

## Effect of Fermentation and Autoclaving on Dietary Fiber Fractions and Antinutritional Factors of Beans (*Phaseolus vulgaris* L.)

MARÍA A. MARTÍN-CABREJAS,\* BEATRIZ SANFIZ, ADRIA VIDAL,  
 ESPERANZA MOLLÁ, ROSA ESTEBAN, AND FRANCISCO J. LÓPEZ-ANDRÉU

Departamento de Química Agrícola, Facultad de Ciencias, Universidad Autónoma de Madrid (UAM),  
 28049 Madrid, Spain

The effect of fermentation on antinutritional factors and also on total dietary fiber (TDF), insoluble (IDF) and soluble (SDF) dietary fiber fractions was studied in beans (*Phaseolus vulgaris* L.). The processes studied were two types of fermentation (lactic acid and natural), and a portion of the obtained flours were processed by autoclaving. The dietary fiber (DF) content and its components were determined using the enzymatic–gravimetric and enzymatic–chemical methods. The TDF content ranged from 24.5% dry matter (DM) in the raw to 25.2% DM in the processed beans. All the processing treatments significantly decreased the SDF content, and irrelevant changes were noticed in the IDF content of processed beans. Cellulose content of all samples was reduced by the processing treatments. Correspondingly, higher amounts of resistant starch was observed in the processed beans, except in the lactic acid fermented ones. However, the levels of pectic polysaccharides and Klason lignin were higher in the samples fermented by *Lactobacillus plantarum*. The action of microorganisms was determinant for the different degradation of the bean cell wall, disrupting the protein–carbohydrate integration, thus reducing the solubility of DF.

**KEYWORDS:** Beans (*Phaseolus vulgaris* L.); antinutritional factors; dietary fiber; carbohydrates; fermentation conditions; autoclaving

### INTRODUCTION

Bean (*Phaseolus vulgaris* L.) seeds are a common human food low in fat and rich in proteins, vitamins, complex carbohydrates, and minerals. Beans as legumes are good sources of “lente” (slow release) carbohydrates, mainly due to their higher soluble dietary fiber when compared to other fiber-rich plant foods such as cereals and tubers. They are beneficial for health, with low glycemic index and the potential to decrease serum cholesterol concentrations (1, 2). However, their wider use is somehow limited by the presence of antinutritional factors in the seeds, which may have adverse effects for human and animal nutrition. Some examples of these compounds are enzyme inhibitors, lectins, phenolics, phytates, and cyanoglycosides (3, 4). Consequently, it is desirable to develop transformation processes that could improve the nutritional quality of legumes and also provide new derived products for the consumers (5).

Fermentation is one of the simple and inexpensive processing techniques to achieve desirable changes in the seed composition and improve palatability, and it is used throughout the world and particularly in developing countries. Age-old technology

such as lactic acid fermentation has been proposed for processing of more digestible and palatable foodstuffs (6). Lactic acid fermentation is used as a major method for processing and preserving vegetables, cereals, and legumes. It is a desirable method for processing and preserving food because of its low cost, low energy requirements, and high yield with acceptable and diversified flavors for human consumption (7).

Several experiments have demonstrated that fermentation of legumes enhances their nutritive value, reduces some antinutritional natural compounds such as phytic acid, trypsin inhibitor, and oligosaccharides (8, 9), and exerts beneficial effects on protein digestibility and biological value of legumes (10). Thus, fermentation seems to be an effective treatment for lowering antinutritional factors in beans, and it could possibly enhance the nutritional quality. On this respect, no information has been found on the effect of lactic acid and natural fermentation in the content of dietary fiber (DF) of beans and the composition of their fractions (soluble and insoluble).

The present investigation was undertaken to study the effect of various processing treatments on the dietary fiber fractions and antinutrients in *Phaseolus vulgaris*, with the aim of utilization of bean seeds using lactic acid and natural fermentations and autoclaving to modify functional, organoleptic, and nutritional properties of the seeds.

\* To whom correspondence should be addressed. Tel.: +34 91 4978678. Fax: +34 91 4973830. E-mail: maria.martin@uam.es.

## MATERIALS AND METHODS

**Samples.** Beans *Phaseolus vulgaris* type *Carilla* were used. Bean seeds were rinsed three times in distilled water and drained. The seeds were finely ground in a ball mill, sieved, and the 0.50 mm fraction collected.

**Fermentation.** Two types of fermentations were carried out:

**Natural Fermentation.** Suspensions of bean flours in sterilized tap water were aseptically prepared and were allowed to ferment naturally (with only the microorganisms on the seeds) at 37 °C for 48 h without aeration, stirred at 450 rpm in a fermentation unit (Fermentor Infors ISF-100, Infors AG, Switzerland). The fermentation started 10–40 min after suspension was prepared, while being stirred and with temperature controlled.

**Lactic Acid Fermentation.** Bean flours were inoculated with *Lactobacillus plantarum* at 10% in saline solution (v/v), and fermentation was carried out as described previously.

**Autoclaving.** A portion of the bean fermented flours were autoclaved at 121 °C for 20 min. The fermented and autoclaving products were freeze-dried and stored at 4 °C until further analysis. Samples were named as follows: LF (lactic acid fermented), NF (natural fermented), LF + A (lactic acid fermented followed by autoclaving) and NF + A (natural fermented followed by autoclaving).

**Analytical Methods.** Proximate composition was determined by AOAC (11) methods in triplicate on raw and fermented material and expressed on dry weight basis.

**Crude Protein.** Nitrogen content was determined according to the method 2055 by the Kjeldahl method ( $N \times 6.25$ ). Ash was determined by combustion for 5 h at 525 °C (method 14006). pH and titratable acidity were measured using AOAC methods 14022 and 22058 (11), respectively. pH was measured on a slurry prepared with 10 g of bean flour in 40 mL of distilled water. Acidity was measured on the same slurry by titration to pH 8.1 with standardized titrant and calculated as g kg<sup>-1</sup> acetic acid. Carbohydrate content was determined by difference.

**Antinutritional Factors.** Bean flours were extracted (1:10, w/v) by stirring with 0.02 M sodium phosphate buffer pH 7.0 containing NaCl (8 g/L) overnight at +1 °C followed by centrifugation at 5000g for 25 min. Clear supernatants were used for antinutritional evaluations. Chymotrypsin inhibitor activity was determined by Grant et al. (12). The  $\alpha$ -amylase inhibitor content was evaluated by the starch/iodine procedure of Piergiovanni (13). The protein inhibitor contents of seed extracts were calculated by comparison of the amount of sample or inhibitor required to cause 50% inhibition of enzyme activity and were expressed as g-equiv of standard inhibitor per kg seed meal. For inhibitor levels, triplicate assays were conducted. Hemagglutinating activity (PHA) was estimated in sodium phosphate extracts by a serial dilution procedure using rat blood cells (14). The amount of material which caused agglutination of 50% of the erythrocytes was defined as that containing 1 haemagglutinating unit (HU). For comparison, values were expressed as HU kg<sup>-1</sup> seed meal. The assays were reproducible to  $\pm 1$  dilution and values in the text were the mean of four separate measurements. Cyanogenetic glycosides were determined by AOAC alkaline titration (11).

**Dietary Fiber Determination.** Mes-Tris AOAC method 991.43 was used for DF determination (15). Two replicates of each sample were taken to complete the six-sample analysis method. The principle of the method was based on the use of three enzymes (heat-stable  $\alpha$ -amylase, protease, and amyloglucosidase) under different incubation conditions to remove starch and protein components. Dietary fiber fractions were obtained as indigestible residues after enzymatic digestion of nondietary fiber components; the insoluble residues were isolated by filtration and soluble fiber was precipitated with ethanol. Dried residues correspond to insoluble dietary fiber (IDF) and soluble dietary fiber (SDF), respectively. Determination of residual ashes and protein (as Kjeldahl N  $\times 6.25$ ) were carried out in the residues for corresponding corrections. Total dietary fiber (TDF) was calculated as sum of IDF and SDF. Kjeldahl nitrogen and ash contents were assayed according to standard procedures (15).

**Chemical Analysis of DF Components.** IDF and SDF fractions were dispersed in H<sub>2</sub>SO<sub>4</sub> as described in Martín-Cabrejas et al. (16). The neutral sugar composition of the DF was determined by HPLC (17)

**Table 1.** Proximate Composition, pH, and Acidity in Raw and Processed Bean Flours

sample	crude protein <sup>a</sup>	ash <sup>a</sup>	pH	acidity <sup>b</sup>	carbohydrate <sup>c</sup>
raw	20.9 ± 0.1	1.6 ± 0.0	6.22 ± 0.03	6.27 ± 0.05	76.3
LF	21.5 ± 0.1	1.7 ± 0.1	3.69 ± 0.02	59.70 ± 0.20	75.4
NF	20.8 ± 0.1	1.7 ± 0.0	4.50 ± 0.01	31.55 ± 0.04	76.0
LF + A	21.5 ± 0.1	1.7 ± 0.3	3.68 ± 0.01	58.60 ± 0.07	75.1
NF + A	21.5 ± 0.1	1.8 ± 0.0	4.47 ± 0.02	31.34 ± 0.02	75.0

<sup>a</sup> Percent dry matter. <sup>b</sup> g kg<sup>-1</sup> of acetic acid. <sup>c</sup> Determined by difference: 100 – protein – ash – lignin.

and uronic acids were determined colorimetrically by adapting the 3-hydroxydiphenyl method of Blumenkrantz and Asboe-Hansen (18) with D-galacturonic acid (Sigma). Klason lignin was obtained from the weight of IDF residue left after hydrolysis with 12 M H<sub>2</sub>SO<sub>4</sub> for 3 h at 20 °C, followed by dilution to 1 M acid and heating at 100 °C for 2.5 h. The insoluble residue was recovered quantitatively over a glass filter (Pyrex N. 2), washed thoroughly with pure water, and dried for 18 h at 105 °C.

**Statistical Analysis.** The data were processed for the analysis of variance according to the standard method of statistical analysis (19).

## RESULTS AND DISCUSSION

The chemical data for the studied bean flours are presented in **Table 1**. The crude protein content of bean flour was 20.9% of dry matter (DM), emphasizing the high protein of this variety of bean and its potential as a food protein source. The total protein content of processed flours was not affected significantly, which is consistent with other findings (9). Tabera et al. (20) reported also a slight increase in protein content during fermentation of lentils in which the fermentation liquid was kept. Lactic acid fermentation has been found to affect the availability of amino acid content in cereals and legumes (6). It is postulated that the increase of protein susceptibility to proteolytic enzymes is due to partial protein denaturation and pH decrease during fermentation (21).

The pH and acidity of bean flours were also measured; raw beans exhibited a neutral or slight acidic pH, in agreement with earlier findings in different bean varieties (22). Fermented flours showed a lower pH in both conditions, the fermentation carried out with *Lactobacillus plantarum* exhibited the lowest values, which agreed with those presented by Vidal-Valverde et al. (23) in fermented lentils. The autoclave process did not affect this chemical parameter. As pH decreased, titratable acidity increased for processed flours. Carbohydrate content was slightly less in processed samples, which was due to the metabolism of microorganisms that produces changes on the different carbohydrates and also on the dietary fiber levels that will be discussed below.

Beans contained low amounts of lectins and  $\alpha$ -amylase inhibitor but significant levels of chymotrypsin inhibitor (**Table 2**) compared to other legumes (24). These antinutritional factors are known to have deleterious or toxic effects for animals and man (1, 25); therefore, they need to be inactivated by a suitable pretreatment before they can be safely used as a food source (3). The NF and LF fermentation processes, led to an important reduction of lectin levels, suggesting hydrolysis of these complex stored proteins in simpler and more soluble available products. This reduction was attributed to degradation by microorganisms. However, no effect or slight increase of  $\alpha$ -amylase inhibitor and chymotrypsin inhibitor contents were observed. Thus, the subsequent autoclave process has to be done to produce an important elimination of above antinutritional factors on these flours.

**Table 2.** Antinutritional Levels in Raw and Processed Bean Flours (g kg<sup>-1</sup> DM)

sample	$\alpha$ -amylase inhibitor	chymotrypsin inhibitor	cyanogenetic glycosides	lectins
raw	0.5 $\pm$ 0.1	4.7 $\pm$ 0.0	n.d. <sup>a</sup>	1.2 $\pm$ 0.6
LF	2.1 $\pm$ 0.1	4.1 $\pm$ 0.1	n.d.	0.6 $\pm$ 0.3
NF	2.1 $\pm$ 0.1	6.0 $\pm$ 0.0	n.d.	0.4 $\pm$ 0.2
LF + A	0.7 $\pm$ 0.1	2.6 $\pm$ 0.3	n.d.	n.d.
NF + A	0.8 $\pm$ 0.1	2.0 $\pm$ 0.0	n.d.	n.d.

<sup>a</sup> n.d., non detected.**Table 3.** Content of Insoluble, Soluble, and Total Dietary Fiber (Percent DM) and Its Distribution in Raw and Processed Bean Flours (Mean  $\pm$  SD ( $n = 6$ ))

sample	IDF	SDF	TDF	IDF/SDF
raw	17.1 $\pm$ 1.4 <sup>a</sup>	7.7 $\pm$ 1.1 <sup>a</sup>	24.5	2.3
LF	18.0 $\pm$ 1.6 <sup>a</sup>	4.6 $\pm$ 0.8 <sup>b</sup>	22.6	3.9
NF	17.7 $\pm$ 1.4 <sup>a</sup>	5.0 $\pm$ 0.9 <sup>b</sup>	22.7	3.5
LF + A	18.1 $\pm$ 1.8 <sup>a</sup>	4.3 $\pm$ 0.9 <sup>b</sup>	22.4	4.2
NF + A	20.3 $\pm$ 2.2 <sup>a</sup>	4.9 $\pm$ 1.0 <sup>b</sup>	25.2	4.1

<sup>a,b</sup> Mean values of each column followed by different superscript letter significantly differ when subjected to Duncan's multiple range test (DMRT) ( $p < 0.05$ ).

Although different studies have been carried out on the effect of different thermal methods and chemical and mechanical processes upon DF components (16, 26), the effect of different types of fermentations (natural and with microorganisms such as *Lactobacillus plantarum*) upon DF fractions of *Phaseolus vulgaris* has not been well documented. Beans exhibited a relevant level of TDF (24.5% of DM) higher than in other important legumes and cereals (27, 28). The processes of fermentation (natural and lactic acid) and autoclaving showed slight decreases of TDF contents (Table 3), except in case of natural fermented flour followed by autoclave process. These results reflected the same trend of DF fractions in all processed samples. The IDF exhibited a slight increase (from 69.8 to 80% of TDF). This could be due to a lower accessibility of microorganisms to the insoluble fiber components during fermentation. SDF fraction decreased significantly from 30 to 19% of TDF. A degradation of SDF of high molecular weight to smaller fragments occurred, and depending on the extent of depolymerization, a fraction of SDF could be incompletely recovered by ethanolic precipitation in the fiber analysis, thus decreasing the fiber content. The effect of both fermentations was similar, although the lactic acid fermentation seemed to produce more impact on these values. In addition, the following thermal treatment on the fermented flours corroborated the above data.

**Table 4.** Composition of Insoluble Dietary Fiber in Raw and Processed Bean Flours (g kg<sup>-1</sup> DM) (Mean  $\pm$  SD ( $n = 6$ ))

sample	H <sub>2</sub> SO <sub>4</sub> hydrolysis	carbohydrates						total sugars	Klason lignin	total	UA/NS
		Glc	Xyl	Gal/Rha	Ara	Man	UA				
raw	12 M	66.9 $\pm$ 6.0	4.7 $\pm$ 0.4	4.6 $\pm$ 0.4	25.6 $\pm$ 2.3	4.1 $\pm$ 0.4	27.6 $\pm$ 2.5	133.6	26.7 $\pm$ 2.4 <sup>a</sup>	160.3	0.26
	0.6 M	31.5 $\pm$ 2.8	3.3 $\pm$ 0.3	4.1 $\pm$ 0.4	20.1 $\pm$ 1.8	2.5 $\pm$ 0.2	13.7 $\pm$ 1.2			75.2	0.22
LF	12 M	50.4 $\pm$ 4.5	7.6 $\pm$ 0.7	5.8 $\pm$ 0.5	32.3 $\pm$ 2.9	4.7 $\pm$ 0.4	32.1 $\pm$ 2.9	132.9	28.1 $\pm$ 2.5 <sup>a</sup>	161.0	0.32
	0.6 M	25.9 $\pm$ 2.3	3.5 $\pm$ 0.3	4.0 $\pm$ 0.4	19.3 $\pm$ 1.7	2.4 $\pm$ 0.2	18.9 $\pm$ 1.7			74.0	0.34
NF	12 M	56.8 $\pm$ 5.1	6.2 $\pm$ 0.6	4.6 $\pm$ 0.4	28.1 $\pm$ 2.5	3.9 $\pm$ 0.3	28.3 $\pm$ 2.5	127.9	13.3 $\pm$ 1.2 <sup>b</sup>	141.2	0.28
	0.6 M	42.2 $\pm$ 3.8	3.3 $\pm$ 0.3	3.5 $\pm$ 0.3	19.7 $\pm$ 1.8	2.0 $\pm$ 0.2	15.5 $\pm$ 1.4			86.2	0.22
LF + A	12 M	52.3 $\pm$ 4.7	6.3 $\pm$ 0.6	4.7 $\pm$ 0.4	29.7 $\pm$ 2.7	4.2 $\pm$ 0.4	26.7 $\pm$ 2.4	123.9	30.7 $\pm$ 2.8 <sup>a</sup>	154.6	0.27
	0.6 M	25.7 $\pm$ 2.3	3.2 $\pm$ 0.3	3.7 $\pm$ 0.3	19.1 $\pm$ 1.7	0.0 $\pm$ 0.0	14.7 $\pm$ 1.3			66.4	0.28
NF + A	12 M	62.7 $\pm$ 5.6	6.2 $\pm$ 0.6	4.9 $\pm$ 0.4	28.9 $\pm$ 2.6	5.7 $\pm$ 0.5	27.8 $\pm$ 2.5	136.2	15.4 $\pm$ 1.4 <sup>b</sup>	151.6	0.26
	0.6 M	43.9 $\pm$ 4.1	3.6 $\pm$ 0.3	3.3 $\pm$ 0.3	20.7 $\pm$ 1.9	2.8 $\pm$ 0.3	17.6 $\pm$ 1.6			91.9	0.24

<sup>a,b</sup> Mean values of each column followed by different superscript letter significantly differ when subjected to DMRT ( $p < 0.05$ ).

The result of this processing could influence in the physiological effects of DF. IDF/SDF fiber ratios are important from both a dietary and a functional perspective. To be acceptable, a dietary fiber ingredient must perform in a satisfactory manner as a food ingredient (29). The ratio of insoluble to soluble fiber is an important variant related to structural and also sensorial properties. The changes promoted by fermentation conditions are reflected in the IDF/SDF ratio. This suggests that the different fermentation treatments might be used to alter the dietary and functional characteristics of the fiber.

The profile of the sugar composition of IDF from bean (Table 4) agreed with previously published data on legumes (16, 27, 28). IDF mainly comprised glucose, uronic acids, and arabinose as the main carbohydrate constituents, followed by xylose, galactose/rhamnose, and mannose, which were found in minor amounts. Half (52%) of the glucose can be inferred to be of cellulosic origin, because it was released only by 12 M H<sub>2</sub>SO<sub>4</sub>. Because the level of cell wall xylose and mannose was much lower than the noncellulosic glucose, no presence of significant amounts of xylan and mannan hemicelluloses can be inferred. Approximately 48% of the glucose was solubilized by 0.6 M H<sub>2</sub>SO<sub>4</sub>, and it is likely that an important quantity of noncellulosic and resistant starch were present in the IDF residues. The remainder of the IDF carbohydrates comprised pectic polysaccharides as indicated by the levels of uronic acids and arabinose that exhibited similar levels, indicating a highly branched pectic polysaccharide.

Thus, comparing mild and strong hydrolysis (0.6 and 12 M H<sub>2</sub>SO<sub>4</sub>, respectively) carried out on IDF residues, 80% of arabinose was solubilized by mild conditions, probably due to arabinans not linked to cellulose matrix. Uronic acids had different behavior, they were only solubilized 50% in mild conditions, and the rest by 12 M H<sub>2</sub>SO<sub>4</sub>. This could be due to homogalacturonans link to cellulose as was observed in other food cell wall materials (30, 31). The low level of hemicelluloses containing xylose and mannose were mostly hydrolyzed under mild acid conditions.

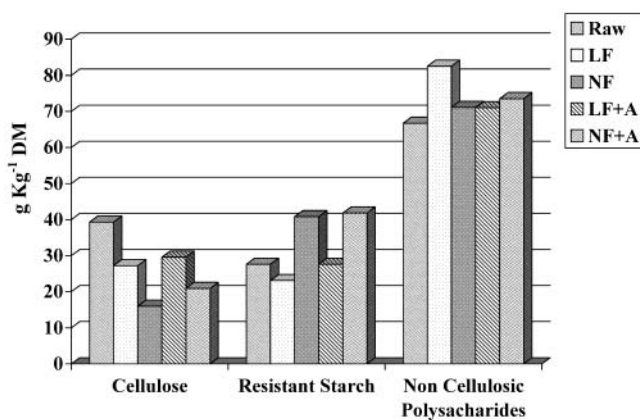
The acid hydrolysis of insoluble fermented residues and their chemical analysis of IDF components as sum of total sugars (neutral sugars + uronic acids) showed the same trend of the enzymatic-gravimetric AOAC method. No significant changes were observed in the total sugar levels. However, samples fermented by *Lactobacillus plantarum* exhibited important decreases of glucose (25%) and increases of arabinose (26%) compared to raw bean. The changes showed by natural fermented beans were lower. Therefore, the fermentation processes induced different changes in the cell wall components. The different microorganisms involved in the experiments probably were able to hydrolyze polysaccharides and the

**Table 5.** Composition of Soluble Dietary Fiber in Raw and Processed Bean Flours ( $\text{g kg}^{-1}$  DM) (Mean  $\pm$  SD ( $n = 6$ ))

sample	carbohydrates						total sugars	UA/NS
	Glc	Xyl	Gal/Rha	Ara	Man	UA		
raw	$2.8 \pm 0.3$	$1.6 \pm 0.1$	$1.5 \pm 0.1$	$5.0 \pm 0.5$	$3.7 \pm 0.3$	$7.2 \pm 0.6$	21.8	0.49
LF	$2.5 \pm 0.2$	$1.2 \pm 0.1$	$1.1 \pm 0.1$	$5.0 \pm 0.5$	$2.1 \pm 0.2$	$6.2 \pm 0.6$	18.1	0.52
NF	$2.1 \pm 0.2$	$0.9 \pm 0.3$	$1.1 \pm 0.1$	$4.1 \pm 0.4$	$3.2 \pm 0.3$	$6.7 \pm 0.6$	18.1	0.59
LF + A	$1.7 \pm 0.1$	$0.7 \pm 0.1$	$0.8 \pm 0.1$	$3.0 \pm 0.3$	$1.4 \pm 0.1$	$7.6 \pm 0.7$	15.2	0.42
NF + A	$2.4 \pm 0.2$	$1.0 \pm 0.1$	$1.4 \pm 0.1$	$4.5 \pm 0.4$	$3.1 \pm 0.3$	$5.7 \pm 0.5$	18.1	0.46

**Table 6.** Composition of Total Dietary Fiber in Raw and Processed Bean Flours ( $\text{g kg}^{-1}$  DM) (Mean  $\pm$  SD ( $n = 6$ ))

sample	carbohydrates						total sugars	total sugars + lignin Klason	UA/NS
	Glc	Xyl	Gal/Rha	Ara	Man	UA			
raw	$69.7 \pm 6.3$	$6.4 \pm 0.5$	$6.1 \pm 0.5$	$30.6 \pm 2.8$	$7.9 \pm 0.7$	$34.8 \pm 3.1$	155.4	182.1	0.29
LF	$52.9 \pm 4.8$	$8.8 \pm 0.8$	$6.9 \pm 0.6$	$37.3 \pm 3.4$	$6.8 \pm 0.6$	$38.3 \pm 3.5$	151.0	179.1	0.34
NF	$58.9 \pm 5.3$	$7.1 \pm 0.6$	$5.7 \pm 0.5$	$32.2 \pm 2.9$	$7.1 \pm 0.6$	$35.0 \pm 3.2$	146.0	159.3	0.32
LF+A	$54.0 \pm 4.8$	$7.0 \pm 0.6$	$5.5 \pm 0.5$	$32.7 \pm 2.9$	$5.6 \pm 0.5$	$31.5 \pm 2.8$	133.0	163.7	0.28
NF+A	$65.1 \pm 5.9$	$7.2 \pm 0.6$	$6.3 \pm 0.6$	$33.4 \pm 3.0$	$8.8 \pm 0.8$	$33.5 \pm 3.0$	154.3	169.7	0.27

**Figure 1.** Distribution of carbohydrates in the IDF fractions in raw and processed bean flours.

products of their degradation (oligosaccharides and monosaccharides) using them for their metabolism. Similar observations were noticed in relation to oligosaccharides content in other legumes (9, 21). Regarding the autoclaving processes, a reduction of glucose was noticed. It is supposed that the loss of these sugars was possibly caused by the Maillard reaction. Reducing carbohydrates formed by hydrolysis during fermentation may interact with suitable protein groups resulting in the reduced release of these sugars and the increased Klason lignin level.

In relation to the Klason lignin level, raw material showed a relatively high value ( $26.7 \text{ g kg}^{-1}$  DM) compared to other bean varieties and legumes (28, 29). However, the acid hydrolysis insoluble residue (Klason lignin) exhibited differences between the different processing. Interestingly, the Klason lignin residues of lactic acid fermentation flours were higher to those of raw flour, although much higher to natural fermentation. It could be concluded that the natural microorganisms and enzymes had a direct effect on the structure of this complex component of cell wall, making it more soluble.

The autoclave processes (LF + A, NF + A) caused slight increases of the Klason lignin compared to fermented and control flours, due to the formation of new insoluble products (Maillard components). These results were in agreement with previously published data on processed food (26, 27).

**Figure 1** shows the decrease of cellulose in the fermented flours, the natural fermented sample exhibiting the highest

decrease (59% respect to raw sample). The decrease observed in IDF might be due to the use of cellulose, the main component of this bean variety, by microorganisms present in the fermentation medium. Resistant starch solubilized by mild acid conditions showed different behavior, lowering by *Lactobacillus plantarum* microorganisms and considerably increasing by natural fermentation (48%) with respect to raw flour. These results reported are similar to those found in natural fermented beans (9) and in other legumes (32). The noncellulosic polysaccharides were the majority; mainly pectic polysaccharides increased during the fermentation, although in lesser extent than the above components. The following autoclaving (LF + A and NF + A) treatments also exhibited the same trends as those found in the fermentation processes.

The carbohydrate composition of SDF of raw beans contained uronic acid as the main sugar component (**Table 5**) followed by arabinose. The pectic polysaccharides thus composed 63% of total sugars. As in previous studies, the level of arabinose found in this fraction was high ( $5.0 \text{ g kg}^{-1}$  of DM) like in other legumes (31). The fermentation process produced a decrease of sugar content like in other processing methods as cooking and toasting (27). Interestingly, both types of fermentation exhibited a 17% of decrease with respect to SDF in raw bean. A generalized decrease of every sugar is responsible for this low level. However, this fraction showed an increase of UA/NS ratio in fermented samples as a consequence of a lower decrease of acid sugars. The use of autoclave processing also caused important changes in SDF fraction, showing higher decreases of uronic acids with respect to fermented samples. However, its effects were different depending on fermentation type: *Lactobacillus plantarum* fermentation was the most affected (41% of decreasing), while natural fermentation followed by autoclave process exhibited the same level of total sugars as fermented flours.

Significant differences were detected in the carbohydrate composition of TDF from different fermentation conditions (**Table 6**). The levels of glucose in any processed samples were lower compared to the raw. This suggests the degradation of polysaccharides carried out by the enzymatic systems of microorganisms responsible of fermentation processes. According to IDF and SDF trends, the TDF of studied samples contained arabinose-rich polysaccharides. The UA/NS ratios of TDF were similar to that observed in IDF, because of the major contribution of this fraction in the global determination of dietary

fiber. It was observed that the insoluble fraction showed higher amounts of branched pectic polysaccharides than the soluble fraction in all samples, because of the minor UA/NS ratios of insoluble fraction. This fact suggests that arabinan chains bring about the insolubilization of pectic polysaccharides (29). Autoclave processes showed the same trend of fermented samples, but had progressively less pectic arabinose.

Hence, TDF showed a relative increase of pectic polysaccharides as cell wall insoluble components, especially in fermented samples. No clear redistribution from soluble to insoluble fiber components was noticed during fermentations, as was observed in other processing such as germination, roasting, and pressure-cooking (29, 32).

This study found differences in DF content between the studied fermentations, hence different physiological and specific nutritional effects should be expected. The physical properties and physiological action of DF is also dependent on the plant cell wall integrity. Processing treatments, particularly the lactic acid fermentation, may modify the structure of both cell wall and storage polysaccharides of legumes possibly by affecting the intactness of tissue histology and disrupting the protein-carbohydrate integration by microorganism action, thus reducing the solubility of DF.

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