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Changes in dietary fiber content and its composition as affected by processing of black beans (*Phaseolus vulgaris*, Tamazulapa variety)

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Abstract. This paper presents the effect that the traditional cooking process of black beans (*Phaseolus vulgaris*, Tamazulapa variety) has on the quantity and composition of soluble (SDF) and insoluble (IDF) dietary fiber of beans, as well as on its protein digestibility and protein quality. There was an increase of IDF from 18.1% in cooked beans to 22.4% in fried beans, and a decrease in SDF from 8.4% to 6.6%, respectively. Starch content decreased from 34.5% to 31.3%. No change was found in lignin. The xylose content was higher in IDF than in SDF and decreased to some extent from cooked to fried beans. Arabinose content was similar in IDF and SDF with no change caused by processing. The fraction containing glucose, mannose and galactose in IDF was higher than in SDF, the content increasing in IDF and decreasing in SDF, with processing. Protein content in IDF was higher than in SDF, with no major change when processing. About 29.5% of the total protein of beans was bound in DF. Protein digestibility and protein quality decreased from cooked to fried beans and was positively related to IDF.

Introduction

Recently scientists have focused public's attention on the dietary fiber (DF) content of foods, since there has been some evidence that diets that are low in DF are related with several modern western diseases such as constipation, diverticulosis, colon cancer and coronary heart diseases [1, 2].

In developing countries diets are low in animal proteins of good quality and high in vegetable proteins of low protein quality. Intake of vegetable biomass is relatively greater in these countries [3]. In Central America for example beans (*Phaseolus vulgaris*) in their different preparations, are widely consumed as part of the basic diets of urban and rural areas. Intakes vary between 50–80 g/person/day [3]. Beans have been recognized as a good source of supplementary proteins for these populations [4], however, they contain between 25–30 TDF of which about 65% is IDF and 35% is SDF [5]. Therefore, intake of DF from beans will range from 12–24 g per person, per day, values that will increase from the consumption of other foods such as maize tortillas [5]. On the other hand, it has been shown that protein

digestibility of beans is low [6]. At the present time various factors have been suggested to be responsible, one of which may be DF.

The different ways in which beans are consumed vary from one region to another, but basically there are three forms: whole water-cooked beans with or without their broth, blended and strained, and fried beans. Changes in chemical composition and nutritive value have been published before [7]. Some studies have indicated an increase in DF when foods are processed [8]. The physiological effect of dietary fiber (DF) is determined by the physical and chemical properties of the fiber's components [9]. At the present time there is no available information of the chemical profile of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) of black beans after heat treatment. This aspect could be of importance, especially in relation to the poor digestibility of bean protein.

The purpose of this study was to analyze the changes in DF, both in quantitative and qualitative terms of black beans, which were processed in the different consumption forms and relate this change to the protein digestibility and quality in rats.

Materials and methods

Sample preparation. Black beans (*Phaseolus vulgaris* Tamazulapa variety) harvested in March 1988 in Jutiapa, Guatemala were kept refrigerated at 4 °C until used in the studies described. Cooked beans were prepared by mixing boiling water with clean seeds (3:1 v/w), cooked at atmospheric pressure for 2 hours, dried with its broth, in an air oven at 40 °C for 18 hours and milled to pass a 0.5 mm metal screen of the Cyclotec Mill (Tecator, Sweden). The blended-mashed beans were first cooked as described above. After the initial cooking, vegetable oil was added to 100 g of cooked bean flour (25% v/w) and cooked at atmospheric pressure for another 15 min then dried and milled as above. Fried beans were prepared as blended beans only that 25% v/w more vegetable oil was added and cooked until most of the water content had evaporated and had reached a paste-like texture. It was then dried and made into a flour. For the chemical analyses, samples were defatted by maceration in ether during 36 hours.

Chemical analysis. The residual moisture content and the ash, fat and protein analyses were carried out by the AOAC procedures [10].

Analysis of dietary fiber. Samples were treated for DF analysis according to Asp [11]. A defatted sample (0.5 g) was incubated in buffer pH 6 and 100 μl of Termamyl 1 (Novo Industries), and kept in agitation at 100 °C during 20 min. After reaching room temperature, pH was adjusted at 1.5 with HCl. One ml of pepsin (Merck No. 7190) 10% v/w solution was added and incubated for 1 hour at 40 °C. After cooling, pH was adjusted to 6.8, and 1 ml of pancreating

10% v/w (Merck) was added and incubated at 40 °C during 1 hour; water was added to reach the 100 ml mark. Insoluble dietary fiber was filtrated over a fritted glass crucible (40–60 μm porosity) with 0.4 g of Celite (Sigma Chemicals) to help filtration, the residue was washed and dried in an air oven at 105 °C overnight, soluble dietary fiber was precipitated with 4 volumes of 95% ethanol at 60 °C, during 1 hour. Soluble dietary fiber was filtered as described above. Half of the crucible contents were used for ash determination, and the other half for crude protein determination by the Kjelhdal semimicro method with a Kjeltec Auto Analyzer (Tecator, Sweden). Isolation of larger portions of each sample IDF and SDF was done using the same method of Asp et al., in proportionally larger quantities, centrifugating and freeze-drying the DF residues.

Neutral sugar analysis. Fractions of lyophilized IDF were hydrolyzed with H_2SO_4 for two hr at room temperature; after cooling, enough water was added to make the acid 1 M, and hydrolyzed in a sealed ampoule for 1 hour at 100 °C. After thermal equilibrium the ampoule's content was filtrated in a preweighed fritted glass crucible. The residue in the crucible was thoroughly washed with diluted acid and water; dried for 18 hours at 105 °C and weighed again, defining this increase in the crucible weight as Klason Lignin [12]. The filtrate was neutralized with solid $Ba(OH)_2$ until a stable pH of 6.0 was attained; centrifugated, and the supernatant passed through a cation exchange resin (Rexin 101 Fisher). The eluate was concentrated under reduced pressure, and injected into the chromatographic system. Portions of the SDF were hydrolyzed as described by Saeman cited in Selvendran & DuPont [13]. One hundred and fifty mg SDF/ml H_2SO_4 1M were placed at 100 °C for 1 hour and treated as above.

Chromatographic system. Hydrolysis of both SDF and IDF was monitored with a TLC system with glass plate 20 × 20 covered with silica gel 60 (25 mm), in a glass saturation chamber and a mobile phase of NbutOH: IsopropOH: HB03 (5 g/1) in the proportion 50:30:20, respectively, using p-Anisidine/n-ButOH 1% v/w, for color development. The chromatographic system used was a Beckman 114M bomb, with a 20 μl injector using a Waters Bondapak C-18 NH2 carbohydrate column and a detector Varian R1–3. The mobile phase was a solution of acetonitrile water (83:17 v/w) (Malinckrodt Chemicals). The samples were run in triplicate using pure sugars as standard (Sigma Chemicals).

Starch. Starch was measured using an enzymatic method described by Holm et al. [14]. A sample of 500 mg and 15 ml water and 100 μl of Termamyl were incubated at 100 °C for 20 min, then transferred to a 50 ml volumetric flask, mixed and diluted to the mark. One ml of this solution was placed in a plastic tube containing 2 ml of a sodium acetate buffer 0.1M, pH 4.75, and 50 μl were added of amyloglucoseidase (Sigma Chemicals). The mixture was incubated for 30 min at 60 °C. The tube contents were transferred to a 100 ml volumetric

flask mixed, and diluted to the mark. One ml of this solution was transferred to a plastic tube adding 1 ml of distilled water. Four ml of glucose-oxidase reagent (Merkotest 3395 Merck) were added and incubated for 60 min at 37 °C. After centrifugation the absorbance was measured at 450 nm in a Varian DMS 90 spectrophotometer. A standard curve was prepared with pure glucose.

Biological experiment. Experimental diets were prepared containing a 10% level of protein from the bean preparations described before. The amount of blends needed was supplemented with vitamins and minerals as described by Pellet & Young [15] corn starch was used to adjust to 100%. White Wistar rats 20–22 days old with an average weight of 46–48 g from INCAP's animal colony were randomly distributed in 6 groups (3 males and 3 females). They were placed in individual all-wire screen cages. Feed and water were supplied ad libitum during a 28-day period. During the last week of the experiment, feces were collected for 5 days, cleaned, dried and milled through a 1 mm metal screen. Nitrogen content was determined as previously described. The total weight gain of the animals and food ingested were used to calculate PER and fecal nitrogen to calculate TD values. For this purpose a nitrogen-free diet was also fed to a similar group of rats during 28 days.

Statistical analysis. Statistical analysis was performed using a SAS Stat Program (SAS Institute), licensed to INCAP; using anova, orthogonal contrasts, linear regression analysis and the Tukey means test.

Results and discussion

The DF analysis of the three preparations are presented in Table 1. The results show an increase in the IDF (18.1, 21.0 and 22.4% dwb for cooked, blended

Table 1. Whole balance of black beans*

	Cooked	Blended	Fried
Protein (6.25)	20.8±0.3	19.2±0.3	19.6±0.2
Ash	3.7±0.1	3.3±0.2	2.3±0.0
Fat	4.3±1.2	4.4±1.1	4.9±1.1
Residual moisture	6.6±0.1	9.7±0.1	7.7±0.1
Klason lignin	4.0±0.5	4.2±0.8	3.9±0.3
Carbohydrates			
Insoluble DF	18.1±0.7	21.0±1.4	22.4±2.5
Soluble DF	8.4±0.5	7.3±0.7	6.6±0.9
Starch	34.5±2.6	30.5±1.9	31.1±3.5
Total	100.05	99.7	98.7

*Expressed as % of dwb.

and fried beans, respectively), a decrease in the SDF content (8.4, 7.3 and 6.6% dwb), and an increase in the ratio SDF/IDF. These changes are definitively related between themselves. The linear regression analysis between the increase of IDF and the decrease of SDF has a relation of 54% ($p<0.05$). These results indicate that the thermal treatment used to prepare the three products does not affect the total amount of the DF, instead a redistribution occurs between IDF and SDF. This can be visualized in the ratio of SDF/IDF, that are opposite to the ones found by Bjork et al. [16] in extruded wheat. This could mean a different effect caused by the type of thermal processing; dry in case of extrusion and moist or wet in the case of black beans. These changes in IDF could also be caused by some thermal degradation of the starch present in the grains (approximately 32%).

Neutral sugar composition. These results are presented in Table 2. With the method of analysis used the peaks of Glu Gal and Man could not be resolved. However, monitoring with the TLC, showed that the major component was the Glu residue. The fractions of Xy and Ara remain basically the same in the processed samples, establishing that the polysaccharides (PS) where they come from, are not subject to any reaction by heat treatment. The portion of sugars containing Glu Gal and Man is the most affected, and from TLC monitoring, the increase of Glu in this fraction is probably due to the starch degradation as proposed by Bjork et al. [16]. The starch values (Table 1) obtained show a slight decrease from 34.5 to 31.3% dwb. This could account in part for the IDF increase, but the method lacks sensitivity to conclude so. Varo et al. [18] found an effect of the thermal treatment on the DF constituents, especially the starch residue. IDF is composed mainly by Glu PS (cellulose) and the SDF residue

Table 2. Whole balance of black beans dietary fiber*

		Cooked	Blended	Fried
Xylose	(INS)	2.5±0.5	1.2±0.2	2.2±0.0
	(SOL)	1.3±0.1	1.2±0.1	0.8±0.1
Arabinose	(INS)	4.2±0.5	3.1±0.7	5.3±0.0
	(SOL)	4.3±1.7	3.1±0.2	4.0±0.4
Glu+Man+Gal	(INS)	16.8±2.3	10.9±0.5	26.5±0.9
	(SOL)	12.0±1.1	7.4±0.5	6.2±0.8
Protein (6.25)	(INS)	25.0±3.4	25.9±0.9	26.5±0.9
	(SOL)	7.7±1.7	10.0±5.1	5.4±0.0
Ash	(INS)	0	0	0
	(SOL)	2.4±1.0	1.3±1.1	2.4±1.1
Lignin	(INS)	15.5±1.6	14.5±0.2	13.8±0.1
	(SOL)	0	0	0
Total		91.7	78.6	93.1

* Expressed as % dw of total DF.

Table 3. Results of the biological experiment with black beans*

Diet	Feed ingested (g)	Weight gain (g)	PER	TD (%)
Cooked beans	281±33	28±10.0	1.09±0.34	64.4±10.7
Blended beans	216±17	16±3.9	0.80±0.17	61.7±3.5
Fried beans	188±22	12±3.4	0.69±0.18	61.1±5.0
Casein	389±17	87±14.3	2.61±0.37	—
Nitrogen-free	129±11	−14±3.2	—	—

* Average data of six rats (three males and three females).

having a relatively larger quantity of Ara and Xy residues, which are according to Selvendran [2], components of hemicellulose and pectin PS.

Crude protein residues. Table 2 also shows data on the crude protein of the IDF and SDF. The content in IDF averaged 20.5%, while in SDF, the content was 7.4%. The presence of this crude protein fraction indicated the following possibilities: incomplete enzymatic hydrolysis protein imbedded in the cell wall, and protein resistant to degradation by physiological enzymes. The latter two possibilities seem more feasible as has been stated in other papers [2, 17]. On the basis of the content of IDF and of SDF and on their respective protein content it was calculated that 26.6% of the total protein in the bean sample was present in IDF and 2.4% in SDF. Dietary fiber then captured 29.5% of the total bean protein. From these data it can be suggested that this amount of the cooked bean protein is not digested using any in vivo method. The presence of crude protein in the DF residue is of special interest and further experiments are being carried out to evaluate the composition of this crude protein and its nature. The biological experiments (Table 3) show lower feed intake, weight gain and a decrease in PER and TD values with respect to processing intensity. It is recognized that the blended bean and fried bean diets contained more fat than the cooked bean diet, which could influence the result. However, all diets contained 10% protein. These data confirm results on fried beans published before [7] in which a loss of around 16% of available lysine was also reported in fried beans. This could explain the decrease in PER which was observed from cooked to fried beans. Likewise, the decrease in protein digestibility is associated with the increase in IDF. As was shown, about 29.5% of the bean protein was bound in the DF which probably was not available at the gastrointestinal level, resulting in lower digestibility. Further studies are needed to take into consideration this bound protein to estimate the protein digestibility of common beans.

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