Laboratory Enhancements to Improve Human Nutrition
CIAT Progress Report to Monsanto Fund

In December 2007, the Monsanto Fund approved a US$281,000 grant to the Centro Internacional de Agricultura Tropical (CIAT) based in Colombia to carry out laboratory enhancements to improve human nutrition. These funds have been used in the course of 2008 to purchase equipment for the newly formed Nutrition Quality Laboratory at CIAT. This document summarizes the advances the Laboratory has had from January 2009 to July 2009 and corresponds to the semi-annual progress report requested by the Monsanto Fund.

As noted in the Laboratory’s 2008 Annual Report, 2009 has been dedicated to five major tasks:

1. Complete protocols and procedures
2. Analyze samples
3. Implement quality-control activities
4. Prepare articles
5. Develop proposals

Advances on these and other tasks are noted below.

Complete protocols and procedures

Three manuals have been drafted in the reporting period: Analytical Methods, General, and Quality. For beans, maize and rice, a homogeneous sample has been developed that will be used in all analyses as a quality-control measure. The Cost Analysis of Research and Breeding Operations (CARBO) methodology has been completed and prices set for Laboratory services.

Analyze samples (Annex 1)

A total of 3923 samples were received during the reporting period; 3895 (99%) of these have been analyzed (Annex 1). During the reporting period, several projects were begun, continued or completed (Annex 2).

Implement quality-control activities

As part of the Laboratory’s efforts to obtain certification by ISO, a quality-control system is being implemented under the leadership of Dayron Gutierrez. New quality-control measures (such as use of the control card) have been implemented during the reporting period for the in vitro iron bioavailability method. With respect to inter-laboratory trials, contacts have been made with several international laboratories that will analyze the same food sample in the second semester of 2009 for tryptophan quantification, in vitro protein digestibility, in vitro iron dialyzability, in vitro carotenoid bioaccessibility, and carotenoid quantification.

Prepare articles
The Laboratory’s first peer-reviewed scientific article was published (Annex 3). Several other manuscripts are in preparation (Annex 2).

Develop proposals

In response to Colciencia’s (Scientific Ministry of the Colombian government) call for concept notes in health, three notes were prepared, of which one was selected to compete in the next round, as a full proposal.

- Evaluation of the carotenoid bioaccessibility of the main vitamin A dietary sources in Colombia’s Atlantic coast, for the purpose of improving pre-schooler’s vitamin A status. Colciencias code PRE00485021329. Selected for next round.
- Evaluation of the antioxidant contribution of fruits and vegetables in 5 regions of Colombia, and their promotion to address cardiovascular diseases. Colciencias code PRE00485021403.
- Evaluating NIRS technology to predict zinc concentration in beans. Colciencias code PRE00485021555.

Develop Laboratory brochures (Annexes 4 and 5)

New Laboratory brochures were developed in English and in Spanish.

Hire Laboratory personnel

Chemist María Luisa Cortés joined the Laboratory in a part-time capacity in 2009. Effective 1 July 2009, Chemist Ingrid Aragón will join the Laboratory as a full-time staff person. During the January-July 2009 period, three students completed their internship or thesis activities in the Laboratory (Annex 6).

Receive or impart trainings

During the reporting period, Dayron Gutiérrez initiated his ISO/IEC 17025 52-hour training course in Certification Auditing. María Luisa Cortés trained Paulo Izquierdo, from the Andean Bean Program at CIAT, and Katherine Loaiza from CIAT’s Rice Breeding Program, on using NIRS.

Offer presentations (Annex 7)

An oral presentation and two poster presentations were given in Colombia during the reporting period. Further, several abstracts were submitted to upcoming conferences; confirmation of the acceptance of these abstracts has not been confirmed. The titles of these abstracts are listed below.
Obtain complementary funding for Laboratory

CIAT’s Tropical Fruits Program provided complementary funding to purchase inputs such as an HPLC column and reagents to establish in the Laboratory a method to quantify sugars (glucose, fructose, sacarose) and organic acids (malic acid, citric acid, oxalic acid).

Host 86 visitors (Annex 8)

Eighty six people visited the Nutrition Quality Laboratory between December 2008 and June 2009, as follows:

- December 15: CIAT’s Board of Directors (7 people)
- December 19: Monsanto’s Head of Research and Development (1 person)
- February 20: Centro de Investigaciones, Escuela de Nutrición y Dietética, Universidad de Antioquia (2 people)
- March 12: Caldono (Cauca Department) community members, Municipal Ministries of Education and Agriculture, representatives of the Department’s PANES program (50 people)
- April 28: Instituto Colombiano de Bienestar Familiar from Cauca Department (7 people)
- May 8: Representatives from ADA Afro-Latino Development Alliance (5 people)
- May 14: Delegation from the Nariño Governor’s office (8 people)
- June 3: CGIAR Executive Committee (6 people)
**Annex 1.** Samples received in the Laboratory during the reporting period.

<table>
<thead>
<tr>
<th>Institution</th>
<th>Number of Samples</th>
<th>Sample Type</th>
<th>Analyses Requested</th>
<th>Analyses Completed</th>
</tr>
</thead>
<tbody>
<tr>
<td>AgroSalud, Universidad del Cauca</td>
<td>360</td>
<td>Maize</td>
<td>- Tryptophan quantification</td>
<td>- Tryptophan quantification</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- In vitro protein digestibility</td>
<td>- In vitro protein digestibility</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Soluble-protein quantification</td>
<td>- Soluble-protein quantification</td>
</tr>
<tr>
<td>CIAT Cassava Improvement Program</td>
<td>1982</td>
<td>Cassava</td>
<td>- Carotenoid quantification</td>
<td>- Carotenoid quantification</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Determination of antioxidant activity</td>
<td>- Determination of antioxidant activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Protein by NIRS</td>
<td></td>
</tr>
<tr>
<td>AgroSalud, Colombia</td>
<td>6</td>
<td>Maize</td>
<td>- In vitro iron dialyzability</td>
<td>- In vitro iron dialyzability</td>
</tr>
<tr>
<td>AgroSalud, Universidad Industrial de Santander</td>
<td>12</td>
<td>Foliar extract</td>
<td>- In vitro iron dialyzability</td>
<td>- In vitro iron dialyzability</td>
</tr>
<tr>
<td>AgroSalud, Empresa PROPOMIEL</td>
<td>57</td>
<td>Meat, dairy products</td>
<td>- In vitro protein digestibility</td>
<td>- In vitro protein digestibility</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Soluble protein quantification</td>
<td>- Soluble protein quantification</td>
</tr>
<tr>
<td>AgroSalud, Clayuca</td>
<td>36</td>
<td>Maize</td>
<td>- Tryptophan quantification</td>
<td>- Tryptophan quantification</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- In vitro protein digestibility</td>
<td>- In vitro protein digestibility</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Soluble-protein quantification</td>
<td>- Soluble-protein quantification</td>
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<tr>
<td>CIAT Biotechnology</td>
<td>24</td>
<td>Cassava</td>
<td>- Carotenoid quantification</td>
<td>- Carotenoid quantification</td>
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<tr>
<td>AgroSalud, Cauca Project</td>
<td>75</td>
<td>Beans, Maize</td>
<td>- Tryptophan quantification</td>
<td>- Tryptophan quantification</td>
</tr>
<tr>
<td>AgroSalud, Effect of cooking on iron bioavailability</td>
<td>36</td>
<td>Rice</td>
<td>- In vitro iron dialyzability</td>
<td>- In vitro iron dialyzability</td>
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<td>CIAT Bean Program</td>
<td>1335</td>
<td>Beans</td>
<td>- Iron quantification</td>
<td>- Iron quantification</td>
</tr>
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<td><strong>Total</strong></td>
<td><strong>3923</strong></td>
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<table>
<thead>
<tr>
<th>Project</th>
<th>Responsible</th>
<th>Collaborators</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Validation of an <em>in vitro</em> iron dialyzability method</td>
<td>Ingrid Aragón</td>
<td>Dayron Gutiérrez, Darwin Ortiz, Universidad del Valle, AgroSalud</td>
<td>Completed</td>
</tr>
<tr>
<td>Comparison of an <em>in vitro</em> iron dialyzability method with <em>in vivo</em> iron bioavailability methods</td>
<td>Ingrid Aragón</td>
<td>Dayron Gutiérrez, Darwin Ortiz, Universidad del Valle, AgroSalud</td>
<td>Thesis completed; manuscript in preparation</td>
</tr>
<tr>
<td>Evaluation of the nutritional quality of leaf extracts prepared from the foliage of different biofortified crops</td>
<td>Sayda Pico</td>
<td>Darwin Ortiz, Ingrid Aragón, Dayron Gutiérrez, María Alejandra Lozano</td>
<td>Completed; manuscript in preparation</td>
</tr>
<tr>
<td>Protein-quality evaluation of different Colombian recipes prepared with biofortified maize</td>
<td>Paola Imbach</td>
<td>Dayron Gutiérrez, CIAT’s Cassava Program, CLAYUCA</td>
<td>Thesis completed; manuscript in preparation</td>
</tr>
<tr>
<td>The quantification of phytates via HPLC of a population of common beans (<em>Phaseolus vulgaris</em>) and identification of QTLs associated with them</td>
<td>María Alejandra Lozano</td>
<td>CIAT’s Bean Program, Universidad del Valle, AgroSalud</td>
<td>Thesis completed</td>
</tr>
<tr>
<td>Protein digestibility of meat and milk products processed with or without sugar-cane honey as a preservative</td>
<td>Dayron Gutierrez</td>
<td>AgroSalud, Empresa PROPMIEL</td>
<td>Completed; manuscript in preparation</td>
</tr>
<tr>
<td>Evaluation of the anti-oxidant capacity of cassava</td>
<td>Hiroko Kunori</td>
<td>CIAT’s Cassava Program</td>
<td>In process</td>
</tr>
<tr>
<td>Developing NIRS equations to evaluate minerals in beans</td>
<td>María Luisa Cortés</td>
<td>CIAT’s Bean Program, CIP</td>
<td>In process; manuscript in preparation</td>
</tr>
<tr>
<td>Evaluation of the phytate concentration of rice lines</td>
<td>Dayron Gutiérrez, Darwin Ortiz</td>
<td>CIAT’s Rice Program</td>
<td>Initiated, in process</td>
</tr>
<tr>
<td>Evaluation of the <em>in vitro</em> iron bioavailability of rice lines</td>
<td>Ingrid Aragón</td>
<td>CIAT’s Rice Program</td>
<td>Initiated, in process</td>
</tr>
<tr>
<td>NIRS analysis of 600 F1 cassava clones to develop calibration curves for crude protein</td>
<td>Darwin Ortiz</td>
<td>CIAT’s Cassava Program</td>
<td>Initiated, in process</td>
</tr>
<tr>
<td>NIRS evaluation of the carotenoid concentration of 80 high-carotenoid cassava clones</td>
<td>Darwin Ortiz</td>
<td>CIAT’s Cassava Program</td>
<td>Initiated, in process</td>
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<td>NIRS carotenoid-concentration evaluation of 350 F1 yellow-fleshed cassava clones</td>
<td>Darwin Ortiz</td>
<td>CIAT’s Cassava Program</td>
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</tbody>
</table>
Iron, Zinc, and Protein Bioavailability Proxy Measures of Meals Prepared with Nutritionally Enhanced Beans and Maize

H. PACHÓN, D.A. ORTIZ, C. ARAUJO, M.W. BLAIR, AND J. RESTREPO

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 > 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% to 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.

Keywords: beans, bioavailability, biofortification, maize, nutrients

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 Intl. 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 (Krivaneck and others 2007). The Intl. 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 and Graham 1993), or compared with casein (Morales and Graham 1993), or skim milk (Reddy and Gupta 1974). Further, a meta-analysis of 8 efficacy trials carried out with preschool 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 preschool children was tested in Colombian daycare centers (Blair 2007). A substudy 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 preschool children aged 2 to 5 y (Blair 2007). A total of 8 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 mo 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) (Figure 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 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 Univ. del Valle which then distributed the foods to the corresponding daycare centers.

Figure 1 – Study design.

Table 1 -- Uncooked bean and maize sample characteristics.

<table>
<thead>
<tr>
<th>Samplea</th>
<th>Name</th>
<th>Fe (mg/kg), n = 3</th>
<th>Zn (mg/kg), n = 3</th>
<th>N (g/kg), n = 3</th>
<th>Tryptophan (% total protein), n = 1</th>
<th>Lysine (% total protein), n = 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beans</td>
<td>NUA35</td>
<td>62.75 (0.127)a</td>
<td>28.74 (0.430)a</td>
<td>30.31 (0.726)a</td>
<td>0.202</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>NUA45</td>
<td>64.75 (1.947)a</td>
<td>23.99 (0.492)a</td>
<td>30.16 (1.365)a</td>
<td>0.203</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>CAL96</td>
<td>57.12 (7.036)a</td>
<td>21.32 (1.086)a</td>
<td>31.3 (0.700)a</td>
<td>0.208</td>
<td>NA</td>
</tr>
<tr>
<td>Maize</td>
<td>CML491</td>
<td>11.50 (0.389)a</td>
<td>14.89 (0.640)a</td>
<td>14.89 (0.868)a</td>
<td>0.084</td>
<td>0.366</td>
</tr>
<tr>
<td></td>
<td>DK777</td>
<td>15.74 (0.788)a</td>
<td>22.82 (0.963)a</td>
<td>14.97 (0.149)a</td>
<td>0.054</td>
<td>0.254</td>
</tr>
</tbody>
</table>

aFor 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 1 value per crop type.

bThe beans were grown in FIDAR and CIAT fields in 5 sites in Colombia and the maize was grown in FIDAR fields in Palmira, Colombia.

NA = not analyzed.
availability of blender). For this analysis, 2 relatively simple preparations were selected, which were considered to require the least amount of modifications by the cooking staff: frijoles sancochados (a bean stew) and maizamorra (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 2 separate occasions, bean and maize meal samples from the 5 centers providing these meals to the children (Figure 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 2 portions of the meal in 2 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 Univ. del Valle, and frozen at −80 °C until transported on dry ice to CIAT for analyses.

Sample preparation

At CIAT, samples were maintained at −80 °C in their plastic containers. Samples were divided in 2 using a stainless steel knife. Half of each sample was lyophilized (Labconco Corp., Kansas City, Mo., U.S.A.) 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., U.S.A.), unless otherwise stated and all water used was 18 MΩ (Synergy, Millipore SAS, Molsheim, 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 ≥ 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 18 MΩ water and the pH adjusted to 2.8 with 6 M HCl; 2 mL aliquots were transferred to 6-well plates (Corning Inc., Corning N.Y., U.S.A.). 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., U.S.A.) at 37 °C for 2 h. Plates were removed from the water bath and dialysis membranes (Spectrum Laboratories, Rancho Dominguez Calif., U.S.A.) of 6000 to 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. A total of 2 mL of pH 6.3 PIPES solution (0.15 M PIPES adjusted to pH 6.3 using concentrated HCl) were 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 10000 × 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 hydroxyamine monohydrchloride, and 100 mL concentrated HCl taken up to 1 L of solution with 18 MΩ water) were 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 Inc.). A total of 0.225 mL of a ferrozine solution (1 part ferrozine solution 5 mg/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, Vt., U.S.A.) at 562 nm. Iron concentration was calculated from a standard curve generated with FeCl₃ standards.

Results were expressed as percent dialyzable iron:

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

The iron concentration of the undigested food sample was determined as described subsequently; this value was multiplied by the weight (expressed in kilograms) 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 were added. The solution was incubated for 30 min in a 37 °C 65 RPM shaking water bath. Then 5 mL HCl 0.075N were added and the pH adjusted to 8. A total of 2 mL of a multienzyme solution (4 mg trypsin, 4.48 mg chymotrypsin, 1.02 mg peptidase) were added. The pH was monitored for 10 min and the percent digestibility was calculated using the formula:

\[
\% \text{ digestibility} = 210.46 - 18.10X, \text{ where } X \text{ is the pH at 10 min}
\]

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.

### Table 2 — Ingredients in standard bean and maize recipes.

<table>
<thead>
<tr>
<th>Bean recipe: Frijoles Sancochados</th>
<th>Maize recipe: Mazamorra</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ingredient</strong></td>
<td><strong>Quantity</strong></td>
</tr>
<tr>
<td>Beans</td>
<td>888 g</td>
</tr>
<tr>
<td>Carrot</td>
<td>80 g</td>
</tr>
<tr>
<td>White onion</td>
<td>120 g</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>80 g</td>
</tr>
<tr>
<td>Oil</td>
<td>10 g</td>
</tr>
<tr>
<td>Scallion and tomato paste</td>
<td>80 and 120 g</td>
</tr>
</tbody>
</table>

*Sugar-cane juice that after repeated boiling solidifies; used as a sweetener.*
Bioavailability of bean and maize meals... 

(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 percent of total protein. Total phytate concentration (mg/100 g) was determined colorimetrically by an adaptation (Blair and others 2009b) of standard methods (Xu and others 1992; Burbano and others 1995). 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.

Phytatezinc molar ratio

The phytatezinc molar ratio was calculated as follows (IZINCG 2004):

\[
\text{Phytate concentration (mg/100g)/660} \over \text{Zinc concentration (mg/100g)/65.4}
\]

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 approximately 95% IP6 (Hambridge and others 2004). Similarly, IP6 constitutes approximately 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 version 9 (StataCorp, Tex., U.S.A.). 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 2 time points, as planned (Table 3). For the 3 daycare centers in the conventional crops group, samples were obtained at 1 time point for all 3, and 2nd samples were obtained for only 1 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 3 ingredients in the bean recipe (carrot, pumpkin, onion), adding up to 2 ingredients to the maize recipe (sodium bicarbonate and cinnamon) and omitting no ingredients in the maize recipe.

Table 3 — Daycares sampled.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Month</th>
<th>Nutrititionally enhanced (( n ))</th>
<th>Conventionally (( n ))</th>
<th>Total (( n ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beans</td>
<td>November 2006</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Beans</td>
<td>December 2006</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Beans</td>
<td>February 2007</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maize</td>
<td>November 2006</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Maize</td>
<td>December 2006</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Maize</td>
<td>February 2007</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

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 (\( P > 0.05 \)). 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 mg/kg) and CAL96 (\( P > 0.05 \)). Mean nitrogen (approximately 30 to 31 g/kg) values were comparable among the 3 bean types (\( P > 0.05 \)); tryptophan values (approximately 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 (approximately 15 g/kg) (\( P > 0.05 \)). 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 (approximately 45 mg/kg) and phytate concentration (approximately 900 mg/100 g) in the cooked recipes prepared with nutritionally enhanced and conventional beans were statistically comparable (\( P > 0.05 \)); 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 prepared 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 phytatezinc molar ratio, a proxy for zinc bioavailability, of the
### Bioavailability of bean and maize meals

#### 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

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mean (SEM)</th>
<th>Phytate-zinc molar ratio</th>
<th>Phytate iron dialyzability (%)</th>
<th>Tryptophan (% total protein)</th>
<th>Lysine (% total protein)</th>
<th>In vitro protein digestibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutritionally enhanced (n = 8)</td>
<td>30.32 (0.76)</td>
<td>NA</td>
<td>NA</td>
<td>84.15 (3.82)</td>
<td>0.54 (0.03)</td>
<td>0.87 (0.03)</td>
</tr>
<tr>
<td>Conventional (n = 8)</td>
<td>26.06 (0.53)</td>
<td>NA</td>
<td>NA</td>
<td>82.31 (0.52)</td>
<td>0.56 (0.02)</td>
<td>0.80 (0.01)</td>
</tr>
<tr>
<td><strong>Maize</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutritionally enhanced (n = 8)</td>
<td>16.66 (1.00)</td>
<td>NA</td>
<td>NA</td>
<td>83.01 (0.35)</td>
<td>0.75 (0.03)</td>
<td>0.67 (0.03)</td>
</tr>
<tr>
<td>Conventional (n = 8)</td>
<td>12.04 (0.12)</td>
<td>NA</td>
<td>NA</td>
<td>82.30 (0.37)</td>
<td>0.75 (0.02)</td>
<td>0.67 (0.03)</td>
</tr>
</tbody>
</table>

**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 to 64.75 mg/kg) were within the range (54 to 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 2 different bean types. In other words, these data suggest that the iron-differentiated bean intervention was not delivered to the preschool 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 to 28.74 mg/kg) for the uncooked beans were at the higher end (17 to 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 to 18.3 mg/kg Fe and 14.5 to 30.3 mg/kg Zn (Bänziger and 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 *frijol 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 2 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 percent dialyzable iron and also 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 and 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 (approximately 23 to 24:1) in both recipes. There is data to suggest that lysine enhances iron bioavailability in rats (Van Campen and Gross 1969); however, there are no in vivo comparisons of high-lysine maize compared with low-lysine maize on iron bioavailability. It is notable that the in vitro iron dialyzability of the maize recipes was 3 to 4 times higher than for the bean recipes; this could be due to the 3 to 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 Intl. Zinc Nutrition Consultative Group (IZINC 2004) for beans and lentils and in the 22:1 to 53:1 range noted by IZINC for whole-grain cereals such as maize.

The in vitro protein digestibility of the maize–milk preparation was in the order of 82% to 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, 690 p), which was added to the maize recipes. For example, the in vitro protein digestibility was 77% to 80% for boiled conventional maize and 80% for boiled QPM (Fufa and others 2000). For nixtamalized 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 Shah (2004) found the in vitro protein digestibility of cooked red and white kidney beans to be approximately 64%, lower than 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% to 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, 689 p). The formula for percent PDCAAS is as follows:

\[
\text{PDCAAS} = \left( \frac{\text{mg of limiting amino acid in 1 g test protein}}{\text{mg of same amino acid in 1 g reference protein}} \times \% \text{true digestibility} \right)
\]

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 5 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% to 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 1 of 3 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 compared with 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 Panama in 2008, and are planned for Guatemala.
Bioavailability of bean and maize meals…

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 2nd 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 (Bebee and others 2000), suggesting that for crops with high levels of 1 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 3rd 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.

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 day-care centers. However, these varied methods better reflect the cooking conditions that these crops will be exposed to in real-life, non-experimental settings.

Acknowledgments

The daycare directors and cooks for allowing us to take samples of the meals they prepared. Researchers from the parent efficacy study who permitted the addition of this meal analysis subsydy. Lydia Child from Cornell Univ. for design of the sampling protocol and for collecting samples. Piedad Murillo from Univ. del Valle for collection of meal samples. Natália Palacios at CIMMYT for conducting experimental settings.

Consejo Directivo del Fondo Regional de Tecnología Agropecuaria (FONTAGRO), año 1. Cali, Colombia: Centro Internacional de Agricultura Tropical (CIAT), 17 p.

Bioavailability of bean and maize meals...}

References


Bioavailability of bean and maize meals . . .


Annex 4. New Laboratory brochure developed in English.
Evaluating the nutritional quality of food.
Bioavailability is the fraction of an ingested nutrient that is available for use by the human body. In other words, the term refers to the percentage of the nutrients the body consumes that can be digested, assimilated, and used for normal biological functions.

To measure bioavailability, the Laboratory simulates the phases of human digestion—oral, gastric, and intestinal—using in vitro methods.

**Examples of research projects conducted**

- Evaluating the in vitro bioavailability of iron and zinc in a typical Colombian bean dish (assessing both biofortified and conventional beans).
- Evaluating the in vitro digestibility of protein in mazamorra, a typical Colombian porridge made with maize and milk (assessing both high-quality protein and conventional maize).
- Validating an in vitro digestion method for evaluating iron bioavailability in crops.
- Evaluating the nutritional value of extracts prepared from foliage of diverse crops.
- Evaluating the protein quality of typical dishes from the Department of Cauca (Colombia) prepared with biofortified or conventional maize.
- Evaluating the in vitro bioavailability of iron and zinc in a typical Colombian bean dish (assessing both biofortified and conventional beans).
- Determining the molar ratio phytate:zinc as an indicator of zinc bioavailability.
- Identifying carotenoids.
- Quantifying phytates, using HPLC, in a population of common beans (Phaseolus vulgaris) and identifying QTLs associated with phytate contents.
- Evaluating the quality of proteins in meat and dairy products treated with honey as a possible preservation method and comparing these with untreated products.
- Implementing a methodology to evaluate antioxidant activity in food.

**Equipment**

The Laboratory has at its disposal the most recent technology. It can obtain results that are precise, reliable, and quick. The technology includes:

- High Performance Liquid Chromatography (HPLC)
- Near Infrared Reflectance Spectroscopy (NIRS)
- UV/Vis spectrophotometer

**Who we are?**

The Nutrition Quality Laboratory (Laboratorio de Calidad Nutricional) is the first in Latin America to offer a set of analytical methods that determine the in vitro concentration and bioavailability of iron, zinc, vitamin A, and protein. The Laboratory:

- Evaluates the nutritional quality of biofortified (i.e., nutritionally improved) crops.
- Evaluates the nutritional quality of natural or industrially processed foods.

Through this Laboratory, foods whose nutrients are more readily assimilated by the human body can be identified.

**Principal activities**

Quantify the concentrations of nutrients such as carotenes, soluble protein, and tryptophan, using chromatographic and spectroscopic techniques.

Determine bioavailability of nutrients, using previously validated in vitro methodologies such as in vitro bioaccessibility of carotenoids; in vitro digestibility of iron, in vitro digestibility of protein, and the molar ratio phytate:zinc as an indicator of zinc bioavailability.

Develop methods for determining bioactive compounds, including their bioavailability, in foods of interest to different audiences.

Provide technical assistance in the evaluation of the nutritional quality of foods according to the needs of individuals, companies or food programs.

Train researchers and students through inter-laboratory trials, internships, thesis proposals, and other research projects.

Ensure the quality of results by implementing a Quality Management System based on the ISO 17025 standard.
Annex 5. New Laboratory brochure developed in Spanish.
Evaluación de la calidad nutricional de alimentos
Biodisponibilidad es la fracción ingestada de un nutriente que es disponible para el cuerpo. Es decir que se refiere al porcentaje de los nutrientes que el cuerpo consume que pueda digerir, asimilar y utilizar en sus funciones biológicas normales.

Para medir la biodisponibilidad en el laboratorio, se simula condiciones de la digestión humana, tanto en su fase oral, como estomacal e intestinal, por métodos in vitro.

¿Quiénes somos?

El Laboratorio de Calidad Nutricional (Nutrition Quality Laboratory) es el primero en Latinoamérica en ofrecer un conjunto de métodos de análisis para determinar la concentración y biodisponibilidad in vitro de hierro, zinc, vitamina A y proteína para:

- Evaluar la calidad nutricional de cultivos nutricionalmente mejorados (biofortificados).
- Evaluar la calidad nutricional de alimentos naturales o procesados industrialmente.

Con este laboratorio es posible identificar los alimentos cuyos nutrientes serán más asimilados por el cuerpo humano.

Principales actividades

Cuantificar la concentración de nutrientes como carotenos, proteína soluble y triptófano, mediante técnicas cromatográficas y espectroscópicas.

Determinar la biodisponibilidad de nutrientes a través de análisis in vitro de metodologías validadas previamente como la bioaccesibilidad in vitro de carotenos, la dializabilidad in vitro de hierro, la digestibilidad in vitro de proteína y la relación molar fitato:zinc como indicador de la biodisponibilidad de zinc.

Desarrollar métodos para determinar compuestos bio-activos, incluyendo su biodisponibilidad, que tengan los alimentos de interés para diferentes públicos.

Brindar acompañamiento técnico en evaluaciones de calidad nutricional de alimentos de acuerdo a las necesidades de grupos de personas, empresas o programas alimentarios.

Capacitar a investigadores y estudiantes a través de intercambios, pruebas inter-laboratorio, pasantías, proyectos de tesis y otros proyectos de investigación.

Asegurar la calidad de los resultados emitidos mediante la implementación de un Sistema de Gestión de Calidad, basado en la norma ISO 17025.

¿Qué es biodisponibilidad?

El laboratorio cuenta con la más reciente tecnología que brinda la capacidad de obtener resultados más precisos, confiables y rápidos. Algunos de ellos:

- HPLC (High Performance Liquid Chromatography)
- NIRS (Near Infrared Reflectance Spectroscopy)
- Espectrofotómetro UVVIS

Servicios de análisis

- Evaluación de la biodisponibilidad in vitro de hierro y zinc en una receta típica colombiana de frijol (biofortificado y convencional) y de digestibilidad in vitro de proteína de una receta típica colombiana de maíz con leche-mazamorra (de alta calidad de proteína y convencional).
- Validación de un método de digestión in vitro para la evaluación de biodisponibilidad de hierro de cultivos.
- Evaluación del valor nutricional de extractos foliares preparados a partir del folaje de diversos cultivos.
- Evaluación de la calidad proteica de recetas típicas del departamento del Cauca (Colombia), elaboradas con maíz biofortificado.
- Cuantificación de fitatos por HPLC en una población de frijol común (Phaseolus vulgaris) e identificación de QTLs asociados a su contenido.
- Evaluación de la calidad proteica en alimentos cárnicos y lácteos tratados con y sin miel, como un método de preservación.
- Implementación de una metodología para evaluar la actividad anti-oxidante en alimentos.

Algunas investigaciones realizadas

- Cuantificación de proteína soluble.
- Cuantificación de triptófano.
- Determinación de digestibilidad in vitro de proteína.
- Determinación de dializabilidad in vitro de hierro.
- Determinación de bioaccesibilidad in vitro de carotenos.
- Identificación de carotenos.
- Cuantificación de carotenos totales.
- Cuantificación de actividad anti-oxidante.
- Cuantificación de ácido fítico.

Darwin Ortiz, Químico: d.a.ortiz@cgiar.org
Helena Pachón, Nutricionista: h.pachon@cgiar.org
**Annex 6.** Students who contributed to the Laboratory’s activities in the first semester of 2009.

<table>
<thead>
<tr>
<th>Name</th>
<th>University</th>
<th>Academic Program</th>
<th>Role</th>
<th>Dates in Laboratory</th>
<th>Project in Laboratory</th>
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</tr>
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<tbody>
<tr>
<td>Ingrid Aragón</td>
<td>Universidad del Valle</td>
<td>Chemistry</td>
<td>Intern, Thesis student</td>
<td>10 March 2008-31 May 2009</td>
<td>Validation of an <em>in vitro</em> method to assess the iron bioavailability of biofortified crops</td>
<td>Validation of an <em>in vitro</em> method to assess the iron bioavailability of biofortified crops, CIAT, 4 March 2009</td>
</tr>
<tr>
<td>Paola Imbachí</td>
<td>Universidad del Cauca</td>
<td>Agroindustrial Engineer</td>
<td>Intern, Thesis student</td>
<td>12 May 2008-31 May 2009</td>
<td>Protein-quality evaluation of different Colombian recipes prepared with biofortified maize</td>
<td>Protein-quality evaluation of different Colombian recipes prepared with biofortified maize, CIAT, 13 May 2009</td>
</tr>
<tr>
<td>Hiroko Kunori</td>
<td>Tokyo University of Agriculture</td>
<td>Tropical Horticulture</td>
<td>Japan CGIAR Fellow</td>
<td>18 November 2008-15 January 2009</td>
<td>Implementation of an analytic method to evaluate the anti-oxidant capacity of biofortified crops</td>
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### Annex 7. Presentations of Laboratory findings in the first semester of 2009.

<table>
<thead>
<tr>
<th>Event</th>
<th>Place</th>
<th>Date</th>
<th>Authors</th>
<th>Presentation Title</th>
</tr>
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<tbody>
<tr>
<td>I Congreso Internacional, XI Nacional &amp; Olimpiadas Académicas y</td>
<td>Valledupar, Colombia</td>
<td>24 April 2009</td>
<td>Darwin Ortiz</td>
<td>Investigaciones de alimentos en pro de la nutrición humana: Ejemplo cultivos</td>
</tr>
<tr>
<td>Deportivas Universidad de Santander Sede Valledupar</td>
<td></td>
<td></td>
<td></td>
<td>biofortificados dentro del marco del Proyecto AgroSalud</td>
</tr>
<tr>
<td>CIAT Knowledge-sharing Week and Board of Trustees Meeting</td>
<td>Palmira, Colombia</td>
<td>18-29 May 2009</td>
<td>Dayron Gutierrez, Darwin Ortiz, Helena</td>
<td>Nutrition Quality Laboratory: Projects and developments</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pachón</td>
<td></td>
</tr>
<tr>
<td>CIAT Knowledge-sharing Week and Board of Trustees Meeting</td>
<td>Palmira, Colombia</td>
<td>18-29 May 2009</td>
<td>Ingrid Aragón, Darwin Ortiz, Helena Pachón</td>
<td>Validación de un Método de Digestión <em>in Vitro</em> para la Evaluación de Biodisponibilidad de Hierro en alimentos</td>
</tr>
</tbody>
</table>


- December 2008 visit of Andrés Laignelet, Monsanto
- April 2009 visit of nutritionists from the Cauca Department’s Instituto Colombiano de Bienestar Familiar
- May 2009 visit of representatives from ADA Afro-Latino Development Alliance