JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Dephytinization and Follow-on Formula Addition on in Vitro Iron, Calcium, and Zinc Availability from Infant Cereals

C. FRONTELA,* J. F. HARO, G. ROS, AND C. MARTÍNEZ

Department of Food Science and Nutrition, Faculty of Veterinary Science and Food Science and Technology, Murcia University, 30071 Murcia, Spain

Cereals are introduced to infants between the ages of 4 and 6 months to supplement breast milk and follow-on formula. Our objectives were to examine the content and in vitro availability of Fe, Ca, and Zn from five commercially available infant cereals mixed with water or follow-on formula before and after dephytinization. We estimated the bioaccessibility by measuring the soluble or dialyzable mineral fraction resulting from in vitro gastrointestinal digestion of the sample. For most infant cereals analyzed, dephytinization increased the in vitro availability of iron and zinc. This finding was especially dramatic among infant cereals mixed with follow-on formula rather than with water. However, the liquid used for reconstitution did not always show a significant ($p < 0.05$) interaction with phytase addition and in vitro mineral availability. The results of this study indicate that adding follow-on formula to infant cereals does not improve the bioaccessibility of iron, calcium, and zinc, despite the increase in mineral content it implies. Results obtained also showed that mineral solubility and dialyzability do not always follow parallel trends.

KEYWORDS: Infant cereals; phytate; iron; calcium; zinc; solubility; dialysis

INTRODUCTION

The intake of dietary minerals is of interest for human beings in general, but it is particularly important for children in the first year of life, when growth is accelerated. Insufficient mineral intake in this period, especially a lack of iron, calcium, and zinc, is responsible for diseases such as iron deficiency anemia, rickets, osteoporosis, and immune diseases. Early mineral deficiency also can lead to an increase in infectious diseases, which cannot only influence immediate health but also may have an important impact on adult health (*1, 2*). Although breast-feeding is considered to be the natural and preferred method for infant feeding, by the range of between 4 and 6 months and 3 years of age, children's increasing nutritional requirements can no longer be met by breast milk alone. Accordingly, pediatric guidelines advocate supplementing milk feeding with complementary foods from the fifth month of life onward (*3*). Cereals are introduced to infants between the ages of 4 and 6 months to supplement breast milk and follow-on formula since this is a period of rapid growth and development (*4*). Infant cereals have a high energetic load, based on their carbohydrate and protein contents of approximately 78 and 13%, respectively. They also provide minerals and vitamins, particularly thiamine. The most common cereals used to formulate infant cereal flours are wheat, barley, rye, oat, rice, and maize. They may be used individually or mixed to obtain multicereal infant flours. Other products such as roots (tapioca and carrots) or legumes (peas) also may be used to prepare such foods (*1, 5*). Moreover, infant flours and follow-on formulas are commonly fortified with vitamins and minerals according to European legislation (Directive 2006/125/EEC (*6*) for infant cereals and Directive 2006/141/EEC (*7*) for follow-on formulas).

Unfortunately, mineral bioavailability from infant cereals is usually low because of the presence of phytic acid (myinositol hexaphosphoric acid), a dietary factor found principally in cereals and legumes that is a potent inhibitor of iron, calcium, and zinc absorption owing to its strong ability to bind multivalent metal ions. This binding may lead to very insoluble salts with a poor bioavailability of minerals. Low absorption of these nutrients from cereals is considered to be a factor in the etiology of mineral deficiency in infants (*8*).

Considering that during the preweaning period, the diet of an infant may be based upon cereal flours as well as upon baby milk formulas (*9, 10*), it is of interest to determine the proportion of minerals that can be absorbed and metabolized through normal pathways (bioavailability). The effect of phytate on mineral bioavailability, however, depends not only on the amount of phytate and minerals in the diet but also on the ratio of phytate/minerals, which can be used as a predictor of the relative bioavailability of minerals. The reported desirable phytate/mineral molar ratios, for mineral absorption, are less than 1 or preferably less than 0.4 for phytate/iron (*8*), less than 0.17 for phytate/calcium (*11*), less than 18 for phytate/zinc (*12*), and less than 200 (*13*) for phytate × calcium/zinc. Although cereal processing is known to reduce phytic acid content, the bioavailability of minerals can be considerably increased by

* Corresponding author. Tel.: +34 968 364798; fax: +34 968 398767; e-mail: carmenfr@um.es.

10.1021/jf073424m CCC: $40.75 © 2008 American Chemical Society
Published on Web 04/24/2008

phytic acid degradation by adding an exogenous phytase (*8*). Bioavailability should preferentially be determined by an in vivo test. However, such tests are time-consuming, expensive, complicated to perform, and unethical in small infants. Therefore, a rapid and valid in vitro model would be a valuable tool for estimating mineral availability. Several in vitro methods have been proposed as alternatives to in vivo methods. Most of the in vitro methods are based on the simulation of gastrointestinal digestion and on measurement of the soluble or dialyzable elements (*14*). These so-called bioaccessibility methods measure only the amount of soluble minerals available in the gastrointestinal tract for absorption and are widely used because of their good correlation with in vivo studies (*15*). Bioaccessibility values involve the simulation of gastrointestinal digestion and measurement of the soluble mineral fraction—the mineral fraction that dialyses across a semipermeable membrane—and must be taken as relative indices of bioavailability. Nonetheless, promising correlations between in vitro dialyzability and in vivo bioavailability have been reported (*16*). The objective of our study was to determine the extent to which dephytinization modifies the in vitro availability of iron, calcium, and zinc—as studied by solubility and dialyzability—from different infant cereals. We also estimated the influence of the matrix used for reconstitution of the cereals, using water or follow-on formula.

MATERIALS AND METHODS

Samples. We analyzed five types of infant cereal commercially available in Spain—eight cereal—honey, gluten-free cereal, rice cream, wheat, and multicereals. The infant cereals were dephytinized using an exogenous phytase from *Aspergillus oryzae* (EC 3.1.3.26 from Stern-Enzym GmbH & Co. KG, Ahrensburg, Germany, 2500 PU/g). The phytase was added to the aqueous slurry at a level of 3.2 U/g of sample and incubated with stirring at 55 °C and pH 5.5 for 20 min. The dephytinized samples were dried in an oven at 120 °C overnight to obtain the dry weight and were ground in an electrical mill to a fine powder similar to that of commercial infant cereals. Infant cereals and follow-on formula were reconstituted according to the recommendations of the manufacturer. For infant cereals, 200 mL of water or follow-on formula was mixed with 35 g of infant cereal (commercial or commercial dephytinized). For follow-on formula, 210 mL of water was mixed with 33 g of follow-on formula.

Materials and Reagents. Digestive enzymes and bile salts were supplied by Sigma Chemical Co. (St. Louis, MO): pepsin (porcine, catalogue no. P-7000), pancreatin (porcine, catalogue no. P-1750), and bile extract (porcine, catalogue no. B-8756). The pepsin solution was prepared by dissolving 1.6 g of pepsin in 10 mL of 0.1 N HCl. The pancreatin-bile extract solution was prepared by dissolving 0.2 g of pancreatin and 1.25 g of bile extract in 50 mL of 0.1 M $NaHCO_3$. Millipore Milli-Q distilled deionized water (Millipore Ibérica S.A., Barcelona, Spain) was used throughout the experiments. The working solutions of these enzymes were prepared immediately before use. The dialysis membranes had a pore size (MMCO) of 12000 Da (dia. inf. 36/32 in. to 28.6 mm, 30 m, Medicell Int. Ltd.). Total Fe, Ca, and Zn contents of the soluble fraction and the dialysate were measured by flame atomic absorption spectroscopy (AAS) (*17*). For mineral determination, the glass material was washed with detergent, soaked in concentrated nitric acid (sp gr of 1.41), and rinsed 3 times with distilled deionized water before use.

Inositol Phosphate Content. Inositol phosphates were determined by HPLC using a Merck Hitachi chromatograph (pump L-7100, refraction index (RI) detector L-7490, and L-7350 column oven) according to the method of Lehrfeld (*18*). The inositol phosphates were extracted from the different samples with 0.5 M HCl at room temperature for 2 h. The molar ratios of phytate to iron, calcium, and zinc were calculated as the millimol of phytate present in the sample divided by the millimol of iron, calcium, and zinc present in the sample, respectively. To find the phytate × (Ca/Zn) molar ratio, the total amount of Ca (mmol) in 100 g of infant cereal was multiplied by the phytate/Zn molar ratio.

In Vitro Digestion. The solubility and dialysis of iron, calcium, and zinc in commercial infant cereals reconstituted with deionized distilled water or follow-on formula before and after dephytinization were determined by the in vitro method described by Miller et al. (*19*) with suitable modifications adapted to the gastrointestinal conditions of infants younger than 6 months. Reduced amounts of enzymes and gastric and intestinal pH values were modified because the gastrointestinal tract in the early stages of life is not fully developed (*14, 9*). Briefly, the method contained two phases: gastric and intestinal. Prior to the gastric stage, the pH of 17.5 g of each infant cereal homogenized with 100 mL of deionized distilled water was lowered to pH 4 with 6 N HCl. Then, 3 g of pepsin solution was added, and the sample was incubated in a shaking water bath at 37 °C and 120 strokes/min for 2 h to allow pepsin digestion. For intestinal digestion, the pH of the gastric digests was raised to 5.0 by the dropwise addition of 1 M $NaHCO_3$. Following this, a freshly prepared pancreatin-bile solution sufficient to provide 0.005 g of pancreatin and 0.03 g of bile salts/g of sample was added, and incubation was continued for an additional 2 h. The pH was then adjusted to 7.2 by dropwise addition of 0.5 M NaOH. Aliquots of 20 g of sample intestinal digest were transferred to centrifuge tubes and centrifuged (Eppendorf 5804-R Centrifuge, Hamburg, Germany) at 3500*g* for 1 h at 4 °C. The supernatants were used to determine the mineral content (soluble fraction). Dialysis comprised the gastric stage already described, followed by an intestinal step in which a dialysis bag containing 50 mL of deionized distilled water and an amount of $NaHCO_3$ equivalent to the titratable acidity (previously measured) was placed in flasks containing 20 g aliquots of the pepsin digest. After 30 min of dialysis, an amount of the pancreatic-bile mixture previously prepared sufficient to yield 0.001 g of pancreatin and 0.006 g of bile salts/g of aliquot was added; dialysis was continued for another 2 h at 37 °C with stirring, after which the dialysate fraction was collected.. The iron, calcium, and zinc present in the dialyzate represent the bioaccessible fraction (expressed as a percentage) of the total mineral present in the sample (*13*).

Mineral Content Determination. The Fe, Ca, and Zn concentrations of the samples and the soluble and dialysate fractions were determined using a flame atomic absorption spectrometer (PerkinElmer, model 2380, Boston, MA). Prior to AAS determination of Fe, Ca, and Zn from samples and soluble mineral fractions, the organic matter was destroyed by ashing in a temperature-programmed furnace (Heraeus M1100/3, Hanau, Germany) at 525 °C for 24 h (the temperature was slowly increased at a rate of 50 °C/h). To the black ashes, 3 mL of HNO_3 (sp gr of 1.38) was added; the sample was then heated to dryness. After cooling, the residue was dissolved with 1 mL of HCl (sp gr of 1.19), and the solution was then transferred to a 10 mL volumetric flask and made to volume with water. The mineral content in the diluted acidified samples was determined against Fe, Ca, and Zn standard solutions. For calcium determination, lanthanum chloride was added to obtain a final content of 0.1% to suppress phosphate interferences. The calibration curve obtained between 1 and 5 ppm for iron and calcium, and 0.1 and 1 ppm for Zn showed an acceptable linearity; the three minerals showed correlation coefficients greater than 0.997.

Validation Criteria for the AAS Technique. The absence of matrix interferences in the AAS determination of iron, calcium, and zinc in the samples was checked by the addition method. The Community Bureau of Reference material CRM-189 (wholemeal flour) (Brussels, Belgium) was used as a control to test the method for accuracy. We analyzed Fe, Ca, and Zn in the tested samples and in the reference sample. For Fe, Ca, and Zn, the measured mean values were 66.9, 519, and 54.9 μg/g, respectively, which were in accordance with the certified range of 68.3 ± 1.9 μg/g for Fe, 520 μg/g (standard deviation (SD) noncertified) for Ca, and 56.5 ± 1.7 μg/g for Zn. The precision of the method was calculated from the results obtained in the analysis of the soluble mineral fraction from five aliquots of a sample. The values, expressed as a coefficient of variation (%), were 1.2 for iron, 0.99 for calcium, and 1 for zinc.

The solubility (%) was calculated as follows: solubility (%) = (soluble mineral content (mg/100 g)/total mineral content of the sample (mg/100 g)) × 100. Dialysis (%) was calculated as follows: dialysis (%) = (dialysate mineral content (mg/100 g)/total mineral content of the sample (mg/100 g)) × 100.

Statistical Analyses. Results are reported as means ± SD of five experiments. After testing for normality and equal variances, the mean

Table 1. Total Mineral Content (per 100 g)[a]

commercial infant cereal	Fe (mg/100 g)	Ca (mg/100 g)	Zn (mg/100 g)
eight cereals−honey	8.3 ± 0.4	137.3 ± 5.6	0.6 ± 0.3
gluten-free cereals	7.5 ± 1	154.4 ± 38.9	1 ± 0.3
multicereals	8.7 ± 0.2	174.4 ± 21.0	1.5 ± 0.4
rice cream	8.8 ± 0.1	283.1 ± 27.7	1.2 ± 0.2
wheat	12 ± 0.7	280 ± 22.3	0.7 ± 0.3
follow-on formula	9 ± 0.2	600.0 ± 49.6	4.7 ± 0.9

[a] Values are means ± SD of five determinations.

Table 2. Molar Ratios of Phytate to Iron, Calcium, and Zinc and Phytate × Ca/Zn of Infant Cereals

commercial infant cereal	Phy/Fe	Phy/Ca	Phy/Zn	Phy × Ca/Zn
eight cereals−honey	3.8	0.16	53.1	182.3
gluten-free cereals	3.5	0.18	31.8	122.7
multicereals	1.4	0.07	9.8	42.7
rice cream	1.6	0.11	14.4	101.9
wheat	2.1	0.06	44.5	311.5

solubility and dialysis of the infant cereals, dephytinized or not and reconstituted with water or follow-on formula, were compared by one-way analysis of variance (ANOVA) with a Tukey post-test for multiple comparisons. A two-way ANOVA was applied: The main effects were the addition of phytase and the type of liquid used for reconstitution (water or follow-on formula) on mineral solubility and dialyzability. A Pearson correlation analysis was performed to investigate the possible correlation between phytate content; Fe, Ca, and Zn contents; and solubility and dialysis of Fe, Ca, and Zn. Values of $p < 0.05$ were considered significant. All statistical analyses were performed with the Statistical Package for the Social Sciences (version 14.0; SPSS).

RESULTS

The values for the Fe, Ca, and Zn content of infant cereals and follow-on formula are listed in **Table 1**.

Table 2 lists the molar ratios of phytate/minerals of infant cereals.

Table 3 shows that the solubility (%) of iron, calcium, and zinc of eight cereals−honey was higher among samples reconstituted with water and given the same enzymatic treatment, except for iron after dephytinization. In most cases, dephytinization did not increase the iron, calcium, or zinc solubility, but we did observe a significant increase ($p < 0.05$) in mineral dialysability (%) after phytase treatment.

Table 4 shows the results obtained for gluten-free cereals. The addition of follow-on formula increased the solubility (%) of iron. Meanwhile, the calcium and zinc solubility was higher from infant cereals mixed with water than from cereals mixed with follow-on formula. In general, dephytinization did not increase the solubility or dialysability. The interaction between follow-on formula addition and phytase treatment was not significant ($p < 0.05$) for the solubility and dialysis of Fe and Ca and the solubility of Zn; however, it was significant for the dialysis of Zn.

As shown in **Table 5**, the effect of adding follow-on formula to multicereals had a positive effect ($p < 0.05$) on the solubility of iron and the dialysis of calcium. Concerning the effects of dephytinization, the dialysis of Ca and Zn increased in infant cereals reconstituted with water or follow-on formula, and the dialysis of iron increased in infant cereals reconstituted with follow-on formula.

Table 6 shows the results obtained for rice cream cereal. The solubility of calcium and zinc and dialysis of iron and zinc from samples reconstituted with water, dephytinized or not, was significantly ($p < 0.05$) higher than that found in samples reconstituted with follow-on formula. The interaction between the liquid used for reconstitution and phytase treatment had no significant effect on the solubility of iron, calcium, and zinc; however, for dialysis, a significant effect ($p = 0.000$) was observed.

The effects of dephytinization and the use of water or follow-on formula for reconstitution on iron, calcium, and zinc solubility and dialyzability from wheat cereal are summarized in **Table 7**. The solubility (%) trends were similar among gluten-free cereals, multicereals, rice cream, and wheat: the solubility of iron was higher in infant cereals reconstituted with follow-on formula, and the solubility of calcium and zinc was higher in samples reconstituted with water. The dialysability percentage of iron and zinc was significantly higher from infant cereals reconstituted with water; meanwhile, for calcium, dialysability values were higher from infant cereals reconstituted with follow-on formula. A significant increase in iron solubility and dialysis as well as in the dialysis of zinc was observed after phytase treatment. The effect of interaction (phytase treatment × liquid reconstitution) was significant ($p < 0.05$) only on Fe and Zn dialysis. A positive correlation between calcium content and calcium dialysis percentage was observed ($r = 0.625$, $p < 0.05$), as well as a negative correlation between calcium content and zinc and iron dialysis percentages ($r = -0.706$, $r = -0.421$). A negative correlation between iron content and zinc dialysis percentage ($r = -0.536$, $p < 0.05$) also was discovered. The amounts of Fe, Ca, and Zn available for infants per intake (200 mL) from infant cereals reconstituted with follow-on formula or water are summarized in **Tables 8** and **9**, respectively.

DISCUSSION

The values found for the three measured minerals were in the ranges recommended by infant cereals and follow-on formula regulations (EEC/2006) (*6, 7*) and also aligned with the contents reported by the manufacturers on the labels. The differences for each mineral found among different infant cereals could be due to the different proportions used or to the ingredients or cereal origin (*20*). Studies in humans indicate that the absorption of iron (*21, 22*), calcium (*23, 22*), and zinc (*24, 23, 25*) from a meal corresponds directly to its phytate content. In this regard, some critical values of the phytate/minerals molar ratio have been reported. All infant cereals analyzed had a phytate/iron molar ratio ≥1.4. Although the critical phytate/iron molar ratio (above which bioavailability of iron in a meal is compromised) has not yet been well-established, according to Hurrell (*8, 26*), values obtained in infant cereals of this study have the potential to compromise iron bioavailability. Note the very low phytate/calcium molar ratio for all samples analyzed. It is apparent that phytate could not compromise Ca availability since all the infant cereals had a phytate/Ca molar ratio below 0.24, the critical value for which the absorption of calcium is reportedly severely compromised (*22*). Four of the five infant cereals showed a phytate/Zn molar ratio above 12: This value has been implicated in interference with zinc bioavailability in humans (*27, 13*). The wheat and eight cereals−honey showed phytate × calcium/zinc molar ratios above the critical value within the range of 150−200, which have been associated with a decrease in zinc bioavailability (*13*). All the infant cereals reconstituted with follow-on formula had phytate/iron, phytate/calcium, and phytate/zinc ratios below the critical values.

The higher solubility percentage for iron observed among infant cereals reconstituted with follow-on formula as compared to the same infant cereals reconstituted with water is likely explained by the different sources of iron used for the enrichment of infant cereals (elemental iron) and follow-on formula (ferrous sulfate heptahydrate). The effect of the type of iron

Table 3. Solubility (%) and Dialyzability (%) of Iron, Calcium, and Zinc from Eight Cereals−Honey Dephytinized or Not and Reconstituted with Water or Follow-on Formula[a]

	Fe				Ca				Zn			
	solubility (%)		dialysis (%)		solubility (%)		dialysis (%)		solubility (%)		dialysis (%)	
liquid reconstitution	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase
water	43.5 ± 4[a]	23 ± 6	1.86 ± 0.03	4.55 ± 0.25[a,*]	85.6 ± 3.2*	80 ± 4*	2.3 ± 0.02	2.26 ± 0.03	36.3 ± 2.6	36.3 ± 2	11.53 ± 0.52*	14.55 ± 0.95[a,*]
follow-on formula	42.2 ± 1	43.3 ± 1*	1.84 ± 0.02	3.89 ± 0.3[a]	17.2 ± 0.8	22.2 ± 1.2[a]	2.7 ± 0*	2.94 ± 0.11[a,*]	23.5 ± 1.8	24.7 ± 2.2	2.92 ± 0.08	6.82 ± 1.51
						Two-way ANOVA						
phytase treatment	0.001		0.000		0.850		0.017		0.645		0.000	
liquid reconstitution	0.002		0.012		0.000		0.000		0.000		0.000	
phytase treatment × liquid reconstitution	0.002		0.016		0.009		0.003		0.645		0.436	

[a] When the interaction phytase treatment × liquid reconstitution was significant, the superscript a indicates significant differences between infant cereals dephytinized or not reconstituted with the same liquid, and the asterisk denotes significant differences between infant cereals within the same enzymatic treatment reconstituted with different liquids.

Table 4. Solubility (%) and Dialyzability (%) of Iron, Calcium, and Zinc from Gluten-Free Cereals Dephytinized or Not and Reconstituted with Water or Follow-on Formula[a]

	Fe				Ca				Zn			
liquid reconstitution	solubility (%)		dialysis (%)		solubility (%)		dialysis (%)		solubility (%)		dialysis (%)	
	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase
water	33.8 ± 1.1[a]	24.6 ± 2.1	1.87 ± 0.77	2.72 ± 0.85*	22.9 ± 1*	24.5 ± 4.4*	2.32 ± 0.38	2.24 ± 0.18	39.5 ± 3*	38.2 ± 3.5*	17.59 ± 0.39[a,*]	13.99 ± 0.55*
follow-on formula	57.1 ± 3.3[a,*]	51.6 ± 3*	1.78 ± 0.14	1.64 ± 0.21	19.2 ± 0.9[a]	15.5 ± 1.7	2.42 ± 0.04	2.61 ± 0.07[a,*]	24.6 ± 1	23.4 ± 1.7	3.77 ± 0.04[a]	2.15 ± 0.78
						Two-way ANOVA						
phytasetreatment	0.010		0.360		0.480		0.668		0.413		0.000	
liquid reconstitution	0.000		0.123		0.002		0.094		0.000		0.000	
phytasetreatment × liquid reconstitution	0.825		0.207		0.098		0.306		0.973		0.010	

[a] When the interaction phytase treatment × liquid reconstitution was significant, the superscript a indicates significant differences between infant cereals dephytinized or not reconstituted with the same liquid, and the asterisk denotes significant differences between infant cereals within the same enzymatic treatment reconstituted with different liquids.

Table 5. Solubility (%) and Dialyzability (%) of Iron, Calcium, and Zinc from Multicereals Dephytinized or Not and Reconstituted with Water or Follow-on Formula[a]

	Fe				Ca				Zn			
liquid reconstitution	solubility (%)		dialysis (%)		solubility (%)		dialysis (%)		solubility (%)		dialysis (%)	
	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase
water	17 ± 1	17 ± 1.3	3.16 ± 0.44[a,*]	1.53 ± 0	30 ± 2.2*	26 ± 6.7	1.88 ± 0.04	2.18 ± 0.11[a]	29 ± 7.7*	29.5 ± 2*	9.02 ± 0.3*	13.54 ± 1.28[a,*]
follow-on formula	37.2 ± 2.4*	46.8 ± 2.9[a,*]	1.91 ± 0.01	2.16 ± 0.05[a,*]	18.4 ± 1.8	19.1 ± 5.1	2.3 ± 0*	2.49 ± 0.08*	11.8 ± 1.8	13 ± 5.6	3.37 ± 0.08	3.88 ± 0.99
						Two-way ANOVA						
phytase treatment	0.004		0.001		0.538		0.000		0.774		0.037	
liquid reconstitution	0.000		0.042		0.007		0.000		0.000y		0.000	
phytase treatment × liquid reconstitution	0.004		0.000		0.386		0.216		0.905		0.095	

[a] When the interaction phytase treatment × liquid reconstitution was significant, the superscript a indicates significant differences between infant cereals dephytinized or not reconstituted with the same liquid, and the asterisk denotes significant differences between infant cereals within the same enzymatic treatment reconstituted with different liquids.

source on iron solubility was previously observed by Kapsokefalou et al. (*28*). The higher solubility of ferrous sulfate (*29*) as well as the poor bioavailability of elemental iron (*30*) have been reported. Nevertheless, it should be noted that after phytase treatment in eight cereals−honey, rice cream, and wheat, a significant increase in iron dialysis was observed when they were reconstituted with water as well as with follow-on formula; higher dialysis percentages were observed in infant cereals reconstituted with water, probably due to the factor of an inhibitory effect of casein (*10*), milk proteins (*31*), and calcium (*32*), as has been reported.

Our results also agree with the observations of other authors (*31–35,*) who observed the inhibitory effect of some components of milk—such as casein, calcium, and/or protein factors—on iron dialyzability. The casein in infant formulas plays an important role in reducing and inhibiting its dialysability since the iron−casein complex could be in an insoluble form (*35*). On the other hand, the reported inhibitory effect of phytate on iron dialysability (*13*) also has been demonstrated in our study since the infant cereals reconstituted with water (with the lowest iron content (see **Table 2**) and the highest molar ratio of phytate/iron (eight cereals−honey (3.8:1) and gluten-free cereals (3.5:1)) showed the lowest values of iron dialyzability (<2%). Nevertheless, we observed great variability, depending on the cereal analyzed.

All the infant cereals analyzed showed the highest calcium solubility (22.9−85.6%) when they were reconstituted with water; the lowest (15.5%−22.2%) was observed in infant cereals reconstituted with follow-on formula. Differences can be attibuted not only to the form of the dietary calcium used as enrichment (calcium chloride in follow-on formula and calcium citrate in infant cereals, the latter being reported to be more

Table 6. Solubility (%) and Dialyzability (%) of Iron, Calcium, and Zinc from Rice Cream Dephytinized or Not and Reconstituted with Water or Follow-on Formula[a]

	Fe				Ca				Zn			
	solubility (%)		dialysis (%)		solubility (%)		dialysis (%)		solubility (%)		dialysis (%)	
lLiquid reconstitution	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	− phytase	+phytase	−phytase	+phytase
water	33.8 ± 1.1	37.5 ± 1.5[a]	2.12 ± 0*	4.24 ± 0.3[a,*]	34.2 ± 1.7*	32.7 ± 3.8*	2.23 ± 0[a]	2.13 ± 0.02	88.2 ± 3.8[a,*]	76.5 ± 7*	9.08 ± 0*	10.08 ± 0[a,*]
follow-on formula	52.2 ± 7*	49.4 ± 6.7*	1.8 ± 0	2.16 ± 0.06[a]	16.7 ± 0.9	18.6 ± 2	2.65 ± 0.14*	2.91 ± 0[a,*]	41.4 ± 2.8	44.2 ± 9.5	2.26 ± 0.11	2.65 ± 0.39
						Two-way ANOVA						
phytase treatment	0.878		0.000		0.887		0.086		0.879		0.000	
liquid reconstitution	0.001		0.000		0.000		0.000		0.000		0.000	
phytase treatment × liquid reconstitution	0.287		0.000		0.246		0.002		0.548		0.001	

[a] When the interaction phytase treatment × liquid reconstitution was significant, the superscript a indicates significant differences between infant cereals dephytinized or not reconstituted with the same liquid, and the asterisk denotes significant differences between infant cereals within the same enzymatic treatment reconstituted with different liquids.

Table 7. Solubility (%) and Dialyzability (%) of Iron, Calcium, and Zinc from Wheat Dephytinized or Not and Reconstituted with Water or Follow-on Formula[a]

	Fe				Ca				Zn			
	solubility (%)		dialysis (%)		solubility (%)		dialysis (%)		solubility (%)		dialysis (%)	
liquid reconstitution	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase	−phytase	+phytase
water	24.8 ± 1	30 ± 1.2[a]	5.07 ± 0*	6.25 ± 0[a,*]	35 ± 2.7*	33.6 ± 3*	2.11 ± 0	2.27 ± 0[a]	50 ± 3.4*	48.5 ± 3*	22.49 ± 0*	23.33 ± 0[a,*]
follow-on formula	43.7 ± 1.8*	51 ± 2[a,*]	2.66 ± 0	4.57 ± 0.06[a]	19.9 ± 1.2	20 ± 3.8	2.75 ± 0[a,*]	2.67 ± 0*	24.1 ± 2	25 ± 4.4	3.45 ± 0.09	6.34 ± 0.16[a]
						Two-way ANOVA						
phytase treatment	0.000		0.000		0.940		0.610		0.879		0.000	
liquid reconstitution	0.000		0.000		0.000		0.016		0.000		0.000	
phytase treatment × liquid reconstitution	0.276		0.000		0.900		0.701		0.548		0.000	

[a] When the interaction phytase treatment × liquid reconstitution was significant, the superscript a indicates significant differences between infant cereals dephytinized or not reconstituted with the same liquid, and the asterisk denotes significant differences between infant cereals within the same enzymatic treatment reconstituted with different liquids.

Table 8. Available Mineral (mg) per Intake (200 mL) from Infant Cereals Reconstituted with Follow-on Formula

	commercial infant cereal			dephytinized commercial infant cereal		
	Fe	Ca	Zn	Fe	Ca	Zn
eight cereals−honey	0.11	6.07	0.39	0.23	6.61	0.92
gluten-free cereals	0.10	5.60	0.52	0.11	6.10	0.29
multicereals	0.12	5.17	0.46	0.13	5.60	0.52
rice cream	0.11	7.50	0.31	0.13	8.24	0.37
wheat	0.20	7.75	0.47	0.34	7.52	0.86

Table 9. Available Mineral (mg) per Intake (200 mL) from Infant Cereals Reconstituted with Water

	commercial infant cereal			dephytinized commercial infant cereal		
	Fe	Ca	Zn	Fe	Ca	Zn
eight cereals−honey	0.05	1.11	0.02	0.13	1.08	0.03
gluten-free cereals	0.05	1.25	0.06	0.07	1.21	0.05
multicereals	0.1	1.14	0.05	0.05	1.33	0.07
rice cream	0.07	2.21	0.04	0.13	2.11	0.04
wheat	0.2	2.07	0.06	0.23	2.22	0.06

soluble (*36*)) but also to differences in the matrix composition since components such as lactose, casein, fat, and total calcium content can affect calcium availability (*32, 36, 37*). Our results of calcium solubility from infant cereals reconstituted with water were higher than those found by Sahuquillo et al. (*38*) studying chickpeas, white beans, and lentils—probably due to the high phytate content in these legumes—and agreed with the results of Cámara et al. (*2*) for different school meals. Moreover, in our study, dephytinization did not seem to improve calcium solubility. This fact can be attributed to the low phytate content of the infant cereals analyzed before phytase treatment since they had a molar ratio of phytate/calcium lower than 0.24:1.

The calcium dialysability percentages among infant cereals reconstituted with follow-on formula (2.3−2.9%) were higher than those obtained from infant cereals reconstituted with water (1.9−2.3%). This fact could be explained this way: When casein is digested by enzymatic hydrolysis, casein phosphopeptides are formed, which inhibit phosphate precipitation and thereby maintain calcium in a soluble form available for absorption (*39–41*). Moreover, the higher calcium levels of infant cereals reconstituted with follow-on formula likely determine that a larger amount of free calcium is able to be dialyzed: This observation agrees with findings reported by Roig et al. (*33*). In relation to the effect of dephytinization on calcium dialysability, the inhibitory effect of phytate is supported by some authors (*42, 43*). However, in our study, a great variability was found since eight cereals−honey, gluten-free cereals, and rice cream showed a high calcium dialysability after phytase treatment when they were reconstituted with follow-on formula, whereas dephytinized multicereals as well as wheat increased in calcium dialysis when they were reconstituted with water. It has been reported that in milk-based infant formulas, the composition of the protein fraction (specifically casein) affects calcium dialyzability (*33*).

Infant cereals were not Zn-fortified; meanwhile, zinc sulfate was used as a source of zinc in follow-on formula. Contrary to expectations, the values of zinc solubility (29−88.2%) from infant cereals reconstituted with water were higher than those (11.8−44.2%) found in infant cereals reconstituted with follow-on formula. In all likelihood, the higher calcium contents of follow-on formula could explain this finding since a negative effect of calcium on zinc solubility has been reported (*32*). Moreover, phosphopeptides resulting from casein digestion bind zinc, thus rendering a significant proportion of zinc as unavail-

able for absorption (*10, 32*). Alternatively, changes in zinc solubility from infant cereals observed in our study seem not to be linked to phytate content since a lack of correlation was observed between phytate/zinc molar ratio and zinc solubility. A lack of positive effect of dephytinization on zinc solubility also was observed, and these results are in agreement with Kayode et al. (*44*), who found that dephytinization did not imply a larger zinc soluble amount (when studying opaque sorghum beer on zinc solubility).

The values of zinc dialysability (11.53−23.33%) from infant cereals reconstituted with water were higher than those found in infant cereals reconstituted with follow-on formula (2.15−6.82%). It is possible that the size of the compound used for zinc fortification in follow-on formula, as well as the low solubility observed in our study, could render it as having lesser dialysability. Moreover, it has been reported that some components of infant formulas, such as phosphopeptide casein from milk digests, bind zinc, which may result in a decrease in zinc dialysability (*32*). Although some studies support that excess calcium in the diet can impair zinc availability (*40, 45, 46*), it has been reported that calcium−zinc interactions seem to be conditioned by the presence of phytic acid. In this regard, we observed a significant effect of dephytinization on mineral dialysability in infant cereals reconstituted with water and with follow-on formula, whereas Perales et al. (*32*) reported a lack of a negative effect of calcium fortified milk on zinc bioavailability, in the absence of phytate. Some authors (*32, 47*) observed a negative effect of phytates from infant formulas on zinc dialysability. The same trend was observed in our study: The zinc dialysis percentage of the dephytinized infant cereals was higher in four infant cereals analyzed than those percentages corresponding to infant cereals not dephytinized. The lowest dialyzability of zinc was found in multicereals and rice cream reconstituted with water and not dephytinized, although they had the highest concentration of this element. This result agrees with those found by Hemalatha et al. (*13*), indicating that zinc dialyzability is not necessarily dependent on its concentration. All the infant cereals reconstituted with water (except gluten-free cereals) showed a higher ($p < 0.05$) zinc dialysability after dephytinization. Thus, our results confirm previous data (*21, 35, 44*) on the inhibitory effect of phytate on Zn dialyzability in cereal-based food. According to Cámara et al. (*2*), the results from the present study demonstrate that high mineral solubility is not always related to a high dialysis percentage, probably because the mineral may be bound to compounds of molecular sizes in excess of the pore size of the dialysis membrane.

Significant interactions ($p < 0.05$) were found between addition of follow-on formula or water and dephytinization in the solubility percentage of calcium for eight cereals−honey, the dialysis percentage of calcium in rice cream and eight cereals−honey, and the dialysis percentage of iron in multicereals. However, no statistically significant effect was found for each treatment separately. The two-way ANOVA also revealed a significant interaction in the solubility percentage of iron in eight cereals−honey and multicereals, the dialysis percentage of iron in rice cream and wheat, and the dialysis percentage of zinc in wheat and gluten-free cereals. As well, significant individual effects of dephytinization and follow-on formula addition also were observed. From these results, it seems that the interaction between dephytinization and addition of water or follow-on formula did not exert a significant effect on mineral solubility or dialysis. In contrast, a positive correlation between calcium content and calcium dialysis percentage was found ($r = 0.625$, $p < 0.05$), as well as a negative correlation between calcium content and zinc and iron dialysis percentages ($r = -0.706$, $r = -0.421$). These results agree with previous reports studying the absorption of trace elements during infancy (*48*) and studies of calcium-fortified milk (*32*). It has been reported that zinc can have a negative effect on iron absorption (*49*). In accordance with these authors, a negative correlation between iron content and zinc dialysis percentage ($r = -0.536$, $p \gtrsim 0.05$) was found in our study.

A better knowledge of the mineral composition of the normal diet of growing infants is required to improve infant nutrition. Infant cereals we analyzed that had been reconstituted with follow-on formula showed a higher increase in the amount of minerals available after phytase treatment than those reconstituted with water. Per intake (200 mL reconstituted according to the recommendations of the manufacturer), availabilities from commercial infant cereals accounted for 2.1, 1.6, and 8.6% of the RDR (recommended daily requirements) for iron, calcium, and zinc, respectively (*50*). Meanwhile, dephytinized commercial infant cereal provided 3.2, 6.8, and 11.8% of RDR for each mineral, respectively, according to our results. In summary, it should be noted that although follow-on formula provided a higher amount of bioaccesible iron, calcium, and zinc, water reconstitution of infant cereals increased the solubility and dialysability percentages of these minerals as compared to using follow-on formula as a liquid for reconstitution. It appears that dephytinization increases the dialysability of iron and zinc; however, more studies are necessary to evaluate the safety of phytase addition and the need for the supplementation of infant cereals with phytase to improve mineral bioavailability. To optimize phytate degradation during food processing, ascorbic acid supplementation or the use of compounds for cereal fortification have been demostrated to be useful methods for improving mineral bioavailability.

ACKNOWLEDGMENT

We thank Hero España S.A. for providing the samples.

LITERATURE CITED

(1) Febles, C. I.; Arias, A.; Hardisson, A.; Rodriguez-Alvarez, C.; Sierra, A. Phytic acid level in infant flours. *Food Chem.* **2001**, *74*, 437–441.

(2) Cámara, F.; Amaro, M. A.; Barberá, R.; Clemente, G. Bioaccesibility of minerals in school meals: Comparison between dialysis and solubility methods. *Food Chem.* **2005**, *92*, 481–489.

(3) Kersting, M.; Dulon, M. Assessment of breast-feeding promotion in hospitals and follow-up survey of mother−infant pairs in Germany: The SuSe Study. *Public Health Nutr.* **2002**, *5*, 547–552.

(4) COMA, Department of Health. Weaning and the weaning diet. *Report on Health and Social Subjects No. 45*; Her Majesty's Stationery Office: London, 1995.

(5) Pérez-Conesa, D.; Ros, G.; Periago, M. J. Protein nutritional quality of infant cereals during processing. *J. Cereal Sci.* **2002**, *36*, 125–133.

(6) OJL 339, 6.12. Commission Directive 2006/125/EC; 2006, p 16.

(7) OJL 401, 30.12. Commission Directive 2006/141/EC; as last amended by Directive 1999/21/EC; 2006, p 1.

(8) Hurrell, R. F. Phytic acid degradation as a means of improving iron absorption. *Int. J. Vitam. Nutr. Res.* **2004**, *74*, 445–452.

(9) Jovaní, M.; Barberá, R.; Farré, R.; Martín de Aguilera, E. Calcium, iron, and zinc uptake from digest of infant formulas by Caco-2 cells. *J. Agric. Food Chem.* **2001**, *49*, 3480–3485.

(10) Drago, S. R.; Valencia, M. E. Influence of components of infant formulas on in vitro iron, zinc, and calcium availability. *J. Agric. Food Chem.* **2004**, *52*, 3202–3207.

(11) Umeta, M.; West, C. E.; Fufa, H. Content of zinc, iron, and calcium and their absorption inhibitors in food commonly consumed in Ethiopia. *J. Food Comp. Anal.* **2005**, *18*, 803–817.
(12) Hotz, C.; Brown, K. International zinc nutrition consultative group (IZiNCG) technical document #1—Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr. Bull.* **2004**, *25*, 116−117.
(13) Hemalatha, S.; Platel, K.; Srinivasan, K. Influence of germination and fermentation on bioaccesibility of zinc and iron from food grains. *Eur. J. Clin. Nutr.* **2007**, *61*, 342–348.
(14) Bosscher, D.; Lu, Z.; Janssens, G.; Van Caillie-Bertrand, M.; Robberecht, H.; De Rycke, H.; De Wilde, R.; Deelstra, H. In vitro availability of zinc from infant foods with increasing phytic acid contents. *Br. J. Nutr.* **2001**, *86*, 241–247.
(15) Wienk, K. J.; Marx, J. J.; Beynen, A. C. The concept of iron bioavailability and its assessment. *Eur. J. Nutr.* **1999**, *38*, 51–75.
(16) Wolters, M. G.; Schreuder, H. A.; van den Heuvel, G.; van Lonkhuijsen, H. J.; Hermus, R. J.; Voragen, A. G. A continuous in vitro method for estimation of the bioavailability of minerals and trace elements in foods: Application to breads varying in phytic acid content. *Br. J. Nutr.* **1993**, *69*, 849–61.
(17) AOAC. *Official Methods of Analysis of AOAC International*, 16th ed.; AOAC International: Gaithersburg, MD, 1999.
(18) Lehrfeld, J. High-performance liquid chromatography analysis of phytic acid on a pH-stable macroporous polymer column. *Cereal Chem.* **1989**, *66*, 510–515.
(19) Miller, D. D.; Schricker, B. R.; Rasmussen, R. R.; Van Campen, D. An in vitro method for estimation of iron bioavailability from meal. *Am. J. Clin. Nutr.* **1981**, *34*, 2248–2256.
(20) Bosscher, D.; Van Caillie-Bertrand, M.; Van Cauwenbergh, R.; Deelstra, H. Availabilities of calcium, iron, and zinc from dairy infant formulas is affected by soluble dietary fibers and modified starch fractions. *Nutrition* **2003**, *19*, 641–645.
(21) Hurrell, R. F.; Reddy, M. B.; Juillerat, M. A.; Cook, J. D. Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *Am. J. Clin. Nutr.* **2003**, *77*, 1213–1219.
(22) Ma, G.; Li, Y.; Jin, Y.; Zhai, F.; Kok, F. J.; Yang, X. Phytate intake and molar ratios of phytate to zinc-iron and calcium in the diets of people in China. *Eur. J. Clin. Nutr.* **2007**, *61*, 368–374.
(23) Fredlund, K.; Isaksson, M.; Rossander-Hulthén, L.; Almgren, A.; Sandberg, A. S. Absorption of zinc and retention of calcium: Dose-dependent inhibition by phytate L. *Trace Elem. Med. Biol.* **2006**, *20*, 49–57.
(24) Wise, A. Phytate and zinc bioavailability. *Int. J. Food Sci. Nutr.* **1995**, *46*, 53–63.
(25) Krebs, N. F. Food choices to meet nutritional needs of breast-fed infants and toddlers on a mixed diet. *J. Nutr.* **2007**, *137*, 511–517.
(26) Hurrell, R. F. Phytic acid degradation as a means of improving iron absorption. *Int. J. Vitam. Nutr. Res.* **2004**, *74*, 445–452.
(27) Kayode, A. P.; Nout, M. J.; Bakker, E. J.; Van Boekel, M. A. Evaluation of the simultaneous effects of processing parameters on the iron and zinc solubility of infant sorghum porridge by response surface methodology. *J. Agric. Food Chem.* **2006**, *14*, 4253–4259.
(28) Kapsokefalou, M.; Alexandropoulou, I.; Komaitis, M.; Politis, I. In vitro evaluation on iron solubility and dialyzability of various iron fortificants and of iron-fortified milk products targeted for infants and toddlers. *Int. J. Food Sci. Nutr.* **2005**, *56*, 293–302.
(29) Kloots, W.; Op Den Kamp, D.; Abrahamse, L. In vitro iron availability from iron-fortified whole-grain wheat flour. *J. Agric. Food Chem.* **2004**, *52*, 8132–8136.
(30) Walter, T.; Pizarro, F.; Boy, E.; Abrams, S. A. The poor bioavailability of elemental iron in corn masa flour is not affected by disodium-EDTA. *J. Nutr.* **2004**, *134*, 380–383.
(31) Hurrell, R.; Lynch, S.; Trinidad, T. Iron absorption in humans as influenced by bovine milk proteins. *Am. J. Clin. Nutr.* **1989**, *49*, 546–552.
(32) Perales, S.; Barberá, R.; Lagarda, M. J.; Farré, R. Fortification of milk with calcium bioavailability and interactions with iron and zinc. *J. Agric. Food Chem.* **2006**, *54*, 4901–4906.
(33) Roig, M. J.; Alegría, A.; Barberá, R.; Farré, R.; Lagarda, M. J. Effects of different infant formula components on calcium dialysability. *Eur. Food Res. Technol.* **1999**, *209*, 93–96.
(34) Pérez-Llamas, F.; Larqué, E.; Marín, J. F.; Zamora, S. Disponibilidad in vitro de minerales en fórmulas infantiles con distinta fuente proteica. *Nutr. Hosp.* **2001**, *5*, 157–161.
(35) De Souza Nogueira, C.; Colli, C.; Silverio-Amancio, O. M. Infant formula iron dialyzability related to other nutrients. *Food Chem.* **2004**, *90*, 779–783.
(36) Singh, G.; Arora, S.; Sharma, G. S.; Kansal, V. K.; Sangwan, R. B. Heat stability and calcium bioavailability of calcium-fortified milk. *Food Sci. Technol.* **2007**, *40*, 625–631.
(37) Cámara, F.; Amaro, M. A. Influence of dietary factors on calcium bioavailability. *Biol. Trace Elem. Res.* **2002**, *89*, 43–52.
(38) Sahuquillo, A.; Barberá, R.; Farré, R. Bioaccesibility of calcium, iron, and zinc from three legume samples. *Nahrung* **2003**, *47*, 438–441.
(39) Hansen, M.; Sandström, B.; Jensen, M.; Sørensen, S. S. Casein phosphopeptides improve zinc and calcium absorption from rice-based but not from whole-grain infant cereal. *J. Pediatr. Gastroent. Nutr.* **1997**, *24*, 56–62.
(40) Jovaní, M.; Barberá, R.; Farré, R. Review: Effect of some components of milk- and soy-based infant formulas on mineral bioavailability. *Food Sci. Technol. Int.* **2001**, *7*, 191–198.
(41) Roig, M. J.; Alegría, A.; Barberá, R.; Farré, R.; Lagarda, M. J. Calcium bioavailability in human milk, cow milk, and infant formulas—Comparison between dialysis and solubility methods. *Food Chem.* **1999**, *65*, 353–357.
(42) Kennefick, S.; Cashman, D. Inhibitory effect of wheat fiber extract on calcium absorption in Caco-2 cells: Evidence for a role of associated phytate rather than fibre per se. *Eur. J. Nutr.* **2000**, *39*, 12–17.
(43) Kamchan, A.; Puwastien, P.; Sirichakwal, P. P.; Kongkachuichai, R. In vitro calcium bioavailability of vegetables, legumes, and seeds. *J. Food Comp. Anal.* **2004**, *17*, 311–320.
(44) Kayode, A. P. P.; Hounhouigan, J. D.; Nout, M. J. R. Impact of brewing process operations on phytate, phenolic compounds, and in vitro solubility of iron and zinc in opaque sorghum beer. *Food Sci. Technol.* **2007**, *40*, 834–841.
(45) Wood, R. J.; Zheng, J. High dietary calcium intales reduce zinc absorption and balance in humans. *Am. J. Clin. Nutr.* **1997**, *65*, 1803–1809.
(46) Cámara, F.; Amaro, M. A. Nutritional aspects of zinc availability. *Int. J. Food Sci. Nutr.* **2004**, *55*, 597–606.
(47) García, R.; Alegría, A.; Barberá, R.; Farré, R.; Lagarda, M. J. Dializability of iron, zinc, and copper of different types of infant formulas marketed in Spain. *Biol. Trace Elem. Res.* **1998**, *65*, 7–17.
(48) Lönnerdal, B. Effects of milk and milk components on calcium, magnesium, and trace element absorption during infancy. *Physiol. Rev.* **1997**, *77*, 643–669.
(49) Lind, T.; Lönnerdal, B.; Stenlund, H.; Ismail, D.; Seswandhana, R.; Ektrön, E. C.; Persson, L. A. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: Interactions between iron and zinc. *Am. J. Clin. Nutr.* **2003**, *77*, 883–890.
(50) National Research Council. *Nutrient Requirements of Recommended Dietary Allowances*; National Academic Press: Washington, DC, 1991.

Received for review November 23, 2007. Revised manuscript received February 18, 2008. Accepted March 13, 2008. This work was supported by Fundación Séneca (BPS 00205/CV/02), Consejería de Trabajo y Política Social y Fondo Social Europeo de la Comunidad Autónoma de la Región de Murcia, and Hero España S.A.

JF073424M