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Impact of processing on Fe dialysability from bean (*Phaseolus vulgaris* L.)

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The influence of dehulling and cooking on *in-vitro* Fe dialysability from two varieties of *Phaseolus vulgaris* L. (mottled and white beans) was investigated. Fe and seed constituents (phytate, tannin, dietary fibre) were differently distributed between hull and cotyledon. Fe dialysability from mottled bean was 2·5%, from white bean 3·6%. Dehulling significantly decreased Fe dialysability (36%, $P < 0{\cdot}025$) from both varieties. Cooking significantly reduced Fe dialysability from mottled bean (59%, $P < 0{\cdot}025$), whereas in white bean no modifications were detected. On the other hand, cooking positively influenced Fe dialysability from cotyledon: compared with the raw samples, both varieties showed a significant increase (54%, $P < 0{\cdot}005$) in Fe dialysability. This study showed that localization of both Fe and some seed constituents in seed affected Fe dialysability. Changes in food constituents (i.e. degree of protein digestion) account for the modifications in Fe dialysability subsequent to cooking.

INTRODUCTION

Legumes are widespread throughout the world and represent a staple food in the diet of many populations, especially in developing countries, where they are the main source of energy and protein. Even though legumes can substantially contribute to an adequate mineral intake, the mineral availability, especially as far as Fe is concerned, is generally poor, since it is affected mainly by phytic acid, tannin and dietary fibre components (Hallberg *et al.*, 1987; Gillooly *et al.*,1983; Rossander, 1992). While the effects of these constituents on Fe absorption have been the subject of numerous studies, little is known about the influence of cooking procedures on Fe availability. Some food treatments can have a marked effect on the composition of food and, therefore, on Fe availability. Dehulling has been shown to reduce the total Fe content of faba bean (Youssef *et al.*, 1987), soyabean (Levine *et al.*, 1982; Latunde-Dada, 1991) and chickpea (Chavan *et al.*, 1986). Food processing — such as cooking, bread-making and fermentation — is known to affect not only the bioavailability of Fe but also the factors that act as promoters or antagonists of mineral absorption (Bjorn-Rasmussen, 1974; Hazell *et al.*, 1987; Navert *et al.*, 1985; Sandberg, 1991). The question is whether these constituents, as well as food processing, are of importance as far as Fe availability is concerned.

The aim of this study was to investigate the influence of two food treatments (dehulling and cooking) on Fe dialysability from two varieties of *Phaseolus vulgaris* (mottled and white bean). In order to understand the influence of the single main chelating agents of legumes, seeds were dehulled and then both whole seeds and cotyledons were cooked. The impact of both treatments, as well as that of some chelating agents, on Fe dilaysability was then evaluated.

MATERIALS AND METHODS

Two varieties of *Phaseolus vulgaris* L. (mottled and white beans) were studied. The experiments were carried out on both raw and cooked whole seeds and cotyledons. Samples were hand-dehulled and then ground in a Cyclotec 1093, Tecator pin mill (< 50 μm). The samples were soaked for 2 h in deionized water (1:3, w:v ratio) at room temperature and then cooked by autoclaving (120°C, 1 Atm, 20 min) in the soaking water. They were then freeze-dried together with the cooking water.

Phytic acid content was determined according to the method of Harland and Oberleas (1986). Total dietary fibre was determined by the method of Prosky *et al.* (1988). The modified vanillin assay (Price *et al.*, 1978) was used to measure the amount of condensed tannins expressed as cathechin equivalents. Total Fe content was determined by Atomic Absorption Spectrometry using a Varian SpectrAA 400. Certified standard BCR 189, wholemeal flour (Community Bureau of Reference, Brussels), was analysed to check the accuracy of the analysis. Experimental values were not statistically different from certified values (69·3 ± 2·3 and 68·3 ± 1·9 μg/g, respectively).

Dialysability

Fe dialysability was determined according to the '*in vitro*' method of Miller *et al.* (1981). Samples (about 10 g) were blended in 0·1 N HCl, the pH was adjusted to 2·0 ± 0·05 and 5 ml of a pepsin solution (10 g pepsin in 100 ml 0·1 N HCl) were added. The final volume of the homogenates was brought to 100 g by adding deionized water and the samples were incubated at 37°C for 2 h in a shaking water bath. Aliquots (20 g) of the pepsin-digests, were transferred into 100 ml beakers. Segments of dialysis sac (cut-off 6–8000) containing 0·5 M $NaHCO_3$ (an amount previously determined in order to titrate a sample aliquot to pH 7·5) and sufficient deionized water to obtain the volume of 20 ml, were placed in each beaker and incubated for 30 min. Five millilitres of a pancreatin–bile solution (0·8 g pancreatin, 5 g bile extract in 200 ml 0·1 M $NaHCO_3$) were added and the incubation continued for a further 2 h. The dialysates were weighed and the amount of Fe in the dialysis bag was determined by bathophenanthroline (Miller *et al.* 1981).

The data were statistically evaluated by analysis of variance (ANOVA) and the differences between means were determined by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Fe content of whole seeds and seed fractions (hull and cotyledon) is given in Table 1. Both bean seeds and seed fractions contained high amounts of Fe. Hull represented 10% and cotyledon 90% of the mottled bean seed, while in white bean the respective percentages were 7·5 and 92·5%. Most of the total seed Fe was therefore found in cotyledon.

Cooking did not induce changes in total Fe content because the beans were cooked in the soaking water and then freeze-dried together with it.

Fe dialysability from whole seed and cotyledon, before and after cooking, is shown in Table 2. Compared with the mottled variety, raw white bean had a higher Fe dialysability. An explanation for this difference might be the presence of tannins in the mottled variety (Table 3), since it is the only compositional difference evidenced between the two bean varieties. Several studies suggested that tannins exert a marked inhibitory effect on Fe absorption (Gillooly *et al.*, 1983; Brune *et al.*, 1989) and, furthermore, a dose-related effect was reported (Siegenberg *et al.*, 1991; Tutanwiroon *et al.* 1991). In order to better-evaluate the influence of tannin on Fe availability, the hulls, which contain almost all the tannin of the bean, were removed and Fe dialysability was assessed.

Surprisingly in comparison with the whole seed, a significantly lower Fe dialysability was detected in both the varieties (36%, $P < 0·025$) after removing the hulls (Table 2). Thus, even if tannins could influence the level of available Fe from the mottled variety, their removal did not improve Fe dialysability.

Phytic acid and dietary fibre are the other two legume constituents analysed in this study. As shown in Table 3, these factors, which are thought to be involved in determining Fe dialysability, were differently distributed in the seed. They are therefore likely to have a different impact on Fe dialysability from whole seed or from cotyledon.

Phytic acid was mainly located in the cotyledon, with the hull containing less than 0·1% of seed phytate (Carnovale *et al.* 1988). Upon dehulling, a higher amount of phytic acid was detected (28% in mottled bean, 17% in white bean), owing to a more efficient phytic acid extraction subsequent to hull removal. As a consequence, in cotyledon an increased influence of phytate on Fe dialysability could not be excluded.

On the other hand, dehulling not only completely removed tannin from mottled bean, but also reduced the total seed fibre in both the beans. The highest loss of dietary fibre was detected in the insoluble fraction — the major component of hulls — whereas the soluble fraction did not show much change.

Another important factor to be considered is that legume hulls are a rich source of Fe, therefore an important consequence of hull removal was the loss in this mineral. The reduction of the total seed Fe content amounted to about 11·3% in mottled bean and to about 9·6% in white bean.

Table 1. Total Fe content of whole seed and of seed fractions (dry basis)

		Fe mg/100g
Mottled bean	Whole seed	8·74 ± 0·2
	Cotyledon	7·64 ± 0·8
	Hull	9·40 ± 1·4
White bean	Whole seed	6·24 ± 0·8
	Cotyledon	6·00 ± 1·0
	Hull	8·00 ± 1·2

Each value represents the mean ± SD for three determinations.

Table 2. Dialysable Fe (%) from raw and cooked samples

	Raw	Cooked
Mottled bean		
Whole seed	2·50 ± 0·3*a*	1·03 ± 0·1*b*
Cotyledon	1·60 ± 0·5*b*	2·59 ± 0·2*c*
White bean		
Whole seed	3·60 ± 1·5*a*	3·89 ± 0·3*a*
Cotyledon	2·29 ± 0·5*b*	3·50 ± 0·2*ac*

Each value represents the mean ± SD of five determinations. Within a group, values with different letters are significantly different (*a* vs *b*, $P < 0·025$; *b* vs *c*, $P < 0·005$).

Table 3. Phytic acid, tannin and dietary fibre content of samples (% dry basis)

	Phytic acid	Tannin	Dietary fibre Total	Soluble	Insoluble
Mottle bean					
Whole					
raw	1·05	0·127	16·88	1·05	15·82
cooked	0·91	0·085	16·87	5·02	11·80
Cotyledon					
raw	1·35	nd	14·18	2·68	11·50
cooked	1·16	nd	15·90	7·99	7·69
White bean					
Whole					
raw	1·02	nd	18·50	2·34	16·08
cooked	0·95	nd	19·34	5·38	14·65
Cotyledon					
raw	1·19	nd	14·36	2·27	12·09
cooked	1·03	nd	14·05	5·43	8·68

Each value represents the mean of three determinations.
nd = not detectable.

Laszlo (1988) reported that most of the iron in soybean hull was in a highly available ferrous state. Lykken *et al.* (1987) showed a high Fe absorption from soyabean hull. We found (unpublished data) that Fe contained in the hull showed a higher dialysability than Fe contained in the cotyledon. This means that the removal of this seed fraction could have been the main cause of the Fe dialysability reduction observed upon dehulling. The effect of cooking on Fe dialysability from whole seed differed from that on Fe dialysability from cotyledon (Table 2). Mottled bean showed a significant reduction in Fe dialysability after cooking (59%; $P < 0{\cdot}025$), whereas cooking did not influence Fe dialysability from the white seed variety. The value of Fe dialysability from cooked mottled bean found in this study is consistent with values reported for Fe absorption from black beans in humans (0·84 and 1·15%) (Lynch *et al.*, 1984; Layrisse *et al.*, 1969). On the other hand, cooking positively influenced Fe dialysability from cotyledon: compared with the raw samples, both varieties showed a remarkable increase in Fe dialysability (about 54%, $P < 0{\cdot}005$).

Changes in seed consituents were another consequence of cooking treatment (Table 3). Upon cooking, 67% of tannins were detectable in mottled bean. This reduction was likely due to interactions of tannins with minerals and other food components, which lead to formation of complexes that might account for the decrease in Fe dialysability detected in mottled bean after cooking.

Cooking also caused a phytate reduction in both whole seed and cotyledon, which amounted to about 13% in all samples. It is likely that the cooking conditions adopted in this study (see method) were not able to induce a significant phytate hydrolysis (Beal *et al.*, 1985; Reddy *et al.*, 1978; Duhan *et al.*, 1989). However, it must also be taken into account that the method utilized for phytate determination did not discriminate between phytic acid and the various inositol phosphates that might form upon cooking (Leherfeld, 1992). This distinction could be important because Fe dialysability is differently influenced by the various inositols. It has been reported that inositol tetra and tri-phosphate enhance Fe availability (Sandberg *et al.*, 1989).

Also the seed composition was affected by cooking, which altered the distribution of the fibre components. The total content of dietary fibre did not change but we observed a decrease of the insoluble fibre content and a marked increase in the soluble fraction.

The effect exerted on Fe availability by the components of these fibre fractions has not yet been clearly ascertained. While some fibre components have been proven to bind Fe *in vitro*, many studies indicate that they have little effect on Fe absorption (Torre *et al.*, 1991; Rossander, 1992). However, the changes in phytate and dietary fibre contents are not sufficient to explain the differences evidenced in Fe dialysability.

The influence of food digestibility is a further aspect that has to be examined in order to understand the overall effect of the various food constituents on Fe availability.

Research carried out on the same bean varieties as utilized in this study (Carbonaro *et al.*, 1992), showed that dehulling *per se* had no effect on protein digestibility, while cooking significantly increased protein digestibility of cotyledon. These findings may contribute to explain the differences in Fe dialysability between whole seed and cotyledon found after cooking. Several studies have already underlined the importance of the protein source for Fe availability. Nelson and Potter (1980) found a relationship between protein digestibility and the release of protein-bound Fe. Keane and Miller (1984) suggested that the properties of undigested or partially digested protein influence Fe availability. Hurrell *et al.* (1989) reported that Fe dialysability increased according to the extent of milk protein hydrolysis. In a previous study (Lombardi-Boccia *et al.*, 1994) we have pointed out that the degree of protein digestion and peptide composition might likely concur to modify Fe availability from legumes.

CONCLUSIONS

Results showed that the localization of both Fe and other constituents in the seed plays an important role in determining Fe dialysability.

As far as raw whole seeds are concerned, for example, the tannin-Fe interaction might be the main cause of the differences in Fe dialysability observed between the two bean varieties. The lower Fe dialysability from raw cotyledons was mainly due to the fact that dehulling led to removal of a potentially available amount of Fe.

Cooking modified the seed composition, influencing Fe dialysability as well. The changes in Fe dialysability caused by cooking cannot be fully explained by the changes occurring in the single seed constituents. Interactions with the protein matrix may play an important role as far as the potential Fe availability is concerned.

In conclusion, food processing may alter the overall balance among the factors involved in determining Fe availability. Further studies are necessary to better understand the changes in food matrix induced by cooking which mostly influence Fe availability.

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