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Cell walls limit *in vitro* protein digestibility in processed legume seeds

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Legume seeds were treated in various ways, e.g. cooking applied either before or after milling, in order to obtain flours with different microstructural properties. *In vitro* protein digestibility was assessed by a pepsin/pancreatin index. In all cases digestibility was enhanced by cooking, although final values varied depending on the flour preparation procedure. Cooked and milled seeds, which retained intact cell walls, showed lower digestibility indices (58–72%) than sonicated or milled/cooked preparations devoid of such structures (77–89%). Results suggest that naturally occurring physical barriers, such as cell walls, may reduce the bioavailability of legume proteins.

INTRODUCTION

The potential nutritional value of legume seeds is limited by a number of factors. Structural features of some of their protein fractions and the presence of antinutrients, such as trypsin inhibitors, lectins and polyphenols, may affect protein digestion (Liener, 1994). In addition, the physical form in which foods are consumed has proven to be an important factor governing the bioavailability of nutrients (Björck *et al.*, 1994). This has been shown recently for the starch fraction of legumes, where both the cotyledon tissue structure and the presence of thick cell walls represent a physical barrier for starch digestion *in vitro* (Tovar *et al.*, 1990, 1991) and *in vivo* (Tovar *et al.*, 1992). Since the seed storage proteins are also located intracellularly, it is likely that cell structural factors affecting starch hydrolysis would exert similar effects on the protein digestibility. In this work the *in vitro* digestibility of legume preparations retaining intact cell walls is compared with that of flours devoid of such structures.

MATERIALS AND METHODS

Green coat lentils (*Lens culinaris*) and common beans (*Phaseolus vulgaris*) of different colours (brown, black, red and white) were obtained (2 kg each) from a local supplier. The seeds were of Colombian origin.

Precooked flours rich in intact cell walls were prepared according to Tovar *et al.* (1990): seeds were soaked, boiled until soft, freeze-dried and milled to pass a 1-mm screen in a Cyclotec 1093 mill (Tecator AB, Höganäs, Sweden). Cooking times were 70 min for lentils and red beans, and 120 min for black, brown and white beans. Microstructural and some nutritional characteristics of these materials are reported elsewhere (Tovar *et al.*, 1990, 1991, 1992). For comparative purposes, disruption of cell walls in these preparations was achieved by sonication: the flours were suspended in water, cooled on ice and subjected to 10 pulses of ultrasonic treatment (30 s each) with a Braunsonic 2000 Sonicator at 54 watts output power. Cell wall damage was assessed by microscopy, while the enzymic measurement of starch release provided an indirect estimate of microstructure alteration.

Flours from raw seeds were prepared by dry milling using the Cyclotec equipment. In some experiments, these raw flours were suspended in water and boiled for the above-mentioned times.

The *in vitro* protein digestibility of the various legume preparations was measured by the pepsin/pancreatin index of Akeson & Stahmann (1964), using enzymes from Sigma Chem. Co., St Louis. Ten independent samples per treatment were run. Means were compared by one-way analysis of variance, followed by the Duncan multiple analysis comparison test, using the Number Cruncher Statistical System (NCSS) 5.1 program.

RESULTS

Table 1 shows the *in vitro* protein digestibility index of the various legume preparations. The lowest digestibility corresponded to flours from raw seeds, although it was relatively high for lentils (74%). Cooking, either before

Table 1. *In vitro* protein digestibility index* of raw and processed legumes

	Flours from raw seeds		Flours from cooked seeds	
	Raw flour (%)	Cooked flour (%)	Cooked flour (%)	Sonicated cooked flour (%)
Legumes				
Lentil	$73{\cdot}5 \pm 0{\cdot}7^{a}$	$88{\cdot}6 \pm 0{\cdot}1^{b}$	$74{\cdot}7 \pm 0{\cdot}5^{a}$	$82{\cdot}6 \pm 0{\cdot}2^{c}$
White bean	$38{\cdot}4 \pm 1{\cdot}6^{a}$	$83{\cdot}4 \pm 0{\cdot}9^{b}$	$57{\cdot}6 \pm 1{\cdot}9^{c}$	$85{\cdot}1 \pm 1{\cdot}6^{b}$
Brown bean	$44{\cdot}0 \pm 0{\cdot}7^{a}$	$76{\cdot}7 \pm 0{\cdot}3^{b,c}$	$75{\cdot}2 \pm 0{\cdot}6^{b}$	$78{\cdot}8 \pm 1{\cdot}2^{c}$
Red kidney bean	n.e.	n.e.	$80{\cdot}9 \pm 1{\cdot}5^{a}$	$86{\cdot}9 \pm 2{\cdot}3^{a}$
Black bean	n.e.	n.e.	$82{\cdot}0 \pm 1{\cdot}1^{a}$	$86{\cdot}8 \pm 1{\cdot}1^{b}$

* Protein hydrolyzed as % of total proteins.
n.e.—Not evaluated.
Values are mean of 10 digestibility observations.
The means for different treatments of each legume (rows) were statistically compared; those sharing the same superscript are not significantly different ($P < 0{\cdot}05$).

or after milling, improved the digestibility. The digestibility index of casein, used as a reference sample, was $95{\cdot}3 \pm 0{\cdot}5$ (mean ± SEM).

Sonication of the flours prepared from whole cooked seeds increased the digestibility index, with the exception of red kidney beans where, in spite of the difference in mean values and the large number of samples analyzed, the change observed was not statistically significant. The greatest enhancement (28%) was registered for white beans.

In accordance with previous studies (Tovar *et al.*, 1990, 1991), light microscopy observations confirmed that flours obtained by milling of raw seeds contained mainly free starch, whereas those prepared from cooked seeds were rich in cell wall structures surrounding starch. These walls were extensively disrupted by the ultrasonic treatment applied here, which released starch and made it available to enzymic hydrolysis to the same extent as the previously used (Tovar *et al.*, 1990) homogenization treatment (results not shown). At least 6×30 s sonication pulses were necessary for complete starch release.

DISCUSSION

The enhancement of legume protein digestibility by cooking is well known (El Faki *et al.*, 1984; Levy-Benshimol *et al.*, 1985), and it was clearly observed here for lentils and the bean varieties studied. The digestibility indices recorded after wet heat-treatment of raw bean flours (77–89%) were similar to those found in the literature (El Faki *et al.*, 1984).

In spite of the general improving effect of cooking, the final protein digestibility seemed to depend on the type of process applied. Particularly interesting is the behaviour of the cell wall-containing flours prepared from whole cooked seeds. In these samples, the digestibility indices showed a moderate increase after ultrasonic treatment. It has been shown that intact cell walls, in common bean and lentil preparations, restrict the accessibility of starch to amylolytic enzymes (Tovar *et al.*, 1990, 1991). Thus, disruption of the physical barrier imposed by cell wall structures in these flours appears to be responsible also for the increased protein digestibility observed after sonication.

Present results suggest that the occurrence of cell wall-surrounded protein fractions is a determinant of the susceptibility of legume proteins to enzymic digestion. So far, evaluation of protein bioavailability in pulses has been performed without paying attention to the method of sample preparation. Some authors have assessed digestibility after heating of powdered raw seeds (Levy-Benshimol *et al.*, 1985; Lanfer-Marquez & Lajolo, 1990; Saharan & Khetarpaul, 1994), while others have looked at cooked and later milled samples (El Faki *et al.*, 1984; Sharma & Sehgal, 1991; Godinez *et al.*, 1992), processes that yield materials of different microstructure (Tovar *et al.*, 1991). Such experimental inconsistencies might contribute to discrepant inter-laboratory results. Furthermore, assessment of digestibility in legume preparations freed from cellular structures may result in overestimated figures.

It is noteworthy that the digestibility recorded after ultrasonic treatment of cell wall-containing flours compares well with that of cooked flours obtained from raw seeds (devoid of cell wall structures). This observation suggests that here reported differences between the maximal protein digestibility of heat-treated legumes (79–87%) and that of the casein reference (95%) may be attributed to factors other than physical inaccessibility.

Finally, it should be stressed that the existence of physically inaccessible reserve biopolymers, i.e. starch and protein, is a common feature of mild processed legume seeds, such as those prepared according to household recipes (Tovar *et al.*, 1992). This factor might therefore be of importance for human nutrition. Further studies are needed for a better understanding of the physiological significance of these findings.

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