Assessing Potential Effects of Inulin and Probiotic Bacteria on Fe Availability from Common Beans (Phaseolus vulgaris L.) to Caco-2 Cells

J.M. Laparra, R.P. Glahn, and D.D. Miller

ABSTRACT: Inulin, a prebiotic, may enhance intestinal Fe absorption. Our objective was to assess the effects of supplemental inulin and 2 probiotic bacteria (B. infantis and L. acidophilus) on Fe availability to Caco-2 cells from common white and red beans (Phaseolus vulgaris L.). Cooked beans were mixed or not with supplemental inulin (4%, w/w), and then subjected to simulated gastrointestinal digestion (pepsin, pH 2; pancreatin, pH 7.2). Subsequently, the digests were incubated overnight with and without B. infantis or L. acidophilus. Ferritin formation in Caco-2 cells was used to evaluate Fe uptake. Total soluble phenols (Folin–Ciocalteau) and phytate (HPLC-electrochemical detection) were quantified, and the flavonoids profile (HPLC-PDA/UV detection) was monitored in the digests. Supplemental inulin did not affect Fe uptake from white nor red beans. Incubation with B. infantis increased total soluble phenols (TSP) in the digests and decreased Fe uptake. Incubation with L. acidophilus decreased TSP in the digest and increased Fe uptake. Variations in Fe uptake were not associated with soluble phytate concentrations in the digests. The largest change in flavonoids profile was found in the digests incubated with L. acidophilus, which decreased the soluble concentration of astragalin (kaempferol-3-O-glucoside). These results suggest that certain probiotics could increase Fe uptake from common beans.

Keywords: beans, Caco-2 cells, inulin, iron availability, probiotic

Introduction

Common beans (Phaseolus vulgaris L.) are a staple food crop for large groups of people in many Latin American and African countries. Beans are often the main source of protein, and a significant source of minerals for low-income populations. In addition, common beans contain significant amounts of dietary fiber, phytic acid, and polyphenols (Brigide and Canniatti-Brazaca 2006; Espinosa-Alonso and others 2006). Phytates and polyphenols are well-known inhibitors of iron (Fe) absorption due to their metal-chelating ability (Brigide and Canniatti-Brazaca 2006; Hsu and others 2006). It is believed that diets high in phytate and polyphenols are primary factors contributing to Fe deficiency, which is one of the most prevalent nutritional deficiencies worldwide (Beard and Stoltzfus 2001).

Presently, in vitro and in vivo data related to Fe availability from beans are contradictory (Welch and others 2000; Hsu and others 2006; Ariza-Nieto and others 2007; Beiseigel and others 2007). Several in vitro studies, using Caco-2 cells as a model of intestinal epithelium, showed low Fe uptake from colored beans relative to white beans (Hsu and others 2006; Ariza-Nieto and others 2007). The Fe-binding ability of polyphenols, mainly present in the hull of beans, has been proposed to play a key role in reducing Fe availability from colored beans (Hsu and others 2006); however, this effect has not been observed in animal (Welch and others 2000) or human (Beiseigel and others 2007) trials.

In recent years, there has been a growing interest in the so-called bioactive food-derived components. For example, positive effects of probiotics such as inulin on bioavailability of dietary calcium and magnesium (Coudray and others 2003, 2005), and copper and zinc (Coudray and others 2006) using in vivo models have been reported. Yasuda and others (2006) showed that supplementing a corn/soybean diet with 4% inulin increased Fe absorption in young pigs. These results appear to conflict with the putative capacity of soluble fiber to bind mineral cations (Coudray and others 2003), and to add viscosity to the gut contents (Roberfroid 1993). Increased viscosity of digesta might limit diffusion of nutrients to the brush border, thereby reducing absorption.

Fructooligosaccharides are classified as prebiotics because they stimulate the growth and metabolism of probiotic bacteria in the gut (Roberfroid 2005). Certain microbial groups are known to benefit the host through the release of metabolic byproducts and successful competition with potentially pathogenic bacteria. Therefore, dietary strategies that favor the growth of these health-promoting intestinal bacteria have considerable promise. The metabolism of inulin leads to the generation of short chain free fatty acids that are utilized as energy sources by the intestinal epithelium and other tissues and are thought to play additional roles, such as enhancing the solubilization and absorption of minerals (Scholz-Ahrens and others 2001; Hooper and others 2002). Fermentation of carrot juice (Berqvist and others 2006) and maize (Proulx and Reddy 2007) by lactic acid bacteria enhanced Fe availability in in vitro models. Also, degradation of Fe absorption inhibitors such as phytate (Haros and others 2005) and flavonoids (Scalbert and Williamson 2000; Marotti and others 2007) in the gut have been suggested to be due to the metabolic activities of bifidobacteria. Lactic bacteria and bifidobacteria, are 2 bacterial genera in the human intestine. They are widely viewed as probiotics, that is, when
Assessing potential effects of inulin and probiotic bacteria... 

ingested in sufficient quantities they confer a health benefit to the host (Roberfroid 2000). The effects of beneficial bacteria in the gut could be expected to positively influence Fe uptake and could be, at least in part, responsible for the differences in Fe bioavailability comparisons of in vitro and in vivo models.

The objective of this study was to evaluate whether supplemental dietary inulin and 2 probiotic bacterial species affect Fe uptake from white and red beans using Caco-2 cells as an intestinal epithelia model.

Materials and Methods

Reagents

Unless otherwise stated, all reagents were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). All glassware was soaked in 1.2 mol/L HCl for 24 h and then rinsed with deionized (18 MΩ cm) water prior to use.

Instruments

Total Fe content was determined with an inductively coupled argon plasma emission spectrometer (ICP-ES, Model 61E Trace Analyzer, Thermo Jarrell Ash Corp., Franklin, Mass., U.S.A.) after wetashing (Laparra and others 2008). Other equipments used included a spectrophotometer (DU 520 UV/vis, Beckman Coulter, Miami, Fla., U.S.A.) and an automatic gamma counter Wizard 3 Wallac 1480 (Perkin Elmer, Norwalk, Conn., U.S.A.).

Sample preparation

Commercial samples of common white (Great Northern) and red (kidney) dry beans (Phaseolus vulgaris L.) were obtained from local supermarkets in Ithaca (N.Y., U.S.A.) in the form of a raw product, and were kept at room temperature. The analyzed samples constitute a mixture of 3 different batches, but all of them came from the same company. To simulate the regular cooking procedure applied in vitro digestion experiment, the DMEM (dulbecco's modified Eagle's medium, Gibco) was added to each well, and the plates were returned to the incubator for an additional 22 h. The next day, the cells from each well were washed twice with isotonic saline solution and harvested in 2 mL of deionized water. Control solutions containing digestive enzymes but no sample were carried through in parallel. Cell ferritin formation was used as a measure of cell Fe uptake.

Incubation with probiotic bacteria

The strain selection, B. infantis (American Type Culture Collection, ATCC 15697) (grown in RCM media, Gibco) and L. acidophilus (ATCC 11974) (grown in MRS media, Gibco), was based on a previous screening for ability to growth in presence of 4% inulin. Aliquots of bacteria stock cultures (100 μL) were inoculated into the digests to yield final bacteria concentrations ranging between 10^6 and 10^8 CFU/mL digest. Negative controls, consisting in bean samples without addition of any probiotic bacteria, were also incubated (37 °C/anaerobic conditions) overnight. After overnight incubation, bacteria viability was confirmed by plate recount. The initial pH (7 to 7.2) of the digests was maintained throughout the incubation.

Cell cultures

Caco-2 cells were obtained from the ATCC (Rockville, Md., U.S.A.) at passage 17 and used in experiments at passage 25 to 33. Cells were seeded at a density of 50000 cells/cm² in collagen-treated 6-well plates (Costar, Cambridge, Mass., U.S.A.) and maintained with Dulbecco's modified Eagle's medium (DMEM) under conditions previously described (Glahn and others 1998). The cells were used for iron bioavailability experiments at 13-d post seeding. On the day prior to the in vitro digestion experiment, the DMEM medium was removed, the cell monolayers were washed with 2 mL of MEM, 2 mL of fresh minimum essential medium (MEM, Gibco) was added to each well, and the plates were returned to the incubator.

Ferritin and total protein assays

Caco-2 cell ferritin assays were performed with a 1-stage, 2-site immunoradiometric assay (FERIRON II ferritin assay, RAMCO Laboratories, Houston, Tex., U.S.A.). A 10-μL sample of each harvested cell culture was used for the ferritin determination.

The ferritin formation was expressed per unit of cell protein (ng/mg of protein). Caco-2 cell protein was determined using the Bio-Rad DC protein assay kit (Bio-Rad Laboratories). Absorbances of the samples were measured at 750 nm. Baseline cell ferritin in cultures grown in MEM averaged 2.1 ng/mg of cell protein. A positive control (50 μmol/L Fe with 500 μmol/L ascorbic acid) was used to verify responsiveness of the Caco-2 cells to available Fe.
Assessing potential effects of inulin and probiotic bacteria...

**Extraction of phytic acid in in vitro digests**

Aliquots (1 mL) of the digests were centrifuged (15000 × g for 10 min) to separate the soluble fraction. Then, the supernatants were transferred to 15 mL tubes and 10 mL of H₂SO₄ (1.25%, v/v) was added to each sample. The mixture was agitated for 2 h in a mechanical shaker, and then centrifuged (2000 × g for 10 min). The supernatants were filtered through a Whatman 0.45 μm nylon membrane filter (GE Healthcare, Chalfont St. Giles, U.K.) prior to injection (0.1 mL of the supernatant diluted in 0.9 mL of deionized water) into the HPLC system.

**HPLC analysis of phytic acid**

Phytic acid contents were analyzed using a Dionex liquid chromatography system (model DX600) (Dionex Corp., Sunnyvale, Calif., U.S.A.) equipped with a conductivity detector (ED50 electrochemical detector), an advance gradient GS50 pump, and data acquisition software (Dionex PeakNet 6.40). The chromatographic separations (injection volume 25 μL) were carried out using an AG11 guard column (Dionex) and AS11 main separating column (Dionex). The elution rate was 1.0 mL/min using a gradient of mobile phases (A: deionized water; B: 200 mmol/L NaOH; gradient program 0 to 3 min: 87% A, 3 to 11 min: linear gradient up to 50% A; 11.1 to 15 min: 87% A). Phytic acid was identified by matching the retention times of the peaks in the sample chromatograms with those obtained from standards and were quantified using calibration curves of aqueous standards containing 0.125% H₂SO₄.

**Total phenolic content in the digests**

To evaluate the total phenol content, the method described by Dewanto and others (2002) was applied. Briefly, 0.5 mL of deionized water and 0.125 mL of the Folin–Ciocalteau reagent were added to 0.125 mL of the supernatant from digested bean samples. The mixture was allowed to stand for 5 min, and then, 0.125 mL of a 7% aqueous Na₂CO₃ solution was added. The final volume of the mixture was adjusted to 3 mL with deionized water and was allowed to stand for 90 min at room temperature. The absorbance was measured at 760 nm against a reagent blank. The amount of total phenolics was expressed as gallic acid equivalents (mg/g of beans). The aqueous calibration curve ranged between 2 and 12 μg gallic acid/mL.

**Flavonoids profile in the digests**

Flavonoids were analyzed according to the method described by Espinosa-Alonso and others (2006) with slight modifications. The analysis was carried out on a HPLC system (Waters, Milford, Mass., U.S.A.) consisting of a 600E multisolvent pump, a 717 plus autosampler and a 996 photodiode array detector set at 254 nm, operated using Empower software. The separation was performed on a Vydac 5u 300A C18 column (Phenomenex) 250 × 4.6 mm. The gradient program started with 100% of solvent A (1% acetonitrile in 20 mmol/L phosphate buffer adjusted to pH 2.20 with concentrated H₃PO₄) and after 5 min solvent B (70:30 [v/v], water:acetonitrile; HPLC grade) was increased linearly to reach 10% in 7 min. From 12 to 19 min, the rate was kept at 90% solvent A and 10% of solvent B. The column was equilibrated using the initial conditions during 10 min. Aliquots (3 mL) of digests were lyophilized and the residue redissolved in 0.5 mL of MeOH/HCl (85/15, v/v) mixture. Subsequently, samples were filtered through a 0.20-μm filter (MilllexGN, Millipore, Billerica, Mass., U.S.A.) and 0.150 mL of the filtrates was injected into HPLC system. UV absorbance at 260 nm was used to detect flavonoids, kaempferol, and astragalin. Total running time for each analysis was 30 min. The identity of the flavonoids was confirmed by comparing the retention time and the photodiode array spectra with those generate by the standards (Hu and others 2006).

**Statistical analysis**

A 1-factor analysis of variance (ANOVA) and the Tukey test (Box and others 1978) were applied to determine statistical differences on ferritin, total phenols and phyate contents in analyzed cultures and digests. The experiments were conducted in triplicate of each of 2 different days (n = 6). A significance level of P < 0.05 was adopted for all comparisons. Statgraphics Plus version 5.0 (Rockville, Md., U.S.A.) was used for the statistical analysis.

**Results and Discussion**

**Effect of inulin**

The concentration of Fe in the whole cooked beans was 67.9 ± 0.6 μg/g (dry basis) and 87.4 ± 1.2 μg/g (dry basis) for white and red beans, respectively. Total Fe concentrations in the digests loaded into the upper chambers in the in vitro system and ferritin contents in exposed Caco-2 cell cultures after 24 h of incubation are shown in Table 1. Although Fe concentrations in the digests from white beans were significantly (P < 0.05) lower than red beans, cell cultures exposed to white bean digests exhibited markedly higher (P < 0.05) ferritin concentrations. These results indicate higher Fe uptake values from white than from red bean digests. Regardless the type of bean, the addition of supplemental 4% inulin had no effect on Fe uptake (P > 0.05).

Iron uptake from white beans was 18-fold higher (P < 0.05) than from red beans. The low Fe uptake from red beans indicate that in the gastrointestinal digests Fe may be bound to high-molecular-weight complexes that do not cross the dialysis membrane (15000 Da). Total phenol content in the soluble fraction of the digests from red beans was higher than from white beans (Table 1). Phenols have been identified as potent inhibitors of Fe absorption presumably forming complexes with Fe³⁺ ions, and in this way impairing Fe bioavailability (Brune and others 1991; Hu and others 2006). Hu and others (2006) attributed the low Fe uptake to the presence of kaempferol in the hulls of colored beans (red, pinto, and black). In foods, the phenol compounds can occur in many forms, from small monomeric phenolic acids to large polymerized polyphenols (condensed tannins), which might be unable to cross through the dialysis membrane used in this study. The formation of phenol-Fe complexes in in vitro media may make the Fe unavailable for absorption, and could explain the lower Fe uptake values from the colored beans. In addition, poor digestibility of cooked colored beans has been directly related to the tannin content of colored bean hulls (Elias and others 1979; Aw and Swanson 1985). This observation might explain, at least in part, the low Fe solubility in the digests from red beans.

It is assumed that Fe bioavailability may also be negatively affected by phytate (Ma and others 2007). In beans, the predominant form of phytate is myo-inositol hexakisphosphate (IP6) (Welch and others 2000). However, in a previous study where several beans genotypes were screened for Fe bioavailability using the Caco-2 cell model it was concluded that polyphenols had a greater inhibitory effect than phytate on Caco-2 Fe bioavailability (Ariza-Nieto and others 2007). In the present study, differences in ferritin formation from each type of bean cannot be attributed to the phytic acid concentrations in the digests (Table 1). As shown, phytic acid content in the digests from red beans (without added inulin) was only about 35.2% relative to the digests from white beans (without added inulin); however, no ferritin formation response was seen in cultures exposed to the red bean digests. These data showing the lower effect
of phytate than phenols on Fe uptake by Caco-2 cells are in accordance with those reported by Ariza-Nieto and others (2007).

**Effect of probiotic bacteria**

*B. infantis* and *L. acidophilus* were chosen because they are preferred probiotics (Roberfroid 2006; Haros and others 2005), and are also present among the usual microflora in the human intestine. The ferritin concentrations in cell cultures exposed to digests incubated overnight with probiotic bacteria are shown in Table 1.

In both types of beans, Fe availability was influenced by the species of bacteria tested but not by added inulin regardless the type of bean, either white or red, cell cultures exposed to digests incubated with *B. infantis* exhibited significantly (*P < 0.05*) lower ferritin concentrations compared to non-inoculated digests. The Fe uptake from white bean digests was decreased by 17.3% and 29.8%, with or without added inulin, respectively. Uptake from the digests incubated with *L. acidophilus* the cell cultures exhibited significantly (*P < 0.05*) higher ferritin concentrations relative to cultures exposed to nonincubated digests. It is important to mention that the enhancing effect observed was not significant (*P > 0.05*) with or without added inulin. Interestingly, in this case the enhancing effect caused by *L. acidophilus* was relatively higher from red beans increasing ferritin values by 72.7% and 83.9% compared to the values obtained from nonincubated digests. The enhancing effect of *L. acidophilus* on Fe uptake is in accordance with previous reports, where the researchers showed increased Fe solubility and uptake by Caco-2 cells from the lactic acid bacteria fermentation of carrot juice (Berqvist and others 2006), and maize (Proulx and Reddy 2007). To the best of our knowledge there are no reports showing a similar effect caused by *Bifidobacterium* sp.

Previous studies demonstrated that degradation of phytate in the stomach and intestine can be caused by dietary phytases and also probably, to the metabolic activity of the colonic microflora (Wise and Gilburt 1982). In the present study, incubation with *B. infantis* caused only slightly (*P > 0.05*) lower soluble phytic acid concentrations in the digests from white beans without added inulin compared to the nonincubated digests. Incubation with *L. acidophilus* produced lower (*P < 0.05*) phytate concentrations in the digests of white bean (0.87 and 1.2 μmol/g beans with and without inulin, respectively), although, the phytic acid contents in the digests from red beans were higher when compared to the nonincubated. These results indicate that incubation with *L. acidophilus* decreased the soluble phytate concentrations by 33% and 53% with respect to the contents quantified in nonincubated digests from white beans with and without added inulin, respectively. However, phytate concentrations in the digests of red beans inoculated with *L. acidophilus* were not decreased relative to content quantified in digests incubated with *B. infantis* and resulted higher than those quantified in the nonincubated digests.

Presently, phytase activity has only been attributed to *Lactobacillus* strains isolated from food fermentations, and not to bacteria from intestinal origin (Palacios and others 2005). However, in several bifidobacteria representative of common intestinal isolates and probiotic species a phytase activity has been reported (Haros and others 2005). The latter researchers demonstrated differences in phytase activities from the same strain as a function of the temperature and phosphorus content in the media. In addition, *B. infantis* exhibited the lowest phytase activity when compared with several other *Bifidobacterium* sp. (Haros and others 2005). This observation would explain why no differences in phytate contents were observed in *in vitro* digests incubated with *B. infantis* however, further studies are necessary to confirm this metabolic feature in *B. infantis* (ATCC 15697).

**Flavonoids HPLC profile**

In the present study, differences in ferritin formation responses were associated with the significant (*P < 0.05*) changes in total soluble phenolics concentration in the digests (Table 1). To establish differences on Fe uptake as a function of potential changes in the flavonoids profile solubilized in *in vitro* process, the digests from white and red beans were analyzed via HPLC (Figure 1 and 2). Inulin did not cause any significant alteration of chromatographic profiles obtained. The most significative changes in the chromatograms were found as a function of the bacteria used for incubation, and especially in the digests incubated with *L. acidophilus*. In the digests of white beans (Figure 1), a chromatographic signal with retention time (rt) 8.5 min was detected in both nonincubated digests and after incubation with *B. infantis*. The latter signal was not detected in digests incubated with *L. acidophilus* (Figure 1C). Another important difference in digests incubated with *B. infantis* was related to contents quantified in nonincubated digests.

### Table 1—Total phenols (mg Gallic acid/g beans, dw) and phytic acid concentrations (μmol/g beans) in the digests of white and red beans with and without added inulin, and Caco-2 cell Fe uptake (determined by cell ferritin formation).

<table>
<thead>
<tr>
<th>Bean sample</th>
<th>Fe in upper chamber (μg)</th>
<th>Total phenols (mg Gallic acid/g)</th>
<th>Phytic acid (μmol/g)</th>
<th>Ferritin (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Noninoculated with bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>2.07 ± 0.02ab</td>
<td>3.03 ± 0.08ab</td>
<td>2.61 ± 0.02ab</td>
<td>62.88 ± 2.29ab</td>
</tr>
<tr>
<td>White + inulin</td>
<td>2.08 ± 0.29ab</td>
<td>3.08 ± 0.24ab</td>
<td>1.33 ± 0.17ab</td>
<td>61.43 ± 4.17ab</td>
</tr>
<tr>
<td>Red</td>
<td>2.65 ± 0.11c</td>
<td>4.17 ± 0.33c</td>
<td>0.92 ± 0.04c</td>
<td>3.43 ± 1.33c</td>
</tr>
<tr>
<td>Red + inulin</td>
<td>2.79 ± 0.19c</td>
<td>4.05 ± 0.48c</td>
<td>1.82 ± 0.06c</td>
<td>3.56 ± 1.09c</td>
</tr>
<tr>
<td><strong>Inoculated with <em>B. infantis</em></strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>2.07 ± 0.01a</td>
<td>3.48 ± 0.12a</td>
<td>2.17 ± 0.05a</td>
<td>44.15 ± 5.21a</td>
</tr>
<tr>
<td>White + inulin</td>
<td>2.10 ± 0.05ab</td>
<td>4.25 ± 0.19a</td>
<td>2.96 ± 0.03a</td>
<td>50.80 ± 8.56a</td>
</tr>
<tr>
<td>Red</td>
<td>2.72 ± 0.10c</td>
<td>5.45 ± 0.33c</td>
<td>2.23 ± 0.01a</td>
<td>1.75 ± 1.99a</td>
</tr>
<tr>
<td>Red + inulin</td>
<td>2.63 ± 0.03c</td>
<td>5.35 ± 0.12c</td>
<td>2.31 ± 0.05c</td>
<td>1.61 ± 0.41c</td>
</tr>
<tr>
<td><strong>Inoculated with <em>L. acidophilus</em></strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>2.04 ± 0.01a</td>
<td>1.85 ± 0.28a</td>
<td>1.22 ± 0.18a</td>
<td>75.55 ± 6.11a</td>
</tr>
<tr>
<td>White + inulin</td>
<td>2.13 ± 0.03a</td>
<td>1.80 ± 0.14a</td>
<td>0.87 ± 0.14a</td>
<td>86.50 ± 7.38a</td>
</tr>
<tr>
<td>Red</td>
<td>2.75 ± 0.04c</td>
<td>3.04 ± 0.25a</td>
<td>2.93 ± 0.02a</td>
<td>6.31 ± 1.89a</td>
</tr>
<tr>
<td>Red + inulin</td>
<td>2.64 ± 0.01c</td>
<td>2.95 ± 0.32a</td>
<td>2.15 ± 0.15c</td>
<td>6.15 ± 0.51c</td>
</tr>
</tbody>
</table>

*The cells grown in MEM alone had baseline ferritin of 2.1 ng/mg protein.*

Data are expressed as means ± standard deviation (n = 6). Different superscript letters within a column indicate statistically (*P < 0.05*) significant differences (white compared to white and red compared to red, both with and without inulin and incubated or not with bacteria).
Assessing potential effects of inulin and probiotic bacteria

(Figure 1B) was the increased UV-absorbance of the chromatographic signal eluted with $rt > 30$ min, with respect to the nonincubated digests (Figure 1A). The latter might be produced by the release of polymerized polyphenols (tannins), because it matched the retention time of a tannic acid (Sigma) standard solution. These polymerized polyphenols were also detected in the digests incubated with *L. acidophilus*, but the area under the curve was lower (Figure 1C) than in nonincubated digests. None of the chromatographic signals was identified as kaempferol or astragalin in the digests of white beans, which is in good agreement with those data reported in a previous study by our group (Hu and others 2006).

In the digests of red beans (Figure 2A), astragalin was identified as the main flavonol solubilized in *in vitro* media. This flavonol was identified by comparing the peak retention time and the photodiode array spectra with the standards. When comparing the chromatograms obtained from the digests that were incubated with probiotic bacteria, incubation with *B. infantis* caused an increased UV-absorbance signal of the chromatographic peak with $rt$ 9.3 min (Figure 2B). However, no significant alteration in astragalin was detected when compared to digests that were not inoculated with probiotic bacteria. In contrast, after incubation with *L. acidophilus* the chromatographic signal at $rt$ 9.3 min seen in samples incubated with *B. infantis* was not detected. Furthermore, astragalin was reduced ($P < 0.05$) in *in vitro* digests incubated with *L. acidophilus* relative to digests not incubated (Figure 2A compared with 2C). There were no chromatographic signals detected in the digests corresponding to kaempferol. It has been suggested that kaempferol has potentially inhibitory effect on Fe uptake by Caco-2 cells (Hu and others 2006). The latter researchers demonstrated that kaempferol is mainly accumulated in red and pinto seed coats, and suggested that certain parts of the flavonoid ring may play an important role in Fe bioavailability inhibition, which is probably due to the formation of insoluble complexes between polyphenols and ferric iron (Hu and others 2006). As previously reported, the concentration of kaempferol was highly variable (3 to 11.1 mg/kg in bean flour) in different varieties of colored wild and weedy Mexican beans (Espinosa-Alonso and others 2006). Maybe this fact would explain that low kaempferol contents quantified in the red beans used (25.4 ± 1.8 μg/g bean, dry basis) were not detected in digests from red bean.

*Bifidobacterium* sp. from human intestinal origin have the ability to metabolize flavonoid glycosides from common beans (*Phaseolus vulgaris* L.) (Marotti and others 2007). Among the different species of bifidobacteria tested, *B. longum* and *B. infantis* exhibit the highest and lowest levels for glucosidase activity, respectively (Marotti and others 2007). The latter researchers indicated that number and
Assessing potential effects of inulin and probiotic bacteria . . .

Figure 2—Typical chromatograms by HPLC-RP-PDA (260 nm) of flavonoids in digests from red beans not inoculated with bacteria (A), or from those inoculated with B. infantis (B) or L. acidophilus (C).

position of hydroxyl groups are important structural characteristics for flavonoid degradation by bifidobacteria isolated from human intestinal contents. The fact that B. infantis exhibits the lowest glucosidase activity is in accordance with our observation that the astragalin (kaempferol-3-O-glucoside) peak was not altered in digests inoculated with B. infantis (compare Figure 1A and 1B). However, to the best of our knowledge, this metabolic ability to degrade flavonoid glycosides has not been shown in intestinal isolates of the genera Lactobacillus. In the present study, we showed an increased Fe uptake by Caco-2 cells, which was accompanied by decreased total phenol content in the digests (Table 1) and a reduced signal for astragalin in the digests inoculated with L. acidophilus. These observations might suggest that L. acidophilus has the ability to metabolize flavonoids; however, further studies are needed to confirm this hypothesis.

Although several in vitro studies using the Caco-2 model showed a lower Fe uptake from colored beans than white beans (Hu and others 2006; Ariza-Nieto and others 2007), the difference was not seen in animal (Welch and others 2000) or human (Beiseigel and others 2007) trials. Beiseigel and others (2007) compared Fe bioavailability from maize and beans in human and the Caco-2 models. The Caco-2 model accurately predicted bioavailability to humans from the maize but not from the beans. The possible degradation of polyphenols by intestinal microbiota that has been suggested in the present study might explain the contrasting results obtained in Caco-2 and human studies.

Conclusions

Inulin did not inhibit or enhance Fe uptake by Caco-2 cells from white and red beans. However, it should not be ruled out that variable data could be obtained due to the bean genotype considered. The probiotic bacteria L. acidophilus had a positive effect on Fe availability in both white and red beans. The latter observation was associated with a decrease in flavonol content in the digests, mainly astragalin. These results suggest that some species of probiotics could increase Fe bioavailability from
Assessing potential effects of inulin and probiotic bacteria . . .

Although the Caco-2 cell model has demonstrated strong correlations with human studies and offers the advantage of good reproducibility, this model does not take into account the potential effect of beneficial bacteria in the gut. Therefore, intestinal microflora and probiotic supplementation are aspects that should be taken into account to more reliably predict in vitro Fe bioavailability from beans that contain well-known Fe inhibitors.

Acknowledgments
This study was supported by the Robert W. Holley Center for Agriculture and Health (USDA-ARS) and the Dept. of Food Science at Cornell Univ. Dr. José Moisés Laparra was sponsored as a Fulbright Scholar and supported by a postdoctoral fellowship from the Spanish government.

References
Bergqvist SW, Andlid T, Sandberg AS. 2006. Lactic acid fermentation stimulated iron absorption by Caco-2 cells is associated with increased soluble iron content in carrot juice. Br J Nutr 96:705–11.