

## Antinutrients and “in vitro” availability of iron in irradiated common beans (*Phaseolus vulgaris*)

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### Abstract

Irradiation and cooking effects were investigated in beans. Determinations of iron and antinutrients and the “in vitro” dialysis of iron were carried out. Cooking caused a decrease in all parameters except for phytate and iron availability. Tannins were inversely correlated with applied irradiation doses. The same thing happened to the dialyzed iron. The 6-kGy-dose irradiation presented positive effects regarding the iron availability and its use is recommended.

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### 1. Introduction

Mineral elements in low concentrations are called trace elements, microelements or microminerals, and are considered essential and beneficial to the human body. Iron is the most abundant microelement in the human organism (Carpenter & Mahoney, 1992; Nielsen, 1986).

When dealing with the absorption of nutrients, their bioavailability must be considered. This is defined as the proportion of the nutrient in the diet or the food that can be used by the organism (Fairweather-Tait, 1992).

Factors such as thermal treatment and/or processing can influence iron bioavailability in foods. Among the dietary factors, there are those that increase the non-heme iron absorption, such as ascorbic acid and amino acids from the meat, and those that decrease its absorption, such as phytates, polyphenols and calcium, commonly called antinutrients, which are part of the food and, having a varied nature, exert a toxic or antinutri-

tional action, when ingested in the native form (uncooked foods or insufficiently cooked). The interactions of all these intensifiers and inhibitors are determinative of the bioavailability of non-heme iron in the meal. Intrinsic factors of the organism, as, for example, the nutritional state of the individual, influence the absorption and, consequently, the bioavailability of the iron (Benito & Miller, 1998; Canniatti-Brazaca, 1997). Therefore, the bioavailability of a nutrient is influenced by distinct factors, which are classified as intrinsic and extrinsic factors (physiological and dietary, respectively) (Souton, Fairweather-Tait, & Hazell, 1988).

According to the National Research Council – NRC (1989), the recommended daily intake of iron is, for males, 12 mg from 11 to 18-years old and 10 mg over 19-years old. For females, the amount is 15 mg for 11–50-year olds, decreasing to 10 mg for 51 or more year-olds.

Diets have been classified as of high (15%), intermediate (10%) and low (5%) iron bioavailability, depending on the proportion of heme iron and the presence of inhibitors and intensifiers of the absorption of non-heme iron (De Mayer, Dallman, & Gurney, 1989; FAO/WHO, 1988).

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According to Martínez, Ros, and Periago (1999), iron in vegetables ranges from 5.3 to 8.5 mg/100 g. The average per capita consumption of beans in Brazil is approximately 16 kg/habitant/year. The highest consumption is in rural areas of the country (Roston, 1990).

Beans are especially important for Brazil, not only because the country is the largest world-wide producer and consumer (2.2–2.5 million tons, in approximately 5 million hectares cultivated), but also because beans are one of the main proteic foods for the Brazilian population (Gonçalves & Souza, 1998; Roston, 1990). The production of grains in 1995 was 2,946,267 tons in Brazil (Fundação, 1996). According to data from IBGE (2001), the total production was 3,038,238 tons in 2000.

However, there are losses of these grains in each harvest due to (among others factors) the attack of insects and rodents, as well as alterations of their physical–chemical characteristics and structural transformations of their components, resulting in beans that are highly resistant to cooking, with modifications of organoleptical and nutritional properties (Mancini-Filho, 1990; Villavicencio, 1998).

To decrease these losses, the irradiation process is a more attractive and healthful alternative, than chemical treatments with, for example, methyl bromide. The application of ionizing irradiation, with the intention of preserving and disinfesting grains, seems to be a promising practice, which can extend their shelf life and reduce the losses of the harvests during storage. The estimated costs of the benefits of commercial irradiation, as treatment, are competitive with of fumigation and other physical and thermal treatments (Nascimento, 1992; Villavicencio, 1998).

The objectives of this research were to:

- evaluate the effects of the different doses of irradiation on the availability of iron in uncooked and cooked beans;
- determine the effect of the irradiation on tannins and phytates present in beans.

## 2. Materials and methods

### 2.1. Materials

The uncooked material used for the analyses of uncooked and cooked grains and *Phaseolus vulgaris* L. was acquired in the Municipal Market of Piracicaba-SP, Brazil. The grains were irradiated with gamma rays from a commercial Cobalt<sup>60</sup> food irradiator – model JS-8900 by Nordion, with an activity of 1,200,000 Ci, at doses of 0, 2, 6 and 10 kGy – by CBE (Brazilian Company of Sterilization), in the city of Jarinu-SP, Brazil. The dose rate for the irradiation of the beans used was

1.294 kGy/h. The dose amount absorbed during the irradiation was measured by a Poly (methyl methacrylate) dosimeter (PMMA-Red perspex). The reading was carried out in a spectrophotometer, and the dose of absorbed irradiation was reported quantitatively at 530 nm intervals.

The samples were packed, in 500 g portions, into polyethylene bags, and submitted to the radiation doses of 0, 2, 6 and 10 kGy. The replicate was three and each one with three samples.

The uncooked grains were ground in a knife grinder, and sieved (30 mesh), obtaining flour for use in chemical analyses. This flour was stored in a polyethylene bag, closed, and stored at 4 °C.

The grains for cooking were macerated in distilled water at the ratio of 1:3; after 16 h of maceration, the water was discarded and distilled water at the ratio of 1:2 was added; grains were cooked for 10 min at 121 °C in an autoclave, according to the methodology suggested by Molina, Fuente, and Bressani (1975). After the cooking, the samples were placed in aluminium trays and dried in an air circulation stove at the temperature of 50–55 °C until constant weight was achieved (approximately 24 h). Then, the material was ground and stored, as described for the uncooked grains. All the analyses were done in triplicate.

### 2.2. Determination of iron

The minerals were determined according to the method described by Sarruge and Haag (1974). The perchloric nitric acid digestion was used and the reading of the atomic absorption was taken in a spectrophotometer. The wavelength used for the iron was 248.3 nm.

### 2.3. “In vitro” availability of iron

Dialysis was carried out in accordance with method proposed by Lutén, Crews, and Flynn (1996). The beans were submitted to “in vitro” digestion. This digestion was initially carried out with pepsin in 0.1 M HCl (160 mg pepsin/ml) at pH 2, incubated at 37 °C for 2 h under agitation and, then, treated with a solution of pancreatic-bile in sodium carbonate (25 mg/ml) at pH 7.5 at 37 °C for 30 min. The resulting material was placed in a dialysis bag (mol weight cut-off 6000–8000, with 23 mm) and incubated for 2 more hours at 37 °C. It was, then, washed with deionized water and the dialyzed iron, determined at 533 nm.

### 2.4. Antinutritional factors

#### 2.4.1. Tannins

Tannins were analyzed by the methodology described by Price, Hagerman, and Butler (1980). Samples (200 mg) were extracted in 10 ml of methanol for

20 min, under agitation. After this they were centrifuged at 4000 rpm for 15 min. Vanillin assay was performed on the methanol extract (1 ml) by addition of a 5 ml solution of vanillin (1%):HCl (8%) in the ratio of 1:1 at 30°C for 20 min, and later, determining at 500 nm. For the construction of the standard curve, catechin solution was used at concentration from 0.0 to 1.0 mg/ml. The results were expressed in% mEq of catechin. Some authors (Bressani, Elías, & Wolzack, 1983) use tannic acid for this purpose. But, since catechin is present in beans, it is more appropriate to use it as the standard.

#### 2.4.2. Phytic acid

The amount of phytic acid was determined by the method of Grynspan and Cheryan (1989). The sample was placed in HCl solution (0.65 N) and, then, centrifuged at 3.000 rpm for 10 min. The supernatant (2 ml) diluted in distilled water (25 ml) was passed through a column of resin and cotton, and, then NaCl solutions (15 ml, 0.1 M and 15 ml, 0.7 M) were added. Wande's reagent (1 ml) was added under agitation for 15 min and the solution read at 500 nm.

#### 2.5. Statistical analysis

The statistical delineation was entirely randomized. A variance analysis (the *F*-test) was carried out and the average comparison of the averages obtained in the different treatments was analyzed by Tukey's test ( $p < 0.05$ ) (Pimentel-Gomes, 1982). The analysis were done for three replicates and each one with three samples.

### 3. Results and discussion

#### 3.1. Tannin

The values of the tannin content in uncooked and cooked beans irradiated at 0, 2, 6 and 10 kGy are presented in Table 1. Cooked beans had low tannin contents that did not alter with irradiation, but uncooked beans contents changed with irradiation. Cooking also alters tannin content.

Villavicencio, Mancini-Filho, and Delincée (2000) found that the amount of catechin in unirradiated uncooked beans was 0.18% mEq. When samples were irradiated at 2.5, 5 and 10 kGy, the contents of catechin were respectively, 0.16%, 0.14% and 0.14% mEq. After cooking, the contents of catechin were 0.15%, 0.13% and 0.10% mEq catechin in the same respective samples. These results were in agreement with Table 1; they show that the tannin content decreased as the dose of irradiation increased.

The same observation was made by Pinn (1992) who irradiated cooked beans (*Phaseolus vulgaris* L.) at doses of 2; 4; 6; 8; 10; 15 and 20 kGy and observed reduction in the tannin content as the irradiation dose increased. The content of tannin in the control was 0.47% mEq catechin as compared to 0.29% observed in samples irradiated at 20 kGy. Likewise, in our results (Table 1) the tannin content of the control was 0.006% mEq catechin, and only trace amounts were observed in the 10 kGy-treated samples.

#### 3.2. Phytate

The phytate content (Table 1) of uncooked and cooked beans, without irradiation, were respectively, 8.28 and 9.55 mg/g. After irradiation with doses of 2, 6 and 10 kGy, values decreased for all doses and for uncooked and cooked.

Pinn (1992) observed a decrease of phytate content, from 0.21 to 0.14 mg/g, when samples were irradiated at 20 kGy.

According to Ologhobo and Fetuga (1984), cooking and autoclaving do not affect the phytate content in beans. As in the research of Ene-Obong and Obizoba (1996), thermal treatment did not affect the phytate content. However, maceration for 12 h was able to reduce the phytate content by 31% and maceration for 24 h was able to reduce it by 16% according to research by Ene-Obong and Obizoba (1996).

Ene-obong (1995) found phytate content variations from 6.30 to 7.45 mg/g, 8.31 to 11.3 mg/g and 8.40 to 9.92 mg/g for *Sphenostylis stenocarpa*, *Cajanus cajan* and *Vigna unguiculata*, respectively.

Table 1  
Tannins (% mEq catechin) and phytic acid (mg/g) in the common beans uncooked and cooked with and without irradiation (dry base)

| Dose of irradiation (kGy) | Tannin (% mEq catechin)                |                           | Phytic acid (mg/g)       |                          |
|---------------------------|--|---------------------------|--------------------------|--------------------------|
|                           | Uncooked                               | Cooked                    | Uncooked                 | Cooked                   |
| 0                         | 2.08 <sup>A</sup> ± 0.02 <sup>BB</sup> | 0.006 ± 0.00 <sup>a</sup> | 8.28 ± 0.01 <sup>a</sup> | 9.55 ± 0.01 <sup>a</sup> |
| 2                         | 2.49 ± 0.11 <sup>a</sup>               | 0.103 ± 0.15 <sup>a</sup> | 4.63 ± 0.27 <sup>c</sup> | 9.43 ± 0.1 <sup>b</sup>  |
| 6                         | 1.93 ± 0.01 <sup>b</sup>               | 0.031 ± 0.00 <sup>a</sup> | 7.58 ± 0.24 <sup>b</sup> | 5.29 ± 0.02 <sup>d</sup> |
| 10                        | 1.56 ± 0.02 <sup>c</sup>               | –                         | 7.25 ± 0.01 <sup>b</sup> | 7.33 ± 0.10 <sup>c</sup> |

<sup>A</sup> Average + SD.

<sup>B</sup> In the same column, means with different superscript are statistically different ( $p \leq 0.05$ ).

Table 2

Total iron (mg/100 g), dialysed iron (%), and amount of available iron (mg/100 g) in non-irradiated and irradiated, uncooked and cooked beans

| Dose of irradiation (kGy) | Cooked beans                          |                         |            | Uncooked beans           |                         |            |
|---------------------------|---------------------------------------|-------------------------|------------|--------------------------|-------------------------|------------|
|                           | Total Iron (mg/100 g)                 | Dialysed Fe (%)         | (mg/100 g) | Total Iron (mg/100 g)    | Dialysed Fe (%)         | (mg/100 g) |
| 0                         | 12.3 <sup>A</sup> ± 0.1 <sup>aB</sup> | 5.33 ± 0.0 <sup>c</sup> | 0.65       | 14.6 ± 3.0 <sup>ab</sup> | 1.16 ± 0.0 <sup>d</sup> | 0.02       |
| 2                         | 12.9 ± 1.3 <sup>a</sup>               | 5.38 ± 0.0 <sup>c</sup> | 0.69       | 15.6 ± 1.6 <sup>ab</sup> | 1.93 ± 0.0 <sup>b</sup> | 0.03       |
| 6                         | 11.7 ± 1.2 <sup>a</sup>               | 8.02 ± 0.1 <sup>a</sup> | 0.94       | 12.0 ± 0.4 <sup>b</sup>  | 2.39 ± 0.0 <sup>a</sup> | 0.29       |
| 10                        | 11.7 ± 0.5 <sup>a</sup>               | 7.84 ± 0.0 <sup>b</sup> | 0.92       | 18.0 ± 0.9 <sup>a</sup>  | 1.61 ± 0.0 <sup>c</sup> | 0.29       |

<sup>A</sup> Average + SD.<sup>B</sup> In the same column, means with different superscript are statistically different ( $p \leq 0.05$ ).

### 3.3. Iron and dialysed iron

Considering that the recommendation for iron intake is, on average, 12.5 mg and, for healthful individuals, absorption is about 15% of the total amount of iron present in the diet, the importance of common beans in the diet of the Brazilian population is verified.

Benefit of the effect of irradiation was observed in uncooked beans, with an increase in the content of the iron dialysis under the effect of irradiation when compared to the control. In cooked beans, maceration and cooking were responsible for the inactivation of antinutritional factors and, consequently, for the better iron dialysis, as well as ensuring the texture, flavour and aroma for consumption.

The total iron contents of uncooked and cooked beans were 12.3 and 14.6 mg/100 g, respectively, and they not alter with irradiation of cooked beans, but only of uncooked beans. Dialysed irons in uncooked and cooked beans were different. The process of cook and irradiation increased dialysed iron. The best dose was 6 kGy, because it gave the higher value of available iron (total iron and dialysed iron), 0.94 and 0.29 mg/100 g, respectively, in cooked and uncooked beans (Table 2).

Pinn (1992) verified that, according to the dose applied, there was an increase in the percentage of dialyzable iron. With doses of 2, 4, 6, 8 and 10 kGy, increases of approximately 11%, 13%, 23%, 57% and 70%, respectively, were observed in relation to the percentage of dialyzable iron of the non-irradiated beans (0.82%).

Martínez et al. (1998) reported that the availability of iron “in vitro” in cooked *Phaseolus vulgaris* averaged 4.1–9.0%, similar to those presented in Table 2.

Amaya, Acevedo, and Bressani (1991) found the following variations for total iron during the processing of black beans (*Phaseolus vulgaris*): brothless beans, 2.9–3.8 mg/100 g, beans with broth, 4.1–5.8 mg/100 g, and broth alone, 12.6–15.5 mg/100 g. The ionizable iron, as an indicator of the bioavailability of iron, ranged from 0.31 to 0.41 mg/100 g for brothless beans and 0.29 to 0.41 mg/100 g for beans with broth, which were very close values. However, the value found in the bean broth ranged from 1.13 to 1.92 mg/100 g.

Using in vivo methodology with anemic rats, with ferrous sulfate as standard, Sgarbieri, Antunes, and Almeida (1979) found 4.05% of bioavailable iron in cooked *Phaseolus vulgaris* beans. The availabilities found in the present research were higher, the in vitro results being comparable to the method of extrinsic iron staining in human beings, according to Miller and Schriker (1982).

## 4. Conclusions

The irradiation process with a 6-kGy dose showed a reduction in the antinutritional factors in cooked beans and was the most effective result for the iron availability.

Thus, uniting irradiation doses (60-kGy doses) and cooking, we achieved an improvement in the iron availability of the grain, consequently improving its nutritional value, and extending its conservation. We also concluded that:

- the tannin content decreased as the dose of irradiation, as well as the cooking, increased;
- the phytate content decreased with the irradiation process.

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