

Estimated Iron and Zinc Bioavailability in Soybean-Maize-Sorghum Ready to Use Foods: Effect of Soy Protein Concentrate and Added Phytase

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Abstract

Efficacy and cost of nutritional supplements are critical in addressing malnutrition. Use of cheaper and locally available ingredients in manufacturing ready-to-use foods (RUF) can potentially reduce cost and increase access to supplements in resource-poor settings. Soy protein concentrate (SPC) is a cheaper source of protein and can potentially replace the more expensive milk powder in RUF. However, SPC contains phytic acid (PA) which inhibits mineral bioavailability. PA may be degraded by the enzyme phytase. This study aimed to determine the effect of replacing skim milk powder (MP) with SPC and of added phytase on bioavailability of iron and zinc in soybean-maize-sorghum RUF.

RUF samples were made using either SPC or MP. Phytase was added to food samples with either low (<5%) or high (>50%) moisture prior to estimation of bioavailability of iron and zinc by *in vitro* dialysability. Compared to samples with MP, SPC-based foods had significantly higher content of PA (0.84 g/100 g vs. 0.57 g/100 g; $p < 0.001$); lower bioavailability of iron (2.79% vs. 4.85%; $p < 0.001$) and lower zinc bioavailability (3.61% vs. 8.69% for zinc; $p < 0.001$). After one hour of incubation at 35°C, 68% of PA in high-moisture foods and 10% of PA in low moisture foods were degraded. The data indicate that replacing MP with SPC in SMS RUF increases PA content with subsequent reduction of bioavailability of iron and zinc. Added phytase significantly reduces PA content in high moisture foods and may potentially remain active in the Stomach where moisture is high. Adding such a phytase could be a promising approach to increase iron and zinc bioavailability from SMS RUFs and provide cheaper locally produced formulations for addressing malnutrition in resource-poor settings.

Keywords: Iron and zinc bioavailability; Ready-to-use foods; Phytase

Introduction

Deficiencies of micronutrients are a major concern among children and adults globally with evidence that such deficiencies affect physical growth, cognitive development, reproduction, physical work capacity and risk of illness [1-3]. Iron and zinc deficiencies are most common in developing countries with the former being more widespread affecting up to 47% of children globally and up to 68% of pre-school age children and pregnant and lactating women in Africa [4-7]. Correlations have been identified between iron and zinc status of individuals. The highest deficiency prevalence of both iron and zinc has been reported in children from low-income families [4,8]. The prevalence of iron deficiency in resource-poor regions is exacerbated by a reliance on staple food crops which have low bioavailability of the minerals [5,9].

Ready to Use Foods (RUF) are fortified nutrient-dense formulations designed for prevention and treatment of malnutrition. In contrast to powdered foods such as Corn Soy Blend (CSB) and Wheat Soy Blend (WSB), RUF are high-energy, low-moisture lipid pastes that do not require further cooking. Their low-moisture characteristic makes them resistant to microbial contamination [10]. RUF formulations differ depending on target group and type of malnutrition to be addressed. Ready-to-use therapeutic food (RUTF) is designed to treat severe acute malnutrition (SAM) while ready-to-use supplementary foods (RUSF) are designed for prevention and treatment of moderate acute malnutrition (MAM). Ready-to-use complementary foods (RUCF) are designed to prevent chronic malnutrition when given to children 6-24 months of age. Peanut-based RUTF (P-RUTF) is the most widely used therapeutic food. P-RUTF is made from peanut paste, milk powder, vegetable oil, sugar, vitamins and minerals. It has been successfully used to address SAM in children and adults [11]. The success of P-RUTF has motivated development not only of other RUTF made from other ingredients but similar lipid-based foods to address other forms of malnutrition. Development of other RUF is aimed at reducing

cost and increasing variety. Soybean-maize-sorghum ready to use foods (SMS RUF) are similar lipid-based products meant to serve as cheaper alternatives for addressing different forms of malnutrition [12]. SMS, RUF are made from soybean, maize, sorghum, little or no milk powder, sugar, vegetable fat and micronutrient premix. Different formulations of SMS RUF have been developed. They include soybean-maize-sorghum ready-to-use therapeutic food (SMS RUTF), soybean-maize-sorghum ready-to-use supplementary foods (SMS RUSF) and soybean-maize-sorghum ready-to-use complementary food (SMS RUCF). Acceptability and efficacy of SMS-RUF have been tested for treatment and prevention of malnutrition [13-15] among children in resource poor settings. The impact of consumption of SMS-RUCF on breast milk intake has also been assessed [16]. A study among Congolese children in reduction of stunting and underweight found no significant difference between an SMS-based complementary food and a fortified blended food commonly referred to as UNIMIX [17]. Another study comparing effectiveness of a milk-free SMS-based therapeutic food with that of standard peanut-based food with milk in treating SAM among Zambian children did not confirm equivalence [14].

Bioavailability of minerals in cereal- and pulse-based foods can be significantly reduced by phytic acid (*myo*-inositol hexakisphosphate).

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Phytic acid occurs naturally as the storage form of phosphorus in plants. It inhibits the bioavailability of the minerals by forming insoluble and indigestible complexes. Reduction in the inhibiting effect of phytic acid has been achieved through use of chelated forms of iron such as sodium iron ethylenediaminetetraacetate (EDTA) and by use of intrinsic or added phytase enzyme to degrade phytic acid [18,19]. The phytate/mineral molar ratio in foods has been used to estimate mineral bioavailability. A phytate/iron ratio of less than one is preferred in order to significantly improve iron absorption in cereal or legume-based foods without iron absorption enhancers [20]. Phytate/Zinc ratios of 15 and above are reported to greatly reduce zinc absorption resulting in negative zinc balance [21].

Cereals and pulses form about 50% by weight of the SMS-RUF. It is likely that the level of phytic acid in the foods is high enough to significantly reduce bioavailability of iron and zinc. Moreover, if SPC is to replace DSMP, mineral bioavailability is of critical interest. To our knowledge, no past studies have investigated bioavailability of the minerals in SMS RUF and how it is affected by added phytase and replacing disodium monophosphate (DSMP) with soy protein concentrate (SPC). Therefore, the aim of this study was to estimate the bioavailability of iron and zinc in SMS-RUF using an *in-vitro* method based on simulated gastric digestion followed by dialysis.

Materials and Methods

Food preparation

Processing of foods was done at Insta Products EPZ, Athi River Kenya. Insta Products EPZ is internationally certified by UNICEF to supply both corn soy blend (CSB) powder and ready to use therapeutic food (RUF). Food preparation was done as described for SMS RUTF [13]. The process involved two main stages: extrusion cooking of a blend of whole soya, maize and sorghum grains and mixing of the blend with sugar, oil, fat, milk powder or soy protein concentrate, and premix containing vitamins and minerals. Figure 1 shows the process flow chart. Grains were cleaned and accurately weighed in the right proportions (Table 1) and blended manually. The full description of the procedure is as outlined by Owino et al. [13]. SPC was obtained from United Soy Board, USA while premixes of vitamins and minerals were obtained from Fortitech Strategic Nutrition, Denmark. According to the certificate of analysis from the supplier, 3 g of each premix contained among other micronutrients 487.7 µg retinol equivalents vitamin A as retinyl acetate, 16.5 mg tocopherol equivalents vitamin E as dl-alpha-tocopheryl acetate, 73 mg vitamin C as ascorbic acid, 7.3 mg iron as NaFeEDTA and 9.2 mg zinc as zinc sulfate. Foods were

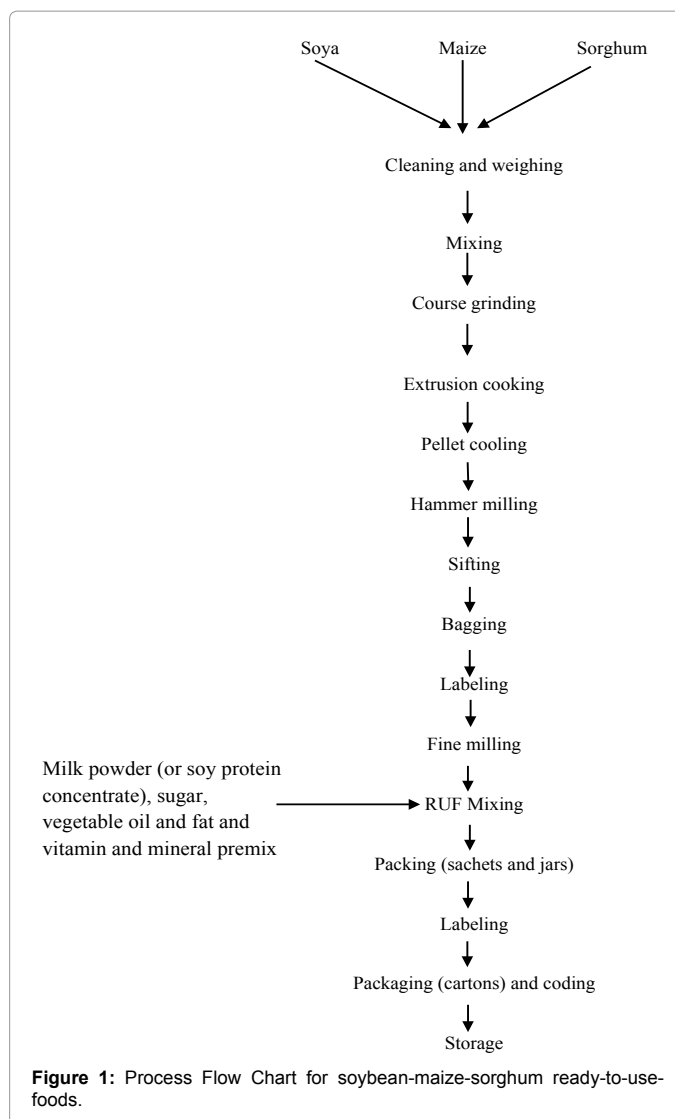


Figure 1: Process Flow Chart for soybean-maize-sorghum ready-to-use foods.

packed in polyethylene terephthalate (PET) jars of 60 g and labeled appropriately.

Determination of iron and zinc dialysability

Analyses of iron and zinc dialysability were done by a modified method of Miller et al. [22] at the Human Nutrition Laboratory, ETH, Zurich, Switzerland. The method uses a simulated gastrointestinal digestion system in which test meals were enzymatically digested and dialyzable iron and zinc pass into a dialysis tube containing a NaHCO₃ solution. The quantity of dialyzed iron and zinc were then measured by atomic absorption spectrometry. The test meal was prepared as follows: 450 ml ultra-pure water (18 MΩ.cm) were added to 39.0 g semolina in a 1 L beaker. The mixture was then heated in a boiling water bath until the temperature of the semolina meal reached 90°C. After cooling down, the pH of the mixture was brought to 2.0 with 1 M HCl and the weight was adjusted to 600 g with ultra-pure water (18 MΩ.cm). Forty grams aliquots of this acidified meal were transferred into 100 ml Erlenmeyer flasks. About 5 g of RUF was weighed at accuracy of 0.1 g and added to the meal. A 1.3 ml volume of a porcine pepsin (Sigma-Aldrich, Buchs, Switzerland) solution (16 g/100 ml in HCl 0.1 M) was added to each

| Ingredient | Grams per 100 g RUF | |
|--------------|---------------------|------------|
| | DSMP recipe | SPC recipe |
| Soybean | 21.4 | 17.5 |
| Maize | 22.2 | 18.1 |
| Sorghum | 5.4 | 4.4 |
| DSMP | 10.0 | - |
| SPC | - | 19.0 |
| Sugar | 14.0 | 14.0 |
| Palmolein | 22.0 | 22.0 |
| Palm stearin | 2.0 | 2.0 |
| Premix | 3.0 | 3.0 |

SMP, Skim milk powder; SPC, Soy protein concentrate

Table 1: RUF recipes

aliquot, and the flasks were put in a shaking water bath at 37°C for 2 hours. For the second part of the digestion, a 28 cm cut of the dialysis tubing (SpectraPor 1, 6000-8000 Da, 20.4 cm Ø, Spectrum), filled with 25 ml of a NaHCO₃ solution was immersed in each flask. The amount of NaHCO₃ solution in the dialysis tubing was just enough to shift the pH of the digested meal to 7.5; this quantity was predetermined by titration of an aliquot of the acidified meal. After 30 min in the shaking water bath at 37°C, 5 ml of a pancreatin solution (containing 20 mg porcine pancreatin (Sigma-Aldrich) and 125 mg porcine bile (Sigma-Aldrich) extract in 0.1 M NaHCO₃) were added to the samples. This was followed by a 2-hour digestion at 37°C in the shaking water bath. Iron and zinc contents of the dialysates were then measured by graphite furnace and flame atomic absorption spectrometry (SpectraAA-240 Z and FS respectively, Varian Inc., Mulgrave, Australia) respectively. Each RUF was tested in duplicate and a reference sample containing about 2 mg Fe as ferrous sulfate was included in each batch of analyses.

Determination of phytic acid content

Analysis of phytic acid was done in triplicate using a modified method by Makower [23]. The method involved extraction, precipitation and mineralization of phytic acid followed by spectrophotometric measurement of the mineralized inorganic phosphate in a micro-titration plate according to Van Veldhoven and Mannaerts [24] and converted into phytate concentrations.

Determination of minerals content

Iron, zinc and calcium analysis was done in triplicate. An internal standard reference sample was used in each batch of analysis to check accuracy. Samples of food and ingredients were first mineralized by microwave digestion (MLS ETHOS plus, MLS GmbH; Leutkirch, Germany) using a nitric acid-hydrogen peroxide mixture (7:3 v/v).

Iron and zinc concentrations were measured by graphite furnace and flame atomic absorption spectrometry respectively, using external calibration curves.

Degradation of phytic acid

Phytase enzyme (20,000 FTU per gram; DSM Nutritional Products, Basel, Switzerland) was used to degrade phytic acid. The enzyme had two pH optima, one at pH 5 and the other just below pH 3 with activity of 100% and 60% respectively. FTU is the amount of enzyme that liberates 1 µmol of inorganic phosphorus per minute under defined conditions. The pH and moisture of three aliquots of RUF were adjusted using 1.2 M ascorbic acid (Table 2). pH 5 was specifically targeted because it is the optimum pH for 100% activity of the enzyme. Phytase was added at the rate of 5000 FTU per 100 g of food. Each aliquot was divided into two portions. One set of the portions was incubated at 25°C and the other at 35°C. Analysis of phytic acid was done to determine the initial and residual levels after 1, 3 and 14 days. To test for effect of food moisture content on the activity of the enzyme, food portion 3 (Table 2) was mixed 1:1 (w/w) with water. The mixture was incubated at 35°C in a water bath with intermittent mixing. Residual phytic acid was analyzed after 1 and 2 hours after stopping the degradation by addition of tetrachloroacetic acid.

Data entry, analysis and statistics

The data was entered in excel sheets (Excel 2010, Microsoft cooperation) from where variances were calculated. The data was subjected to analysis of variance using Genstat statistical package 13th edition (VSN International Limited, UK). Least significance difference (LSD) analysis was carried out at 5% level of significance to determine differences in treatment means. Zinc, iron and phytic acid levels were used to calculate phytate/mineral molar ratios in order to estimate their bioavailability according to Hallberg et al. [20] and Turnlund et al. [21].

Results and Discussion

Table 3 shows the compositional characteristics of ingredients of SMS RUF while Table 4 shows phytic acid content and iron and zinc bioavailability in SMS RUF. Soybean had significantly higher levels of iron, zinc and calcium (p<0.001) than maize and sorghum grains. PA content in soybean (1.61 g/100 g) was about three times higher than that in maize (0.59 g/100 g) and sorghum (0.56 g/100 g). DSMP was nearly five times higher in calcium content and twenty times lower in iron than SPC. The results of raw material composition are consistent

| Food aliquot | pH | Added water | Added phytase (per 100g of food) |
|--------------|--------------|-------------|----------------------------------|
| 1 | Native (6.8) | Nil | 5000 FTU |
| 2 | 5.1 | Nil | 5000 FTU |
| 3 | 5.1 | 100% w/w | 5000 FTU |

FTU, phytase units defined as amount of enzyme that liberates 1 µmol of inorganic phosphorus per minute.

Table 2: Treatments for phytase study.

| Parameter | Ingredient | | | | | | |
|-------------------|--------------------------|---------------------------|--------------------------|--------------------------|---------------------------|--------------------------|-------------------------|
| | Soybean | Maize | Sorghum | Raw SMS | Extruded SMS | SPC | DSMP |
| Fe, mg/100 g | 6.8 ± 0.16 ^a | 1.8 ± 0.03 ^{b,c} | 3.9 ± 0.15 ^c | 4.3 ± 0.13 ^c | 15.0 ± 3.42 ^d | 10.1 ± 0.21 ^e | 0.6 ± 0.02 ^b |
| Zn, mg/100 g | 4.8 ± 0.02 ^a | 1.7 ± 0.03 ^b | 1.8 ± 0.04 ^c | 3.0 ± 0.02 ^d | 3.0 ± 0.03 ^d | 3.3 ± 0.00 ^e | 3.5 ± 0.06 ^f |
| PA, g/100 g | 1.61 ± 0.08 ^a | 0.59 ± 0.02 ^b | 0.56 ± 0.06 ^b | 1.09 ± 0.06 ^c | 0.897 ± 0.03 ^d | 2.09 ± 0.13 ^e | Not detectable |
| PA/Fe molar ratio | 20.0 | 27.9 | 12.3 | 21.6 | 1.60 | 17.5 | - |
| PA/Zn molar ratio | 32.9 | 35.1 | 30.4 | 36.3 | 29.2 | 63.0 | - |

SMS: Soybean-maize-sorghum blend; SPC: Soy protein concentrate; DSMP: Dry skim milk powder; PA: Phytic acid. Values with different superscripts in same row are significantly different (p ≤ 0.05).

Table 3: Compositional characteristics of ingredients of soybean-maize-sorghum ready-to-use foods (values are mean ± SD; n = 3).

| RUF Recipe | Fe, mg/100 g | Zn, mg/100 g | PA, g/100 g | PA/Fe molar ratio | PA/Zn molar ratio | Fe dialysability, % | Zn dialysability, % |
|------------------|---------------------------|--------------------------|--------------------------|-------------------|--------------------|--------------------------|--------------------------|
| DSMP | 11.90 ± 2.83 ^d | 5.84 ± 3.16 ^d | 0.57 ± 0.05 ^c | 4.05 ^a | 9.67 ^a | 4.85 ± 0.46 ^c | 8.69 ± 1.81 ^c |
| SPC | 15.11 ± 5.93 ^e | 5.60 ± 2.71 ^e | 0.84 ± 0.02 ^d | 4.70 ^a | 14.86 ^a | 2.79 ± 0.59 ^d | 3.61 ± 0.37 ^d |
| Reference sample | 2.00 ± 0.55 | - | - | - | - | 7.44 ± 2.86 | - |

SPC: Soy protein concentrate; DSMP: Dry skim milk powder; PA: Phytic acid. Values with different superscripts in same column are significantly different (p<0.05).

Table 4: Phytic acid content and iron and zinc dialysability in RUF.

with those in literature [25]. The PA/mineral molar ratios observed for soybean, maize, sorghum and the raw and extruded SMS were higher than those preferred for adequate mineral absorption [20,21] and thus indicate that all the grains are inhibitory for absorption of both iron and zinc. Moreover, bioavailability of iron in sorghum is also likely to be adversely affected by high content of polyphenols (2700 mg/100 g) [26]. Extruded SMS had significantly higher amounts of iron (15.03 mg/100 g; $p < 0.001$) but lower amount of PA (0.897 g/100 g; $p < 0.001$) than raw SMS (4.27 mg/100 g and 1.09 g/100 g for iron and PA respectively) (Table 3). Iron contamination from equipment during milling has been reported and is attributed to frictional wear and tear of the moving mill parts [27]. Because SMS processing was done in equipment traditionally used for fortified blended foods, the process steps that involve friction such as extrusion and milling are likely to have led to iron contamination, with consequent elevation of iron content in extruded SMS. The contaminant iron however was not studied for solubility and its bioavailability was unknown. It has been reported that heat treatment degrades some PA and is likely to explain the lower content of PA in extruded SMS compared to that in raw SMS.

Iron content was significantly higher in foods made from SPC recipe (15.11 mg/100 g) than in those from DSMP (11.90 mg/100 g; $p < 0.001$) (Table 4). The significant difference in iron content between the recipes is attributed to higher iron content in SPC (10.07 mg/100 g) than in DSMP (0.55 mg/100 g; Table 4). PA was also significantly higher ($p < 0.001$) in foods from SPC recipe (0.84 g/100 g) than those from DSMP recipe (0.57 g/100 g; Table 4). On the contrary, the PA/Fe molar ratios for the two recipes were statistically similar indicating that the high content of PA in SPC seems to be offset by the similarly higher content of iron. On the basis of PA/Fe ratios, it would be expected that iron bioavailability in both recipes would be similar. However, bioavailability of iron was significantly higher in foods made from DSMP (4.85%) than those made from SPC (2.79%; $p < 0.001$; Table 4). The significantly lower iron bioavailability from SPC recipe can be explained in two ways. Firstly, because the iron in SPC and extruded SMS is non-heme of unknown form including contaminant iron, it appears that a large proportion did not enter the common iron pool for absorption. There is evidence that many forms of iron do not enter the iron common pool and remain unavailable for absorption. Secondly, soy protein [28] and milk proteins [29,30] have been associated with lower iron bioavailability even in the absence of phytic acid, but from these results, it appears that soy protein in SPC played a more significant role than milk proteins in DSMP to and further reduced iron bioavailability in SPC recipe. For adequate bioavailability of iron, the PA/Fe molar ratio should be < 1 [30]. None of the recipes was of adequate iron bioavailability hence methods to improve iron bioavailability in the foods are needed.

Zinc bioavailability was significantly higher in foods from DSMP recipe (8.69; $p < 0.001$) than in foods of SPC recipe (3.61%; Table 4). This was expected because DSMP foods had a lower PA/Zn molar ratio (9.67) than SPC foods (14.86; Table 4). SPC and DSMP had comparable zinc contents (3.29 and 3.46 mg/100 g respectively). However, the significantly higher PA content in SPC gives a much higher PA/Zn molar ratio in SPC foods than in DSMP foods, predicting higher inhibition in SPC foods. However, WHO [31] classifies bioavailability of zinc as low, moderate or high if absorption is 15%, 30-35% and 50-55%, respectively. Both DSMP and SPC foods therefore fall in the same category of low bioavailability and thus do not differ much nutritionally. However, due to their slightly higher zinc bioavailability, foods from DSMP recipe may offer better zinc complement than those from SPC recipe. The difference between 8.7% (for DSMP recipe) and 3.6% (for SPC recipe) is important in aiming to reach the Recommended Dietary Allowance.

Addition of phytase resulted in a significant reduction of phytic acid in high moisture foods ($p < 0.001$). However, there was no effect of incubation time and temperature on PA content in foods without added water (Table 5). Phytic acid content of food with added water reduced from 0.68 to 0.22 g/100 g in the first one hour of incubation (Table 5). The reduction in PA content has significant potential to enhance iron and zinc bioavailability in the foods. As an enzyme, phytase requires moist environment to be active. Because one optimum of the enzyme is at pH 2, the enzyme can be utilized to degrade phytic acid in RUF in the gut. Such application of the enzyme in other inhibitory meals has been successfully studied [18]. However, retention of enzyme activity in the food matrix up to the end of shelf life needs to be established.

Conclusions and Recommendations

Replacing DSMP with SPC increased content of PA thereby decreasing the bioavailability of iron and zinc in SMS RUF. Adding phytase to the RUF resulted in degradation of PA and improved bioavailability of the minerals. However, phytase requires high moisture to be active hence its activity would predominantly take place in the gut at a second optimum pH of around 2. In settings where SPC is more affordable than MP, replacing MP with SPC in SMS RUF has potential of reducing cost and addition of phytase may bring the benefit of improving iron and zinc bioavailability in the foods. Research on retention of phytase activity in SMS RUF during storage is recommended and to verify improvement of bioavailability of the minerals in human absorption studies. Aspects of formulation would need to be appropriately considered to achieve target micronutrient and amino acid profiles since the two ingredients differ in nutritional composition.

| Food aliquot | Food characteristics | Initial PA content (g/100 g food) | Residual PA content (g/100 g) | | | | | | | | |
|--------------|----------------------|-----------------------------------|-------------------------------|--------------------------|----------------------------|----------------------------|--------------------------|----------------------------|----------------------------|----------------------------|--|
| | | | Incubated at 35°C | | Incubated at 25°C | | | Incubated at 35°C | | | |
| | | | 1 hr | 2 hr | 1 day | 3 days | 14 days | 1 day | 3 days | 14 days | |
| 1 | pH 5.9, no water | 0.68 ± 0.03 ^c | n.t. | n.t. | 0.67 ± 0.02 ^{b,c} | 0.61 ± 0.01 ^{a,b} | 0.64 ± 0.00 ^b | 0.59 ± 0.04 ^a | 0.63 ± 0.01 ^{a,b} | 0.61 ± 0.00 ^{a,b} | |
| 2 | pH 5.1, no water | 0.68 ± 0.03 ^d | 0.61 ± 0.01 ^b | 0.62 ± 2.81 ^b | 0.55 ± 0.01 ^a | 0.64 ± 0.05 ^{b,c} | 0.71 ± 0.02 | 0.67 ± 0.02 ^{c,d} | 0.60 ± 0.02 ^b | 0.58 ± 0.01 ^a | |
| 3 | pH 5.1, + water | 0.68 ± 0.03 ^b | 0.22 ± 0.00 ^a | 0.19 ± 2.88 ^a | n.t. | n.t. | n.t. | n.t. | n.t. | n.t. | |

PA: phytic acid; n.t.: not tested. Values with different superscripts in same row are significantly different ($p < 0.05$).

Table 5: Initial and residual contents of phytic acid in foods incubated with phytase (values are mean ± SD; n = 3)

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