Iron, Zinc and Protein Bioavailability Proxy Measures of Meals Prepared with Nutritionally Enhanced Beans and Maize

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Abstract

Nutritionally enhanced beans (NEB) with more Fe and Zn than conventional beans (CB) and nutritionally enhanced maize (NEM) with more tryptophan and lysine than conventional maize (CM) were developed as part of a crop-biofortification strategy to improve human nutrition. Proxy measures were used to assess Fe and Zn bioavailability and protein digestibility of a bean recipe (*fríjol sancochado*) and a maize-milk recipe (*mazamorra*) prepared with enhanced or conventional crops in Colombia. Fe concentration was similar in the cooked NEB and CB and in NEM and CM ($P \geq 0.05$); *in vitro* Fe dialyzability was similar in cooked NEB (9.52%) and CB (9.72%) and greater for NEM (37.01%) than CM (32.24%). Zn concentration was higher in the uncooked and cooked NEB than in the CB ($P<0.05$); phytate:Zn molar ratios were high in cooked NEB (36:1) and CB (47:1), suggesting low Zn bioavailability, and not different from each other ($P=0.07$). There were no differences in Zn concentration or phytate:Zn molar ratio in the maize recipes. Nitrogen, tryptophan and lysine concentrations were higher in the cooked NEM than CM; nitrogen was higher in the cooked NEB than CB ($P<0.05$). *In vitro* protein digestibility was comparable (82-83%) for NEM and CM and higher for NEB (84%) than for CB (82%). The higher nutrient concentrations + similar bioavailability (protein in NEM, Zn in NEB), same nutrient concentrations + higher bioavailability (Fe in NEM) or higher nutrient concentrations + higher bioavailability (protein in NEB) can translate into more nutrients absorbed and utilized by the body.

Key words: Bioavailability, maize, beans, nutrients, biofortification

Introduction

Food-based approaches for addressing nutrient deficiencies include food fortification, dietary diversity, and more recently, crop biofortification. With biofortification, the nutrient levels of staple crops are naturally increased through conventional plant breeding and modern biotechnology (Nestel and others, 2006). To achieve biofortified crops, high-nutrient plants are crossed with commercially successful, locally important and/or agronomically superior plants. Through a succession of crosses that are closely monitored by plant breeders, progeny are selected which maintain the desirable characteristics of the parent plants, such as high nutrient levels and agronomically favorable traits. The International Center for Maize and Wheat Improvement (CIMMYT) in Mexico has followed this path to develop maize with twice the levels of tryptophan and lysine found in conventional maize; this maize is known as quality protein maize (QPM) or, its predecessor, opaque-2 (Krivanek and others, 2007). The International Center for Tropical Agriculture (CIAT) in Colombia has also used conventional plant breeding to develop beans with elevated iron and zinc levels in comparison with conventional beans (Blair and others, 2009a).

Biofortified crops are those with higher nutrient levels and proven efficacy in improving human nutrition. The QPM used in this study meets this criteria; opaque-2 or QPM has been shown to improve the protein status of severely malnourished children or children recuperating from severe malnutrition, either compared with conventional maize (Graham and others, 1989; Morales & Graham, 1993) or compared with casein (Morales & Graham, 1993) or skim milk (Reddy & Gupta, 1974). Further, a meta-analysis of eight efficacy trials carried out with pre-school children in Latin America or Africa estimated an 8 and 9% improvement in children's height and weight, respectively, during the intervention period when they consumed QPM compared with conventional maize (Gunaratna, 2007). The higher-mineral beans have not been evaluated for their efficacy in improving human nutrition but have shown 25 mg/kg and 10 mg/kg increments in iron and zinc concentration, respectively, over conventional beans (MW

Blair, unpublished data). In this manuscript, the QPM and higher-mineral beans will be referred to as nutritionally enhanced maize and beans, respectively.

The efficacy of the combination of these nutritionally enhanced crops in improving the nutritional status of pre-school children was tested in Colombian daycare centers (Blair, 2007). A sub-study was carried out to evaluate nutrient bioavailability in meals prepared in the daycare centers with nutritionally enhanced or conventional beans and maize. The purpose was to explore if there were differences in nutrient concentrations and bioavailability in the meals served to the study children which could explain the impact of the crops on the children's nutritional status. Proxies were used for bioavailability of zinc (phytate:zinc molar ratio), iron (*in vitro* iron dialyzability) and protein (*in vitro* protein digestibility).

Materials and Methods

Study context

This study took place in the context of a larger efficacy study whose objective was to evaluate the nutritional impact of nutritionally enhanced beans and maize on pre-school children aged 2 to 5 y (Blair, 2007). Eight daycare centers from socioeconomic strata 1 and 2 (where 1 is the lowest and 6 is the highest) in a large Colombian city were randomly assigned to receive for 6 months high-mineral beans and quality protein maize (n=2), conventional beans and maize (n=3), or an iron supplement providing 10 mg of iron (n=3) (Fig. 1). The beans and maize were produced and provided by the study team and distributed monthly to the centers; other ingredients for the meals prepared at the center were purchased with government-provided funds (through the *Instituto Colombiano de Bienestar Familiar*) or with private funds. The centers receiving beans and maize prepared bean and maize meals or snacks 2 times per week. The centers receiving the supplement provided the iron to the children 1 time per week.

Beans and maize

The beans (*Phaseolus vulgaris*) and maize (*Zea mays*) used in the study were developed by CIAT and CIMMYT, respectively, and were multiplied for the efficacy study by the Fundación para la Investigación y Desarrollo Agrícola (FIDAR). The nutritionally enhanced beans provided during the time the meals were sampled were primarily NUA35 with some NUA45 (Table 1). Previous analyses suggested that these beans had mean iron concentrations of 77.7 mg/kg (NUA35) and 73.7 mg/kg (NUA45) and mean zinc concentrations of 33.2 mg/kg (NUA35) and 28.7 mg/kg (NUA45) (Carolina Astudillo, CIAT, personal communication), while the conventional beans were CAL96 which had been characterized as having 60.4 mg/kg and 30.9 mg/kg mean iron and zinc, respectively (Carolina Astudillo, CIAT, personal communication). The nutritionally enhanced quality protein maize CML491 was selected for its higher tryptophan (0.092%) and lysine (0.421%) content than conventional maize DK777 (tryptophan 0.054%, lysine 0.254%) (José Restrepo, unpublished observations). Beans and maize were harvested by FIDAR, dried to 13% and 14% humidity for maize and beans, respectively, sorted in 1 kg batches, packaged in polypropylene bags at the start of the efficacy trial and subsequently in paper bags for maize alone, labeled, and delivered to the Universidad del Valle which then distributed the foods to the corresponding daycare centers.

Meals prepared with beans and maize

All daycare centers receiving beans and maize were provided with a recipe book and training to standardize preparation of meals with these foods. For two of the centers, meals and snacks were prepared, on a rotating basis, by mothers of children attending the daycare. For three of the centers, food preparation was done by cooking staff. Both of these groups will be referred to as cooking staff. Daycare centers often had to make adjustments to the standardized recipes given limitations in the kitchen facilities (for example, availability of blender). For this analysis, two relatively simple preparations were selected, which were considered to require the least amount of modifications by the cooking staff: *fríjoles sancochados* (a bean stew) and *mazamorra* (a maize-milk combination) (Table 2). These, as well as the other bean and maize recipes prepared by the cooking staff, were prepared at most 4 times per month, to avoid monotony and rejection of these foods by the children.

Meal sampling at daycare centers

The study was designed to collect, on two separate occasions, bean and maize meal samples from the five centers providing these meals to the children (Fig. 1). At each sampling point, two 75 g samples were obtained as follows. In the pots originally used to cook the bean and maize recipes, the cooked meals were stirred thoroughly by the cooking staff. The cooking staff served two portions of the meal in two separate acid-washed plastic containers (with 80 mL capacity). Staff were asked what ingredients they used in the recipe; these were noted. Samples were refrigerated on ice, transported to the Universidad del Valle, and frozen at -80ºC until transported on dry ice to CIAT for analyses.

Sample preparation

At CIAT, samples were maintained at -80ºC in their plastic containers. Samples were divided in two using a stainless steel knife. Half of each sample was lyophilized (Labconco

Corporation, Kansas City, MO) over 4 d and then ground to a homogenous flour with a locally produced zirconium-ball mill to avoid contamination with minerals. Two aliquots of each sample were used in subsequent analyses. All chemicals and enzymes were purchased from Sigma Chemical Co. (St. Louis, MO, USA), unless otherwise stated and all water used was 18MΩ (Synergy, Millipore SAS, Molshein, France).

***In vitro* iron dialyzability of cooked bean recipes**

Dialyzable iron was measured using the method by Argyri and others (2009) which is an adaptation of the method developed by Kapsokefalou and Miller (1991). *In vitro* iron dialyzability methods are highly correlated with *in vivo* iron bioavailability measures ($r \geq 0.92$) and are considered appropriate for screening purposes (Sandberg, 2005). In the adapted method, 1 g of the cooked recipes was dissolved in 10 mL 18MΩ water and the pH adjusted to 2.8 with 6 M HCl; 2 mL aliquots were transferred to 6-well plates (Corning Incorporated, Corning NY). 1 mL of a pepsin solution (4 g porcine pepsin suspended in 0.1 M HCl) was added to each well. Covered plates were placed in a 65 RPM reciprocal shaking water bath (Thermo Fisher Scientific, Marietta, Ohio) at 37°C for 2 h. Plates were removed from the water bath and dialysis membrane (Spectrum Laboratories, Rancho Dominguez CA, USA) of 6000-8000 molecular weight cut-off was secured to an insert ring placed over each well, allowing the membrane to have contact with the well contents. 2 mL of pH 6.3 PIPES solution (0.15 M PIPES adjusted to pH 6.3 using concentrated HCl) was added on top of each insert, gradually diffusing through the membrane and adjusting the pH of samples to 6.3. After 30 min in the 37°C shaking water bath, the inserts were temporarily lifted to add 0.5 mL of a pancreatin-bile solution (0.2 g porcine pancreatin and 1.2 g bile extract suspended in 100 mL 0.1 M NaHCO3) to each well. Inserts were placed over the wells again and the plates were put in the 37°C shaking water bath for 2 h. Plates were removed from the water bath, inserts were removed, dialysates centrifuged (Eppendorf AG, Hamburg, Germany) at 10,000 *g* for 20 min, and supernatants placed in 15 mL tubes.

To prepare the samples for iron concentration analysis, 0.25 mL reducing protein precipitant solution (100 g trichloroacetic acid, 50 g hydroxylamine monohydrochloride and 100 mL concentrated HCl taken up to 1 L of solution with 18MΩ water) was added to 0.5 mL of the supernatant. After overnight storage at room temperature, the samples were centrifuged at 5000 *g* for 10 min, and 0.1 mL aliquots of the supernatant were transferred to a 96-well plate (Corning Incorporated, Corning, NY). 0.225 mL of a ferrozine solution (1 part ferrozine solution 5mg/mL and 8 parts HEPES buffer 0.3 M, pH 7.5) was added to each well. After 1 h, absorbances were read in a spectrophotometer (µQuant, Biotek Instrument, Winooski, Vermont) at 562 nm. Iron concentration was calculated from a standard curve generated with $FeCl_3$ standards.

Results were expressed as % dialyzable iron:

$$\frac{\text{Total [Fe] in dialysate (mg/mL) x Total volume dialysate (mL)}}{\text{Total Fe in food sample (mg)}} \text{ X } 100$$

The iron concentration of the undigested food sample was determined as described below; this value was multiplied by the weight (expressed in kg) of the bean or maize sample to generate the denominator in the equation. The numerator was calculated from 10 replicates per sample, the denominator was calculated from 1 replicate per sample.

In vitro protein digestibility of cooked maize recipes

The method of Hsu and others (1977), modified by McDonough and others (1990), was used. This method yields data that are highly correlated (r=0.90) with *in vivo* results in rats (Hsu and others, 1977). Briefly, samples or a casein-sodium salt from bovine milk containing 10 mg of N were dissolved in 2.5 mL of water. To this, 2.5 mL NaOH 0.2N was added. The solution was incubated for 30 min in a 37°C 65 RPM shaking water bath. Then 5.0 mL HCl 0.075N was added and the pH adjusted to 8.0. 2 mL of a multi-enzyme solution (4 mg trypsin, 4.48 mg chymotrypsin, 1.02 mg peptidase) was added. The pH was monitored for 10 min and the percent digestibility was calculated using the formula:

% digestibility = 210.46 – 18.10X, where X is the pH at 10 min.

4 replicates were run for each cooked recipe.

Nutrient determinations

The iron and zinc concentrations (mg/kg) of uncooked maize and beans and cooked maize and bean recipes were determined in 2 replicates using atomic absorption spectrophotometry (Benton-Jones and others, 1991). After acid digestion of the samples, nitrogen (g/kg) was determined colorimetrically (Skalar Analytical BV, 1995). Colorimetric methods were also used to measure tryptophan (Villegas and others, 1992 as modified by Nurit and others (2008)) and lysine (Tsai and others, 1972); these were determined in duplicate and expressed as % of total protein. Total phytate concentration (mg/100 g) was determined colorimetrically by an adaptation (Blair and others, 2009b) of standard methods (Burbano and others 1995; Xu and others 1992). The intention was not to discriminate among inositol phosphates (IPs), but rather to quantify total phytate concentration. For this purpose, the use of a colorimetric method is appropriate.

Phytate:zinc molar ratio

The phytate:zinc molar ratio was calculated as follows (IZiNCG, 2004):

$$\frac{\text{Phytate concentration (mg/100 g)/660}}{\text{Zinc concentration (mg/100 g)/65.4}}$$

This molar ratio is considered a proxy zinc bioavailability measure by several international organizations (WHO/FAO/IAEA, 1996). Other researchers have used the molecular weight of IP6 (660) to estimate the molar ratio of total phytates to zinc for maize because maize is composed of ~95% IP6 (Hambidge and others 2004). Similarly, IP6 constitutes ~96% of the IPs in common bean (Alonso and others 2001). Therefore, it is appropriate to use 660 as the molecular weight for total phytates for these crops because IP6 is the main IP.

Statistical analyses

Statistical analyses were completed with Stata v9 (StataCorp, Texas, USA). All values were log-transformed to better approximate a normal distribution and Student's t-test was performed between the recipes prepared with nutritionally enhanced and conventional crops. Means were considered to be statistically significantly different if $P<0.05$.

Results

Meal samples

For both daycare centers in the nutritionally enhanced group, bean and maize meal samples were obtained at two time points, as planned (Table 3). For the three day care centers in the conventional crops group, samples were obtained at one time point for all three, and second samples were obtained for only one of the centers. Deviations from the standard recipes, based on cooking staff's report of what ingredients were used in the recipes, are summarized in Table 4. Staff added a variety of ingredients (n=10) to the bean meal as compared to the standardized recipe, omitting up to three ingredients in the bean recipe (carrot, pumpkin, onion), adding up to two ingredients to the maize recipe (sodium bicarbonate and cinnamon) and omitting no ingredients in the maize recipe.

Nutrient profile of uncooked beans and maize

The iron, zinc, nitrogen, tryptophan and lysine profile of the uncooked nutritionally enhanced beans and maize are listed in Table 1. The mean iron value for the conventional beans (57.1 mg/kg) was lower than the nutritionally enhanced beans (62.8 mg/kg for NUA35 and 64.8 mg/kg for NUA45); but this difference was not statistically significantly different ($P \geq 0.05$). Mean zinc was higher for NUA35 (28.7 mg/kg) than for CAL96 (21.3 mg/kg) ($P<0.05$); there were no differences in zinc values between NUA45 (24.0 mg/kg) and CAL96 ($P \geq 0.05$). Mean nitrogen (~30-31 g/kg) values were comparable among the three bean types ($P \geq 0.05$); tryptophan values (~0.20%) were similar among the bean types.

For maize, the nutritionally enhanced CML491 had lower mean iron (11.5 mg/kg) and zinc (14.9 mg/kg) values than the conventional maize (15.7 and 22.8 mg/kg, respectively) ($P<0.05$). Nitrogen levels were comparable in both maize types (~15 g/kg) ($P \geq 0.05$) while tryptophan and lysine levels were higher in CML491 (0.084 and 0.366%, respectively) than in DK777 (0.054 and 0.254%, respectively) ($P<0.05$).

Nutrient profile of cooked bean and maize recipes

The iron (~45 mg/kg) and phytate concentration (~900 mg/100 g) in the cooked recipes prepared with nutritionally enhanced and conventional beans were statistically comparable ($P \geq 0.05$); the nitrogen and zinc concentrations were higher ($P<0.01$) in the recipes prepared with nutritionally enhanced beans as compared with conventional beans (Table 5).

In the cooked recipes, nitrogen, tryptophan and lysine were statistically higher ($P<0.05$) in the maize recipe prepared with nutritionally enhanced maize than in the recipe prepared with conventional maize; there was no difference ($P>0.05$) in the iron, zinc and phytate concentration of the cooked recipes prepare with both maize types.

Proxy bioavailability measures for iron, zinc, and protein

In vitro iron dialyzability was not different between the bean recipes prepared with enhanced (9.52%) or conventional (9.72%) beans ($P>0.05$) (Table 5). *In vitro* iron dialyzability was higher for the recipes prepared with enhanced maize (37.01%) compared with conventional maize (32.24%) ($P<0.01$). There was a trend for the phytate:zinc molar ratio, a proxy for zinc bioavailability, of the bean and maize recipes cooked with nutritionally enhanced crops to be lower than the recipes prepared with the conventional crops; however these values were not statistically different ($P>0.05$). *In vitro* protein digestibility was higher ($P=0.01$) in the cooked recipes prepared with nutritionally enhanced beans (84.15%) than with conventional beans (82.31%). *In vitro* protein digestibility was comparable ($P=0.19$) in the cooked recipes prepared with nutritionally enhanced (83.01%) and conventional maize (82.30%).

Discussion

Nutrient concentrations in uncooked crops and in cooked recipes

Unexpectedly, the iron levels in the uncooked beans were not different between the conventional and nutritionally enhanced samples and the values (57.12-64.75 mg/kg) were within the range (54-74 mg/kg) observed by Ariza-Nieto and others (2007) for beans of the same Andean typology. These similarities carried over to the cooked recipes where there were no differences in the iron levels of the cooked bean recipe prepared with the two different bean types. In other words, these data suggest that the iron-differentiated bean intervention was not delivered to the pre-school children.

In contrast, the higher zinc levels in the uncooked nutritionally enhanced beans did result in higher zinc levels in the cooked beans prepared with the nutritionally enhanced beans. The zinc values observed (21.32-28.74 mg/kg) for the uncooked beans were at the higher end (17-25 mg/kg) observed for other Andean-type beans (Ariza-Nieto and others, 2007).

Iron and zinc concentrations were higher in the uncooked conventional maize than in the nutritionally enhanced maize; however the iron and zinc concentrations in the cooked recipes did not differ between the maize types. The uncooked values were similar to those found in CIMMYT germplasm pools and populations: 9.6-18.3 mg/kg Fe and 14.5-30.3 mg/kg Zn (Bänzinger & Long, 2000).

The higher nitrogen concentration in the recipes prepared with nutritionally enhanced beans was unexpected as the uncooked bean values were not different. This suggests that nitrogen-contributing ingredients in the *fríjol sancochado* recipe were disproportionately used when the nutritionally enhanced beans were cooked. The data collected on ingredients added or omitted to the recipe do not bear this out; however, amounts used in the recipes were not quantified.

Tryptophan and lysine levels were higher in the raw nutritionally enhanced maize as compared to the conventional maize; nitrogen levels were similar between the two maize types. As with the zinc in beans, for the amino acids this translated into higher tryptophan and lysine levels in the cooked maize recipes. Unexpectedly, nitrogen levels were also higher in the cooked maize recipes prepared with nutritionally enhanced maize. This difference is unlikely due to systematically more milk being added to the recipe prepared with the nutritionally enhanced maize, unless the cooking staff noted a difference in cooking with this maize and made adjustments to the recipe accordingly. Cooking amounts were not recorded; thus this hypothesis cannot be tested.

Bioavailability proxy measures in cooked recipes

There was no difference in the percent dialyzable iron in the cooked bean recipes prepared with enhanced or conventional beans, suggesting similar iron bioavailability. Using a similar *in vitro* methodology to the one we used, Lombardi and colleagues (1991, 1995) found the iron dialyzability of extruded mottled bean flour and cooked mottled beans to be ≤1.2% and of cooked white beans to be 3.89%, lower than what we observed. This difference could be due to the contamination iron from extrusion used in the 1991 Lombardi study which increased the denominator in the dialyzability equation thus decreasing the % dialyzable iron and also that in contrast to the Lombardi studies which used no ingredients other than beans, the carotenoid- and ascorbic acid-contributing ingredients in the current study could have increased the dialyzability in the bean meals (García-Casal and others, 1998; Cook & Reddy, 2001).

In contrast, the *in vitro* iron dialyzability of maize was higher in the recipe prepared with nutritionally enhanced compared with conventional maize. This was not driven by differences in the phytate:iron molar ratio which was comparable (~23-24:1) in both recipes. There is data to suggest that lysine enhances iron bioavailability in rats (Van Campen & Gross, 1969); however there are no *in vivo* comparisons of high- compared with low-lysine maize on iron bioavailability. It is notable that the *in vitro* iron dialyzability of the maize recipes was 3-4 times higher than for the bean recipes; this could be due to the 3-4 times lower phytate concentration in the maize recipes compared with the bean recipes.

Lower phytate:zinc molar ratios are suggestive of greater zinc bioavailability. Lower phytate:zinc molar ratios were observed for the recipes prepared with nutritionally enhanced crops compared with the recipes prepared with the conventional crops; however, these differences were not statistically significant. Several international organizations offer a classification system for estimating zinc bioavailability based on phytate:zinc molar ratio: <5:1 suggests high bioavailability, 5:1 to 15:1 medium, and >15:1 low (WHO/FAO/IAEA, 1996). Thus, the recipes analyzed with either type of maize or beans would be classified as low bioavailability. The phytate:zinc molar ratios observed for recipes prepared in this study are in the 19:1 to 56:1 range noted by the International Zinc Nutrition Consultative Group (IZiNCG, 2004) for beans and lentils and in the 22:1 to 53:1 range noted by IZiNCG for whole-grain cereals such as maize.

The *in vitro* protein digestibility of the maize-milk preparation was in the order of 82-83%, regardless of the maize type used. These values are higher than other digestibility studies of QPM alone; this is not unexpected given the higher digestibility of milk (IOM, 2005, p 690), which was added to the maize recipes. For example, the *in vitro* protein digestibility was 77-80% for boiled conventional maize and 80% for boiled QPM (Fufa and others, 2003). For nixtamilized QPM flour, *in vitro* protein digestibility ranged from 73 to 79% depending on the different processing conditions examined (Milán-Carrillo and others, 2004). The *in vitro* protein digestibility of cooked recipes was higher for the nutritionally enhanced beans than the conventional beans, but in the same order of magnitude as the maize. Rehman and colleagues (2004) found the *in vitro* protein digestibility of cooked red and white kidney beans to be ~64%, lower then what we found. However, the methodology they used was different: they digested the samples with pepsin alone, incubated for 23 h, filtered the residue through Celite and used nitrogen content to determine digestibility (Price and others, 1979). Another study that used the same *in vitro* methodology for protein digestibility as in the current study, reported protein digestibility values in the 81-83% range for extruded whole pinto bean flour (Balandrán-Quintana and others, 1998).

The protein digestibility-corrected amino acid score (PDCAAS) is one way to measure quality protein in a meal or diet (IOM, 2005, p 689). The formula for % PDCAAS is as follows:

$$\frac{\text{mg of limiting amino acid in 1-g test protein}}{\text{mg of same amino acid in 1-g reference protein}} \times \text{\% true digestibility}$$

Assuming that in the maize recipes the only amino acids with different values between those prepared with nutritionally enhanced and conventional maize are tryptophan and lysine, and that protein (N * 6.25), tryptophan, lysine and digestibility values are as listed in Table 5, the PDCAAS is 64.1% for the enhanced maize and 43.6% for the conventional maize recipes. These values are consistent with those obtained by researchers who calculated the PDCAAS of 15 QPM and five commercial maize cultivars (Zarkadas and others, 2000); for those investigators, the digestibility portion of the equation was taken from published data, not data generated with these varieties. In that study, PDCAAS ranged from 54 to 72% in the lyophilized QPM varieties and 30-50% in the lyophilized commercial maize.

Potential of enhanced crops to improve human nutrition

Nutritionally enhanced crops can improve human nutrition if they translate into more nutrients absorbed and utilized by the body. This can be achieved in one of three ways: higher nutrient concentrations but same bioavailability as conventional crops, same nutrient concentrations but higher bioavailability as conventional crops, or higher nutrient concentrations combined with higher bioavailability.

The first option for improving human nutrition through enhanced crops (higher nutrient concentration, same nutrient bioavailability) most closely describes the results observed in this study with zinc in beans and quality protein in maize. Zinc concentration was higher in the bean recipes prepared with enhanced versus conventional beans, and zinc bioavailability, as proxied by phytate:zinc molar ratio, was similar in the bean recipes prepared with both bean types. Given the high phytate:zinc molar ratio, it is unlikely that statistically different ratios would lead to greater zinc bioavailability, unless the ratio could be reduced to below 15:1 for the enhanced bean recipe. Breeding strategies should focus on increasing the zinc content in the enhanced beans and reducing the phytate:zinc molar ratio.

For protein quality, the same trend was observed: higher amino acid and protein levels in the cooked maize recipes prepared with enhanced maize yet similar *in vitro* protein digestibility values as maize recipes cooked with conventional maize. The PDCAAS calculation of the cooked recipes supports the assertion that higher amino acid levels from enhanced maize coupled with similar digestibility values as conventional maize yield more quality protein in the diet. This enhanced maize is likely to benefit children who consume a low proportion of dietary protein from animal-source foods. Using FAO food-balance data, the Latin American and Caribbean countries with the lowest proportion of dietary protein from animal sources from 2001 to 2003 were as follows (FAO, 2007a), where the total proportion of animal and plant sources of protein was approximately 90% (not 100%): El Salvador (28%), Guatemala (22%), Haiti (14%), Honduras (33%) and Nicaragua (22%). With the exception of Haiti, these countries are also high maize-consuming (FAO, 2007b), as defined by the proportion of per capita energy intake consumed from maize: El Salvador (31%), Guatemala (39%), Honduras (31%) and Nicaragua (21%). QPM cultivars have been commercially released in Nicaragua in 2007, in El Salvador, Haiti, Honduras and Panamá in 2008, and are planned for Guatemala in 2009 (Gary Atlin, CIMMYT, personal communication). The nutritional impact of these QPM cultivar releases on young children's maize intake and nutritional status should be monitored.

The second option for improving human nutrition through enhanced crops (same nutrient concentration, higher nutrient bioavailability) describes the results observed in this study with iron in the cooked maize recipe. The greater *in vitro* iron dialyzability may have more to do with the other ingredients in the recipe, or the cooking preparation, than with the maize *per se*, however, it highlights the importance of examining the bioavailability of biofortified crops that are cooked using local recipes and methods. Further, it is worthwhile mentioning that during the years-long process of developing biofortified crops through conventional plant breeding, there may be unintended consequences, positive or negative, of selecting for crops that are agronomically and nutritionally superior. For example, a high correlation between iron and zinc concentration is found in beans (Beebe and others, 2000), suggesting that selection for crops with high levels of one of these nutrients will yield crops with high levels of the other nutrient. Therefore, it is possible that selection for maize with higher tryptophan and lysine can unintentionally influence other maize constituents that lead to greater iron dialyzability; this requires further study.

The third option for improving human nutrition through enhanced crops (higher nutrient concentration, higher nutrient bioavailability) describes the results obtained with nutritionally enhanced beans for protein. As with nutritionally enhanced maize, these beans can be promoted in those countries where they contribute importantly to protein intakes.

Study strengths and limitations

The small sample size limited the statistical power to detect differences in nutrient values between nutritionally enhanced and conventional crops. While attempts were made to standardize preparation methods and ingredients, these varied among the daycare centers. However, these varied methods better reflect the cooking conditions that these crops will be exposed to in real-life, non-experimental settings.

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Figure 1. Study design.

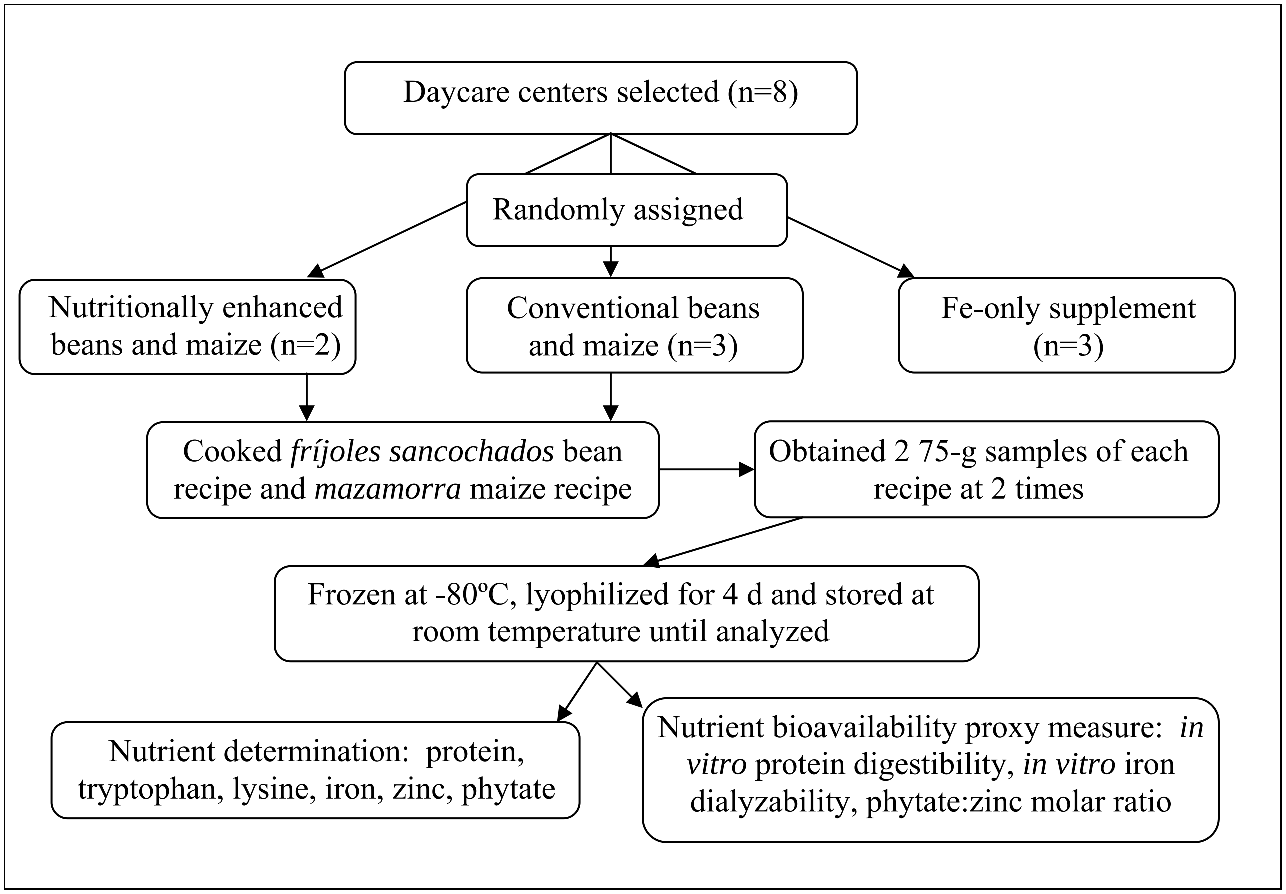

Table 1. Uncooked bean and maize sample characteristics.

Sample[2]	Name	Mean (SEM)[1] Fe (mg/kg), n=3	Zn (mg/kg), n=3	N (g/kg), n=3	Tryptophan (% total protein), n=1	Lysine (% total protein), n=1
Beans						
Nutritionally enhanced	NUA35	62.75 (0.127)^{a}	28.74 (0.430)^{b}	30.31 (0.726)^{a}	0.202	NA[3]
	NUA45	64.75 (1.947)^{a}	23.99 (0.492)^{a}	30.16 (1.365)^{a}	0.203	NA
Conventional	CAL96	57.12 (7.036)^{a}	21.32 (1.086)^{a}	31.3 (0.700)^{a}	0.208	NA
Maize						
Nutritionally enhanced	CML491	11.50 (0.388)^{a}	14.89 (0.640)^{a}	14.89 (0.868)^{a}	0.084	0.366
Conventional	DK777	15.74 (0.788)^{b}	22.82 (0.963)^{b}	14.97 (0.149)^{a}	0.054	0.254

[1] For each crop and nutrient, values with no letters in common are statistically significantly different (*P*<0.05). For beans, NUA35 and NUA45 were each compared using Student's t-test to CAL96. No statistical tests were run for tryptophan and lysine as there was only one value per crop type.

[2] The beans were grown in FIDAR and CIAT fields in 5 sites in Colombia and the maize was grown in FIDAR fields in Palmira, Colombia.

[3] NA = Not analyzed

Table 2. Ingredients in standard bean and maize recipes.

Bean recipe: *Fríjoles Sancochados*		**Maize recipe: *Mazamorra***	
Ingredient	**Quantity (g)**	**Ingredient**	**Quantity (g)**
Beans	888 g	Maize	500 g
Carrot	80 g	Water	1200 mL
White onion	120 g	Whole milk	880 mL
Pumpkin	80 g	Sugar or *panela*[1]	60 g
Oil	10 g		
Tomato	180 g		
Scallion and tomato paste	80 g and 120 g		
Salt, pepper and cumin	To taste		

[1] Sugar-cane juice that after repeated boiling solidifies; used as a sweetener.

Table 3. Daycares sampled.

Crop	Month	Nutritionally enhanced (n)	Conventional (n)	Total (n)
Beans	November 2006	2	3	5
Beans	December 2006	2	1	3
Beans	February 2007	0	0	0
Maize	November 2006	2	3	5
Maize	December 2006	1	1	2
Maize	February 2007	1	0	1

1 Table 4. Ingredients reported by cooking staff, compared with standardized recipe.

Ingredients added (n reported)		**Ingredients omitted (n reported)**	
Bean recipe	**Maize recipe**	**Bean recipe**	**Maize recipe**
Potato (6)	Sodium bicarbonate (2)	Carrot (7)	None reported
Cimarrón[1] (1)	Cinnamon (1)	Pumpkin (3)	
Cilantro (4)		Onion (2)	
Red or green pepper (5)			
Garlic (6)			
Plantain (5)			
Artificial color (5)			
Bouillon cube (5)			
Spinach (1)			
Tomillo[1] (1)			

2 [1] Aromatic spices

3

Table 5. Nutrient values and *in vitro* proxy measures for protein, iron and zinc bioavailability for nutritionally enhanced and conventional beans and maize, in cooked recipes.

	Mean (SEM)								
	N (g/kg)	**Tryptophan (% total protein)**	**Lysine (% total protein)**	***In vitro* protein digestibility (%)**	**Fe (mg/kg)**	**Zn (mg/kg)**	**Phytate (mg/100g)**	***In vitro* iron dialyzabillity (%)**	**Phytate:zinc molar ratio**
Beans									
Nutritionally enhanced (n=8)	30.32 (0.76)	NA[1]	NA	84.15 (0.38)	45.15 (3.79)	24.28 (1.29)	871.29 (90.29)	9.52 (0.66)	36.28 (4.35)
Conventional (n=8)	26.06 (0.53)	NA	NA	82.31 (0.52)	45.83 (2.59)	20.15 (0.33)	949.29 (67.28)	9.72 (1.32)	46.75 (3.41)
T-test *P*-value	0.0004	NA	NA	0.01	0.76	0.005	0.43	0.46	0.07
Maize									
Nutritionally	16.66	0.13 (0.01)	0.54	83.01 (0.35)	7.55	9.25	217.59	37.01 (3.48)	22.59 (1.78)

enhanced (n=8)	(0.99)		(0.03)		(0.72)	(0.93)	(32.96)		
Conventional (n=8)	12.04 (0.12)	0.09 (0.01)	0.31 (0.02)	82.30 (0.37)	8.19 (0.87)	7.16 (0.82)	226.58 (37.54)	32.24 (3.28)	33.29 (5.47)
T-test *P*-value	0.0001	0.02	< 0.0001	0.19	0.60	0.11	0.87	0.003	0.12

[1] NA = Not analyzed