98.1	103.5
15	0.2	8	3	47.3	40.2	101.2	97.2	117
10	0.6	8	3	38.5	66.9	60.5	82.2	107.8
8	0.06	6	2	108.3	60.2	275.7	196.9	134.2
2	0.74	6	2	36	92	69	105.7	99.6
9	0.4	2.64	2	35.8	107.2	63.9	91.1	106.5
6	0.4	9.36	2	46.6	94.2	80.5	88.8	104.9
13	0.4	6	0.32	68.1	116.1	77.6	99.6	109.9
7	0.4	6	3.68	32.3	51.9	79.3	87.9	105.4
1	0.4	6	2	56.4	85.1	70.3	90.9	102.3
16	0.4	6	2	52.3	88.2	88.7	95.4	115.5
11	0.4	6	2	58.9	78.7	68.1	90.2	105.4
12	0.4	6	2	66.6	79.6	70.9	85	105.3
4	0.4	6	2	104.1	103	97.3	128.9	108.7
18	0.4	6	2	49.7	98.6	78.2	97.4	110

Table 3: Detailed design matrix with actual values and obtained recoveries for some of the elements after open-wet digestion.

among the measured and certified values of reference material after microwave digestion, volatilization of these elements in some cases may be a major problem during open-wet digestion as seen in Table 3. This effect could also be screened for Ni and Pb. If the achieved recoveries for total determination leads to lower recoveries, higher values could be calculated by using total elemental levels to calculate percent bioaccessibilities. In addition, matrix interferences that may be originating from the incomplete digestion could be also confirmed within the runs of optimization process depending on the results of certified reference material that leads to 140% percent As recoveries as an example. To avoid mentioned important points and correct the achieved results, the results of elemental levels in water fractions obtained after water fractionation studies were highlighted for some elements in Table 4 for further evaluations within this scope. Thus, elemental levels in water fractions were added for Ni, As and Cd to Table 4 and highlighted for Pb. Although, using water fractions may lead to interpret more realistic bioaccessibilities taking into consideration the feeding conditions, it must be noted that these fractions were evaluated for their suitability to calculate percent bioaccessibilities by considering the water-soluble levels as total concentrations. Nevertheless, if water fractions were evaluated as total levels and thus, ready fraction for digestion in human body, higher levels could be achieved in contrast to elemental levels obtained after open wet-digestion.

CCD was also applied to find the optimum enzyme amounts before *in vitro* bioaccessibility studies. For each of the enzymes (pepsin or pancreatin/bile) the volumes of enzyme solution (mL), the volumes of water (mL) and the amounts of sample (g) were optimized separately for the released levels of elements from matrix using a three factor and five-level central composite design (Table 5) as detailed for open-wet digestion.

Analysis of the quadratic model achieved for some of the elements (appropriate to quadratic model) by Design Expert software gave the optimum enzymatic digestion conditions of 0.2 g of sample amount, 4 mL of pepsin (5 mL of pancreatin/bile) and 10 mL of water that were further used for bioaccessibility studies. Optimum conditions found separately for the two enzymes and were combined to simulate gastro-intestinal digestion. Briefly, 5 mL of pancreatin/bile was used after addition of 4 mL of pepsin to samples. The bioaccessible levels and percent bioaccessibilities of selected elements from the samples were represented in Table 6.

Determining the total levels for the elements that may form insoluble chloride precipitates in fractionation studies (water or enzyme) as well as volatile chloride forms in wet-digestion procedure will be problematic for calculating percent bioaccessibilities. Total levels either in fractions or digested samples may be much smaller in terms of solubility. For example, Pb in water fractions is likely to produce small soluble total amounts compared with bioaccessible levels. Thus, comparison of bioaccessible levels with total levels may lead to higher percent bioaccessibilities which is above 100%. On the contrary, these tendency is meaningful depending on the matrix effects given as an

	A	B	C	D	E	F	G
Mn	2.85 ± 0.13	0.60 ± 0.00	1.06 ± 0.38	0.91 ± 0.04	0.95 ± 0.02	2.32 ± 0.06	0.90 ± 0.01
Zn	0.25 ± 0.00	0.22 ± 0.00	0.41 ± 0.29	0.36 ± 0.01	0.34 ± 0.01	0.33 ± 0.01	0.69 ± 0.01
Cu	0.31 ± 0.00	0.43 ± 0.00	0.28 ± 0.10	0.28 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.31 ± 0.01
Fe	2.15 ± 0.04	0.88 ± 0.03	1.38 ± 0.80	1.30 ± 0.05	1.30 ± 0.03	1.17 ± 0.03	1.17 ± 0.04
Mg	66.7 ± 1.64	50.8 ± 0.75	71.4 ± 25.8	49.5 ± 1.94	60.9 ± 1.27	59.4 ± 1.53	78.29 ± 0.93
Ni[c]	0.46 ± 0.02	0.52 ± 0.13	2.09 ± 0.14	0.39 ± 0.05	1.00 ± 0.01	0.53 ± 0.01	0.58 ± 0.21
Cr	0.48 ± 0.01	0.35 ± 0.01	0.16 ± 0.07	0.28 ± 0.01	0.28 ± 0.01	0.25 ± 0.01	0.32 ± 0.04
Cd[c]	<LOD	0.04 ± 0.01	0.11 ± 0.01	0.08 ± 0.07	0.24 ± 0.01	0.02 ± 0.01	<LOD
As[c]	0.04 ± 0.01	0.03 ± 0.02	0.15 ± 0.02	<LOD	0.11 ± 0.01	<LOD	0.11 ± 0.05
Pb	<LOD	0.05 ± 0.01	<LOD	<LOD	<LOD	<LOD	0.10 ± 0.01
Pb[c]	0.02 ± 0.01	<LOD	0.16 ± 0.08	0.02 ± 0.01	0.08 ± 0.01	0.02 ± 0.01	<LOD

[a]Results represented as mean values (mg kg^{-1}) ± standard deviation (sd), (n=3)
[b] <LOD: Under Detection Level
[c]Results represented as elemental levels (µg L^{-1} ± sd) in water fractions

Table 4: Total elemental contents of studied samples[a].

Variable	Unit	Symbol	Coded and actual values				
			(-α)	-1	0	+1	(+α) (α=1.682)
Sample	g	X_1	0.06	0.2	0.4	0.6	0.74
Water	mL	X_2	6.59	10	15	20	23.41
Enzyme (pepsin)	mL	X_3	1.32	2	3	4	4.68

Table 5: Variables, coded and actual values in optimization of Enzyme (pepsin) amounts for the *in vitro* method.

	A	%	B	%	C	%	D	%	E	%	F	%	G	%
Mn	0.15 ± 0.03	5	0.13 ± 0.02	22	0.18 ± 0.01	17	0.13 ± 0.02	14	0.21 ± 0.02	22	0.15 ± 0.04	6	0.14 ± 0.03	16
Zn	0.25 ± 0.03	100	0.20 ± 0.02	91	0.28 ± 0.02	68	0.20 ± 0.03	56	0.32 ± 0.02	94	0.24 ± 0.06	73	0.23 ± 0.03	33
Cu	0.21 ± 0.02	68	0.16 ± 0.02	37	0.22 ± 0.03	79	0.16 ± 0.02	57	0.27 ± 0.03	93	0.20 ± 0.06	67	0.20 ± 0.04	65
Fe	<LOD[b]	-	<LOD	-	<LOD	-	<LOD	-	<LOD	-	<LOD	-	<LOD	-
Mg	0.24 ± 0.02	0.4	0.18 ± 0.03	0.4	0.25 ± 0.01	0.4	0.19 ± 0.02	0.4	0.29 ± 0.02	0.5	0.23 ± 0.05	0.4	0.23 ± 0.03	0.3
Cr	0.02 ± 0.01	4	<LOD	-	0.02 ± 0.01	13	<LOD	-	0.02 ± 0.01	7	0.03 ± 0.01	12	0.02 ± 0.01	6

[a]Results represented as mean values (mg kg^{-1}) ± standard deviation, within triplicate results
[b]<LOD: Under Detection Level

Table 6: Bioaccessibilities of selected elements from samples under optimized conditions[a].

example for interactions with chloride constituent in a matrix as well as the characteristics of element. Finally, the bioaccessible levels of Ni, Cd, As and Pb that are important in this context were shown in Table 7 without their percent bioaccessibilities.

0.13 to 0.21 mg kg^{-1}, 0.20 to 0.32 mg kg^{-1}, 0.16 to 0.27 mg kg^{-1}, 0.23 to 0.29 mg kg^{-1}, 0.02 to 0.04 mg kg^{-1}, <LOD (under detection level) to 0.04 mg kg^{-1}, <LOD to 0.05 mg kg^{-1} bioaccessible levels were found for Mn, Zn, Cu, Mg, As, Cd and Ni respectively (Tables 6 and 7). The mean bioaccessibilities of Mn, Zn, Cu, Mg and Cr were in the range of 5%-22%, 33%-100%, 37%-93%, 0.3%-0.5%, <LOD-13%, respectively (Table 6). Bioaccessibility results of Fe shows that it would not meet the demand of infants in terms of availability. Additionally, elements in Table 7 may conclude to higher percent bioaccessible levels (above from 100%) as specified above and a ratio within bioaccessible levels to total ones was not taken into consideration for these elements. When taken into account to total Pb levels, it could be concluded that under detection levels were found for bioaccessibility (Table 6). Finally, Cr and Cd levels were also varied among samples as under detection levels were achieved for some samples (Table 7).

As shown in Table 6, achieved percent bioaccessibilities were quite similar among the samples for some of the elements (Mg, Fe and Pb). The others showed differences among the samples even within the same matrices with different brands. This difference can be explained by fruit varieties, maturation, distribution of elements in the soil at the place of origin and processing method as well as different constituents and additives of samples as a result of manufacturing processes. Mentioned constituents were also highlighted on the labels of samples. As contribution of elemental-containing macromolecular structures to the differences in bioaccessibility was highlighted by Erdemir and Gucer [17], different bound forms in fruit matrices could be concluding to represent for this tendency. Moreover, organic labeled samples (three of them) showed no distinct content for the bioaccessible levels of especially toxic elements (As, Cr, Cd and Pb) when compared to the unlabeled ones. Zn and Cu are the elements with high percent bioaccessibilities (Table 6) contrary to expectation from a macro element-Mg herein. Mg is indeed found to be as element with the smallest bioaccessibility among the whole detectable elements. Mn could be seen in moderate group in terms of bioaccessibility compared with Zn and Cu. Fe that being one of the most important element for baby feeding was under detection levels for most of the samples and showed no bioaccessibility. The bioaccessibility of non-haem iron is strong influenced by dietary components, which bind iron in the gastrointestinal system. The complexes formed can be either insoluble or so tightly bound that the iron is prevented from being absorbed. One of the main inhibitory substance is phytic acid from cereal grains [18,19]. Notably, total chromium contents may be assessed in toxic elemental group depending on its chemical form, although chromium could be considered as essential part of the diet. It is mentioned by Noel, Leblanc and Guerin [20] that Cr is mostly present in food in the essential trivalent form despite the toxic hexavalent form that is not normally found in food.

All of the studied samples contain some cereal products namely rice, corn, or wheat starch up to 5% level. In a general tendency, cereals highlighted as one of the main contributors to elemental uptake according to Noel, Leblanc and Guerin [20]. Although it is known that natural presence in raw materials, contamination, or manufacturing processes may lead to presence of toxic elements [21], the fact that rice/rice-based products even intended to use for children were reported to contain As and Cd [21,22]. Depending on this fact, As and Cd could be originating from the cereal constituents of the samples especially rice starch as this starch is also detailed on the labels of fruit purees. In addition, bioaccessibility of some elements can be affected significantly by a variety of factors. Especially food component that can have significant effect on non-heam iron bioaccessibility are the phytates in cereal. Bioaccessibility is also affected by interactions with other elements [19]. Thus, grain contents of the samples in addition to carbohydrate and protein contents that will detailed below may be considered as some important contributors or could be also a part of total bioaccessibility for Zn and Cu, also important for the elements in a moderate group of bioaccessibility (Mn). Noel, Leblanc and Guerin [20] also propose that sugar or protein content could be associated with its effect to intake of some elements. In this context, the manufacturer's label for the end-products could be assume as important baseline to attach the macromolecular composition with determined elemental bioaccessibilities shown in Table 6.

Fruits represented a part of the human diet as an important nutritional source, with high water content and relatively high amount of carbohydrates unlike the low contents of fat and protein among others (vitamins, minerals, dietary fiber or antioxidants) [23]. Although, vitamin C in the samples may be associated with the changing elemental bioaccessibility according to Erdemir and Gucer [17], mainly carbohydrates as well as proteins may be taken into account as two important macromolecular structures according to labels of products to assess the bioaccessible levels. Erdemir and Gucer [17] also observed the increased bioaccessible levels of lithium with the addition of sugar which show the important effect of carbohydrates namely sugars to evaluate the matrix effects.

Even for the same fruit-based purees, the quantity of the declared fruits on labels as a macro component found to be differentiate in percentages. Thus, instead of taking into account the carbohydrate and protein levels of products as in labels, the major different and macro-levelled fruit component between samples were selected as pear, carrot and banana and the contents of the carbohydrate (mainly sugar) and protein levels of these fruits as well as their percent quantities in the samples were further evaluated for bioaccessibility conjunction. It must be noted that apple was considered as constant component as it was the same fruit among samples. When sugar contents as a part of carbohydrates are taken into account, the following assessments could be achieved from literature data. Firstly, carrots contain carbohydrates in the form of free sugars at 8.17% level in fresh form, while only a small amount is in the form of starch [23]. In addition, half of the dry matter content was attributed to the soluble sugar mainly consist of

	A	B	C	D	E	F	G
Ni	<LOD[b]	0.03 ± 0.01	<LOD	<LOD	0.05 ± 0.02	0.03 ± 0.01	0.03 ± 0.01
Cd	0.04 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	<LOD
As	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Pb	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

[a]Results represented as mean values (mg kg^{-1}) ± standard deviation, within triplicate results
[b]<LOD: Under Detection Level

Table 7: Bioaccessibilities of selected elements from samples under optimized conditions[a].

the sucrose within the concentration range of 30 and 70% of the dry weight [23,24]. Total sugar was represented as 12% to 15 in banana fruit mainly as sucrose at 8.9% level [25]. Finally, 5.3 to 7.4% of total sugar represented in Deveci cultivar of pear which is the main one in Turkey, with fructose content of 4.2% to 3.2% and sucrose content of 0.2% to 0.5% [26]. However, protein contents among the fruits were showed smaller tendencies compared with sugar content, as the amount of protein is between 5 and 10% of the dry matter in carrots [24], 1-2.5% for bananas [27] and 0.3% for pear [28]. Additionally, the other molecular structures were not evaluated as they found much less compared to other constituents herein. For example, the total amount of organic acids and lipids were mentioned as about 0.2% and 0.3%, respectively for carrots [24]. It must be considered that variety and cultivation conditions among the other factors that may differ the corresponding macro constituents as highlighted by Mohapatra, Mishra and Sutar [27] and detailed such as the genome type, variety, altitude and climate for banana fruit.

Briefly, selected fruits may be put in order as carrot, banana and pear from the highest total sugar content to lowest. Additionally, when comparing the same type of sugar i.e. sucrose contents of carrot and banana, higher elemental bioaccessibilities in carrot-containing samples may be associated with the carbohydrates-associated elements that may show changing intake levels by these type of carbohydrate as a result of water soluble complex formation. Mn, Zn, Cu and Cr could be seen in this group with their higher percent bioaccessibilities in carrot containing samples when comparing sucrose levels of carrot and banana. It must be noted that apple contents have to be similar between two kinds of purees to conclude such a result and this necessity was taken into consideration. While Cd and As showed just the opposite tendency by decreasing percent bioaccessibilities in carrot-type puree, Mg and Ni were the unaffected ones. Cd and As could be attributed to the insoluble form of carbohydrate-attached elemental structure, at that time insoluble sugar content i.e. starch may be more important. The second cause for this observation could be assessed as these elements were not attached to sugar components. The last option was evaluated by comparing the total protein contents of samples in manufacturer's label. Banana and pear type-samples were shown this kind of tendency while in carrots two options may be possible. Fructose may be more important to for Zn association as it is the main part of the sugar content of pear with highest Zn bioaccessibility.

The soluble proteins with molecular weights in the range of 50-100 kDa, require Mn or Mg as cofactors and the inhibitory effect of zinc was highlighted on the activity of some type of proteins [23]. The inhibitory effect of Zn could be seen in pear samples with lowest protein content as well as 100% bioaccessibility. Also, the contribution of Mg for the soluble proteins could be assumed as same with the obtained same bioaccessibilities. Finally, Mn could be concluded to be conjunction with both carbohydrate and protein structures.

In conclusion, although the variety for the fruits is important for its macro molecular structures and contents, total levels depending on literature data was evaluated for the obtained bioaccessibilities as one of the macro structure, sugar has been mentioned to intake of elements. Thus, carbohydrate-attached structures were assumed and evaluated as if they affect the elemental bioaccessibilities. Mn, Zn and Cu could be associated with the sugar/protein content when compared banana and carrot containing samples while Mg, Ni and Cr not associated. As the inhibitory effect of Zn is highlighted for its soluble protein synthase, the samples with the low protein contents could be rich with Zn content and it is confirmed by 100% bioaccessibility from pear samples compared with carrot and banana forms of purees.

Risk assessment studies are considered important as high intakes of any nutrient may result toxicity as well as low intakes ending with nutritional problems [29,30]. For this reason, some definitions such as recommended daily allowance (RDA), recommended daily intake (RDI), recommended nutrient intake (RNI), adequate intake (AI) and the estimated average requirement (EAR) could be used to evaluate the essential elements while some other definitions such as provisional tolerable weekly intake (PTWI) or tolerable daily intake (TDI) could be attributed to non-essential or toxic ones [1,2,21,30]. These reference values may also be used as a part of risk assessment studies. The RDA, RDI or AI levels of studied elements were specified in numerous papers depending on the different age group of infants [1,2,21]. In this context, recommended dietary allowances were specified as: Fe (11 mg/day) and Zn (3 mg/day), while adequate Intake levels were mentioned as: Cu (220 µg/day), Mg (75 mg/day), Mn (0.6 mg/day), Cr (5.5 µg/day) for infants aged 6-12 months [31]. When considered these numeric values, none of this could be achieved for Fe, as the bioaccessible levels were under detection levels among samples. On the contrary, 1.3% level of RDA of Zn, 15.3% AI level of Cu, 0.04% AI level of Mg, 4.4% AI level of Mn and 68% AI level of Cr could be contributing to diet by the consumption of one jar of fruit based complementary baby food. Most of the bioaccessible levels contribute differently to the recommended dietary intakes/adequate intakes of elements. Thus, it may have concluded that the results may point a risk for some essential elements (Zn, Mn and Mg) in terms of sufficiently intake by consuming fruit based complementary foods studied herein. This result also shows the importance of breast feeding for infants and the importance of the bioavailability studies regarding milk for mentioned elements.

Additionally, 2.5 µg/kg body weight (bw), 25 µg/kg bw and 11 µg/kg bw tolerable weekly intake (TWI), PTWI or TDI values were highlighted for non-essential elements Cd, Pb and Ni, respectively [2] with TDI level of 2.1 µg/kg bw for As [21]. For 12-month-old infants weighing 10 kg, consuming additionally puréed infant foods and cereals-based formulae [32], these levels may be calculated as 25, 250, 110 and 210 µg for Cd, Pb, Ni and As respectively. Finally, 20% level of TWI for Cd, 6% level of TDI for Ni and 2% level of TDI for As could be achieved for infants aged 12 moths by the consumption of one jar of complementary baby food while Pb levels were under detection. The large increase in daily intakes of some elements such as As, Cd from foods at early life period of infants is possibly attributed to cereals origin [21] as the selected food types include cereals as detailed above. It should be noted that *in vitro* methods could be used to assess the potential availability [33] and the presence of all constituents with their synergistic or antagonistic effects are important in this case of evaluation [3]. Chemical interferences for bioaccessibility measurements are still a challenging task for solving the effects of carbon constituents that may cause interfered results depending on the remaining levels of organic constituents that may change plasma temperatures while ionization. These interferences could be screen while measuring different isotopes of elements, by analyzing the certified reference materials and also different dilution ratios for the samples of *in vitro* analysis. Considering the organic constituents of the samples conducted to *in vitro* analysis obtained results were assessed for any interference in this context.

Conclusion

Bioaccessible data of elements with improved quality were used for the risk assessment of baby foods. Depending on the percent bioaccessibilities, Fe and Mg will not contribute the diet significantly and shows inadequate contribution in terms of risk assessment for baby feeding. Although some elements such as Pb strictly controlled

for baby foods and its concentrations were found as under detection levels, fast and reliable screening strategies are needed for the market. For volatile elements, different strategies such as fractionation studies and chemometric methods will seem to contribute additional comments to obtain precise interpretations regarding consumption way. Considering the tolerable intake values of some elements and achieved results, further investigations for fractionation and speciation studies will be meaningful as well as considering whole diet for infants. Cr could contribute the 68% of the RDA levels and seems as the most important element in terms of risk assessment and thus, further investigations needed for speciation analysis. Cd on the other hand seems as one of the important elements in toxicity characteristics that resulted in 20% level of tolerable intake values.

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