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XVII. INTERNATIONAL PLANT NUTRITION COLLOQUIUM & BORON SATELLITE MEETING

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Iron Concentration and Bioavailability in Cooked vs. Uncooked Beans of a Traditional Portuguese *Phaseolus vulgaris* Variety

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INTRODUCTION

Iron (Fe) deficiency, the most common micronutrient deficiency, affects approximately 30% of the population. Non-heme Fe, which is obtained from plant foods, is not as easily absorbed as heme Fe, which is obtained from animal foods. Hence, Fe deficiency is higher in developing countries which, due to cultural and economic factors that inhibit meat consumption, rely instead on plant-based diets. In industrialized countries, meat consumption is common (Vasconcelos & Grusak, 2006). However, an increasing number of individuals in these countries are substituting meat-based with plant-based diets due to several motives: adherence to vegetarian lifestyles (perceived to be healthy) and economic limitations resulting from the recent economic crisis. Legumes, which contribute significantly to human nutrition and health, constitute a major source of protein and essential minerals including Fe. In this study, we analyzed the Fe concentration and bioavailability of both cooked and uncooked common beans of a traditional Portuguese variety (*Papo-de-Rola*).

METHODS

Preparation of bean samples

Phaseolus vulgaris "Papo-de-Rola" seeds (8 g) were ground to a fine powder. Four grams were stored at -20 ^{o}C and the other 4 g were mixed with ultra-pure water (1:4 w/v) and cooked for 30 min at 121 ºC in a steam sterilizer (to simulate the cooked boiled beans). The cooked bean sample was stored at -20 ^{o}C for at least 24 h. Both cooked and uncooked samples were freeze-dried for 72 h. Samples were kept at -20 ^{o}C until further assays.

Inductively coupled plasma optical emission spectrometry (ICP-OES) analysis

Cooked and uncooked samples were weighed into glass tubes. First, they were digested overnight in 2 ml of nitric acid and then at 125 ^{o}C for 2.5 h. Then 1.5 ml of 30% H_2O_2 was added and the samples were digested for 1 h; this step was repeated twice. The temperature was then increased to 200 ^{o}C and samples were evaporated to dryness. Residues were dissolved in 15 ml of 2% nitric acid. Fe concentration was determined by ICP-OES.

In vitro digestion/Caco-2 cell assay

Fe as $FeCl_3$ was added to half of the cooked and uncooked samples before the *in vitro* digestion/Caco-2 cell assay. Enough Fe was added so that the final concentration in the digested sample was 80 ppm. A gastric digestion of the samples was simulated by adding pepsin (1 hr at pH 2) followed by the neutralization of the digest with Na_2HCO_3 (0.5 h at pH 7). The digest was added to the Caco-2 cells, maintained in MEM (an Fe-deficient media) for 24 h prior to the assay. Following 24 h of incubation (5% CO_2, 37 ^{o}C), the cells were harvested and later analyzed for ferritin (a proxy of Fe bioavailability) and cell protein.

Statistical analysis

Data was analyzed using R statistical package (http://www.r-project.org/). Means were compared by *t*-tests; statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

The ICP-OES analysis of "Papo-de-Rola" beans revealed a significantly higher Fe concentration ($p < 0.05$) in the cooked beans than in the uncooked ones (Fig. 1A). An *in vitro* digestion/Caco-2 cell model was used to assess Fe bioavailability from the cooked and uncooked beans. While no difference in Fe bioavailability between the cooked and uncooked beans was detected (data not shown), when samples were spiked with 80

ppm Fe, the cooked beans showed a tendency ($p < 0.06$) towards increased Fe uptake in the Caco-2 cells (Fig. 1B). This suggests that after cooking, some Fe-enhancing components emerge or some Fe-inhibiting components are lost from the food matrix.

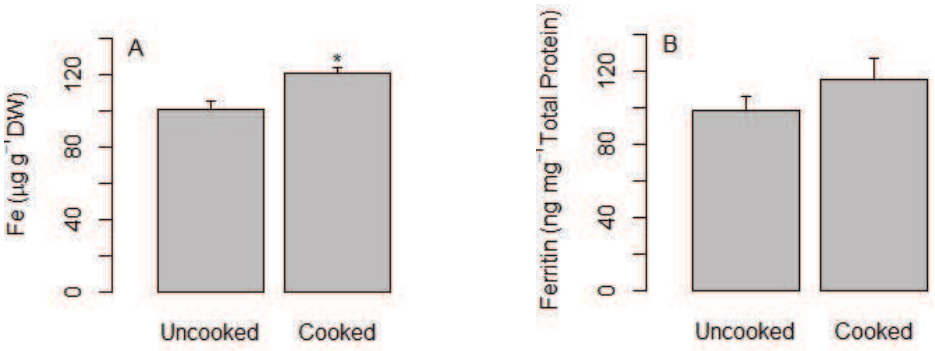

Fig. 1. A - Iron concentration in the uncooked and cooked bean samples. B - Caco-2 cell ferritin formation from the uncooked and cooked bean samples spiked with 80 ppm Fe, as determined by the *in vitro* digestion/Caco-2 cell model. Values are mean ± standard deviation; asterisk indicates statistical significant difference ($p < 0.05$).

CONCLUSIONS

The cooked "Papo-de-Rola" beans had a higher Fe concentration than the uncooked samples. Also, compared to the uncooked beans, cooked beans appeared to increase the uptake of exogenous Fe in the Caco-2 cells. Additional studies should focus on identifying components in the beans that affect Fe bioavailability and how these are affected with boiling and other possible cooking methods. Identifying cooking methods that would enhance Fe bioavailability would be of immense value for Fe-deficient populations whose staple diet revolves around beans. Identifying bean components that enhance Fe absorption could potentially be used in fortification and supplementation of foods.

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