

# CHEMICAL CHARACTERIZATION OF BRAZILIAN HULLESS BARLEY VARIETIES, FLOUR FRACTIONATION, AND PROTEIN CONCENTRATION

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**ABSTRACT:** Even though hulless barley is widely known due to its nutritional potential, in Brazil it is only grown at a few agricultural experimental stations. There is no published data about the chemical composition of Brazilian hulless barley varieties; however, research laboratories have studied their agronomical characteristics. The objectives of this study were to present the chemical characterization and effect of flour fractionation on protein concentration of six Brazilian hulless barley varieties, namely IAC IBON 214/82, IAC 8612/421, IAC 8501/31, IAC 8501/12, IAPAR 39-Acumaí, and IAC 8501/22. The analyses included: ash, ether extract, total protein, starch, total insoluble and soluble dietary fiber, and  $\beta$ -glucans. Flour fractionation was carried out by sieving. The flour fractions were evaluated for crude protein, protein, and protein and non-protein nitrogen. Chemical composition varied ( $P < 0.05$ ) among all the varieties. IAC 8501/22, IAC 8501/31, and IAC 8501/12 showed the highest protein content (15.69, 15.25, and 14.94% respectively). Differences ( $P < 0.05$ ) among the protein of the fractionated flours were detected, and might be attributed primarily to genetic background since all varieties were grown under the same environmental conditions. Fractionating the flour increased the total protein content, in some fractions, by up to 2%. These results may be useful in the food industry for the selection of hulless barley varieties for human consumption and to produce substantially protein-enriched flour fractions.

Key words: cereals, naked barley, protein fractionation, sieving, chemical composition

## CARACTERIZAÇÃO QUÍMICA DE VARIEDADES BRASILEIRAS DE CEVADA NUA, FRACIONAMENTO DA FARINHA E CONCENTRAÇÃO DE PROTEÍNA

**RESUMO:** Apesar de a cevada nua ser amplamente conhecida por seu potencial nutricional, no Brasil é apenas cultivada em poucas estações experimentais agrônomicas. Em relação as variedades brasileiras de cevada nua, não se têm dados sobre a composição química, entretanto instituições de pesquisa têm estudado suas características agrônomicas. Os objetivos deste estudo foram apresentar a caracterização química e o efeito no fracionamento da farinha obtida visando à concentração de proteína de seis variedades brasileiras de cevada nua: IAC IBON 214/82, IAC 8612/421, IAC 8501/31, IAC 8501/12, IAPAR 39-Acumaí, e IAC 8501/22. Foram realizadas análises de: cinzas, extrato etéreo, proteína total, amido, fibra alimentar total, solúvel e insolúvel e  $\beta$ -glucanas. As frações das farinhas foram obtidas por peneiramento e avaliadas quanto aos teores de proteína bruta, proteína, nitrogênio protéico e não protéico. Houve variação entre as variedades testadas ( $P < 0,05$ ). As variedades IAC 8501/22, IAC 8501/31 e IAC 8501/12 apresentaram o maior teor de proteína (15,69; 15,25 e 14,94% respectivamente). Foi observada diferença ( $P < 0,05$ ) quanto ao teor de proteína nas frações de farinha de cada variedade, a qual pode ser atribuída principalmente às características genéticas, uma vez que todas as variedades foram cultivadas sob as mesmas condições ambientais. Após o fracionamento, foi observada concentração, em algumas frações, de no máximo 2% em relação a farinha integral. As variedades brasileiras de cevada nua apresentam potencial para consumo humano bem como para a produção de farinhas enriquecidas com alta proteína.

Palavras-chave: cereais, cevada nua, fracionamento de proteína, peneiramento, composição química

### INTRODUCTION

Barley (*Hordeum vulgare* L.) is used mainly for brewing and as animal feed but there is a growing interest in it for human food and industrial uses (Oscarson et

al., 1996). It contributes significantly to the human food supply as malt products, and to animal livestock feed (Elfverson et al., 1999). Many spontaneous and induced barley varieties occur, and this offers the potential to select particular genotypes for specific uses (Nilan &

Ullrich, 1993). Hulless (HB) or naked barley is a genetically improved variety that allows easier removal of the hull and a fairly new industry has developed around uses of selected HB in order to increase the digestible energy of the grain, especially for swine and poultry (Bhatty, 1999b). HB has been investigated for several potential new applications as whole grain, and for its value-added products. These include bran and flour for multiple food applications (Bhatty, 1999a).

In Brazil, barley is grown in the Southern states, which produced 235,150 tons in 2002 (IBGE, 2003). Almost 100% of the Brazilian covered barley production is used for malting. Hulless barley is still being cultivated only in experimental stations in order to evaluate its possible use as food. The Canadian production of HB was the highest in 1998, with a barley grain yield of around 800,000 tons (Bhatty, 1999b).

Interest in hulless barley has increased due to its soluble dietary fiber,  $\beta$ -glucan, and high protein contents. Even when compared to oats, a  $\beta$ -glucan-rich cereal, barley presents higher amounts. (Lapvetelainen & Aro, 1994; Marconi et al., 2000; De Francisco & De Sá, 2001).  $\beta$ -glucan is particularly interesting for human consumers because it decreases blood cholesterol and glucose levels (Newman et al., 1998).

In relation to other grains, barley and wheat are similar in their protein content (11-12%); however, both are higher in protein than corn (9.5%) and rice (7.5%) (Lockhart & Hurt, 1986). Hulless barley usually has higher total protein, amino acid, and digestible energy contents than hulled barley. Some varieties are high in lysine, the essential amino acid that is most limiting in cereals (Munck, 1992; Shewry, 1993).

The objectives of this study were to determine the chemical composition of flour from six hulless barley varieties and to evaluate the effects of its fractionation on protein content.

## MATERIAL AND METHODS

### Plant material

Six Brazilian hulless barley varieties (IAC-IBON 214-82, IAC 8612-421, IAC8501-31, IAC 8501-12, IAPAR 39 ACUMAI, IAC8501-22) were analyzed. All varieties were grown in experimental fields in 2001, at Mauá da Serra, PR, Brazil (23°49'21"S; 51°14'35"W). Before analysis, 50 g of each barley cultivar was ground in a Cyclone (3010-019, Udy Corporation, Fort Collins, Colorado, USA) sample mill to pass a 0.5 mm screen. Samples were stored at -18°C.

### Chemical analysis

Dry matter content and ash were determined by weight lost upon heating at 105 and 550°C, for 12 and 5 h, respectively. Ether extract was determined by extrac-

tion with diethyl ether in a Soxtec System (Te-044-5/50-8/50 Model, Tecnal, Piracicaba, SP, Brazil) after acid hydrolysis with 8 mol L<sup>-1</sup> HCl. Total protein (Total Nitrogen  $\times$  6.25) was determined by the conventional Kjeldahl method. The above-mentioned analyses were performed according to the standard Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 1995). Total insoluble and soluble dietary fiber,  $\beta$ -glucan, and total starch were determined using Megazyme assay kits (Megazyme International Ireland Ltd, Wicklow, Ireland) according to Approved Methods of the American Association of Cereal Chemists (AACC, 2000). Data were reported on a dry weight basis.

### Fractionation of varieties

The varieties were hand-cleaned to remove foreign material and damaged kernels. Five hundred grams of each were milled with a Cyclone (3010-019, Udy Corporation, Fort Collins, Colorado, USA) laboratory mill using a 1.0 mm screen. Duplicate 100 g samples of each ground sample were fractionated to concentrate protein using a rotary-tapping shaker (Ro-Tap RX 29-16 II-724, WS Tyler, Mentor, OH, USA) equipped with 80 (180  $\mu$ m), 100 (150  $\mu$ m), and 140 mesh (106  $\mu$ m) sieves (ASTME-11 Specification, Tyler Equivalent WS Tyler, Mentor, OH, USA) for 30 minutes. The flour fractions retained on each sieve were weighed and the percentage of the total weight was calculated for each fraction (as-is basis). Crude protein content was determined by the conventional Kjeldahl method, and protein and non-protein nitrogen were determined according to Novoa et al. (1993).

### Statistical analyses

Differences in chemical composition and protein fractions among the varieties were evaluated by analysis of variance and Tukey's HSD multiple pairwise comparisons using the Statistical computational software (version 6.0). Data correspond to the average of three replicates grown in the field.

## RESULTS AND DISCUSSION

The chemical composition of the six Brazilian hulless barley varieties can be summarized as follows: Ash contents (%) were found in the ranges from 1.51-2.27, ether extract 2.91-4.00, protein 12.55-15.92, starch 57.46-63.14, total dietary fiber 12.37-17.39, insoluble dietary fiber 8.07-12.16, soluble dietary fiber 4.30-6.45 and  $\beta$ -glucans 3.70-5.77 (Table 1). The highest constituents were starch (57.5-63.1%), total protein (12.5-15.9%) and total dietary fiber (12.4-17.4%). Starch (58.59-67.46%) and total protein (12.55-16.17%) contents are in agreement with those previously reported for Swedish (Elfverson et al., 1999) and Canadian (Li et al., 2001) varieties. Total dietary fiber was not reported in the above studies; however, our results (12.37-17.39%) are in ac-

Table 1 - Chemical composition (% , w/w dry basis) of Brazilian hulless barley varieties.

Varieties	Ash	Ether Extract	Total Protein <sup>1</sup>	Starch	TDF <sup>2</sup>	IDF <sup>3</sup>	SDF <sup>4</sup>	$\beta$ - Glucans
IAC-IBON- 214/82	2.27 <sup>5</sup> $\pm$ 0.02 <sup>6c</sup>	3.20 $\pm$ 0.16 <sup>ab</sup>	15.83 $\pm$ 0.11 <sup>d</sup>	60.76 $\pm$ 1.63 <sup>ab</sup>	16.89 $\pm$ 1.42 <sup>cd</sup>	10.43 $\pm$ 0.90 <sup>bc</sup>	6.45 $\pm$ 0.59 <sup>c</sup>	5.02 $\pm$ 0.19 <sup>bc</sup>
IAC- 8612/421	2.26 $\pm$ 0.02 <sup>c</sup>	2.91 $\pm$ 0.22 <sup>a</sup>	13.65 $\pm$ 0.11 <sup>b</sup>	60.40 $\pm$ 1.32 <sup>ab</sup>	15.85 $\pm$ 0.47 <sup>bc</sup>	10.77 $\pm$ 0.70 <sup>bc</sup>	5.07 $\pm$ 0.25 <sup>ab</sup>	4.41 $\pm$ 0.21 <sup>ab</sup>
IAC- 8501/31	1.87 $\pm$ 0.01 <sup>b</sup>	4.00 $\pm$ 0.21 <sup>c</sup>	15.17 $\pm$ 0.12 <sup>c</sup>	63.14 $\pm$ 0.91 <sup>b</sup>	13.51 $\pm$ 1.11 <sup>ab</sup>	8.72 $\pm$ 0.87 <sup>ab</sup>	4.78 $\pm$ 0.23 <sup>ab</sup>	3.70 $\pm$ 0.12 <sup>a</sup>
IAC-8501/12	1.61 $\pm$ 0.04 <sup>a</sup>	3.19 $\pm$ 0.15 <sup>ab</sup>	15.92 $\pm$ 0.18 <sup>d</sup>	62.39 $\pm$ 1.40 <sup>b</sup>	12.37 $\pm$ 0.38 <sup>a</sup>	8.07 $\pm$ 0.30 <sup>a</sup>	4.30 $\pm$ 0.07 <sup>a</sup>	4.42 $\pm$ 0.22 <sup>ab</sup>
IAPAR-39-ACUMAI	1.51 $\pm$ 0.08 <sup>a</sup>	3.22 $\pm$ 0.24 <sup>ab</sup>	12.55 $\pm$ 0.13 <sup>a</sup>	57.46 $\pm$ 0.47 <sup>a</sup>	17.39 $\pm$ 0.63 <sup>d</sup>	12.16 $\pm$ 0.12 <sup>c</sup>	5.23 $\pm$ 0.51 <sup>a</sup>	5.77 $\pm$ 0.31 <sup>c</sup>
IAC-8501/22	1.81 $\pm$ 0.09 <sup>b</sup>	3.43 $\pm$ 0.06 <sup>b</sup>	15.61 $\pm$ 0.03 <sup>d</sup>	58.59 $\pm$ 1.95 <sup>a</sup>	14.56 $\pm$ 1.17 <sup>ab,c</sup>	8.67 $\pm$ 0.93 <sup>a</sup>	5.88 $\pm$ 0.23 <sup>bc</sup>	5.40 $\pm$ 1.00 <sup>bc</sup>

<sup>1</sup>Total Nitrogen  $\times$  6.25

<sup>2</sup>TDF=Total Dietary Fiber; <sup>3</sup> IDF=Insoluble Dietary Fiber;

<sup>4</sup>SDF=Soluble Dietary Fiber

<sup>5</sup>Means of three trials followed by standard deviations

<sup>6</sup>Tukey test

cordance to the description for an American HB waxy cultivar (Anderson et al., 1999).

These three constituents together make up more than 90% of the dry matter. According to Oscarsson et al. (1996), the contents of starch, total protein, and total dietary fiber may be affected by both genetic and environmental factors. Bhatti & Rosnagel (1998) showed that there were differences in protein, starch, and total dietary fiber contents between the Japanese and Canadian barley varieties tested. The differences ( $P < 0.05$ ) among the six varieties may be attributed primarily to genetic background, since all varieties were grown under the same environmental conditions.

Covered and hulless barley genotypes were differentiated on the basis of their average contents of constituents that are enriched in the husk. Correa (2003) studied seven different Brazilian covered barley cultivars and showed that the protein contents ranged from 11.14-12.53% while the Brazilian HB varieties showed a broad range of total protein contents (12.55-15.92%). High total protein contents may be attributed to a concentration effect caused by the lack of hulls and/or to the result of breeding for increased protein content in feed barley (Edney et al., 1992; Li et al., 2003). The addition of high protein cereal for enrichment is one way to increase protein intake.

The ash (1.61-2.27%) and ether extract (2.91-4.00%) contents were in agreement with results by Oscarsson et al. (1996), who reported ash and ether extract ranges from 1.3-2.1 and 2.1-3.7%, respectively. Ash consists mainly of inorganic compounds. The major mineral compounds in barley flour are phosphorus and potassium, while iron and zinc are the major trace minerals (Bhatti, 1993).

The total fiber content is represented by both soluble and insoluble fibers. The ratio of soluble dietary fiber and total dietary fiber was 1:3 in all HB varieties.

The  $\beta$ -glucan contents ranged from 3.70 to 5.77%, as previously reported (Bhatti, 1997; Knuckles et al., 1992; MacGregor & Fincher, 1993). Several papers have shown that soluble dietary fiber and  $\beta$ -glucan are of particular interest to consumers due to their effects on blood cholesterol and blood glucose. Thus, the Brazilian HB varieties showed high potential as sources of soluble dietary fiber and  $\beta$ -glucan, and are vital ingredients in health-promoting food products (Wu et al., 1994; Bhatti, 1999b).

### Fractionation of the protein

The yield of the HB flour fractions obtained was 29.43 (+180  $\mu$ m), 6.10 (+150  $\mu$ m), 7.34 (+106  $\mu$ m), and 57.14% (-106  $\mu$ m). The concentrations of crude protein, protein, and protein and non-protein nitrogen is shown in Table 2. Differences ( $P < 0.05$ ) were detected among the total protein of the fractionated flour. The 150-106  $\mu$ m fraction presented the highest total protein content for all varieties, while the lowest total protein content was obtained for unfractionated flour and the  $< 106$   $\mu$ m fraction. Protein nitrogen was approximately 80% higher than non-protein nitrogen, indicating that Brazilian HB varieties may be considered nutritionally valuable. Linko et al. (1989) evaluated the protein composition of a high protein barley flour and reported similar results for protein nitrogen. The ability to prepare products with different protein contents could increase the economic value of Brazilian HB by opening the possibility of tailoring fractions to specific foods.

## CONCLUSIONS

The differences in chemical composition of barley varieties compared to previous studies may be explained by genetic make-up, since all varieties were grown under the same environmental conditions. It is important to consider these differences in chemical composition when comparing results from varieties grown in

Table 2 - Contents of total protein, total nitrogen, and protein and non-protein nitrogen (% w/w dry basis) of Brazilian hulless barley flour fractionation.

Fraction	IAC-IBON-214/82				IAC-8601/421			
	CP <sup>1</sup>	P <sup>2</sup>	PN <sup>3</sup>	NPN <sup>4</sup>	CP	P	PN	NPN
< 1 mm	15.11 <sup>5</sup> ± 0.03 <sup>6,a</sup>	12.69 ± 0.00 <sup>a,b</sup>	2.03 ± 0.03 <sup>a,b</sup>	0.38 ± 0.02 <sup>a</sup>	13.94 ± 0.21 <sup>b</sup>	11.31 ± 0.03 <sup>a,b</sup>	1.81 ± 0.10 <sup>a,b</sup>	0.42 ± 0.07 <sup>a</sup>
>180 µm	16.60 ± 0.06 <sup>b</sup>	13.69 ± 0.01 <sup>a,b</sup>	2.19 ± 0.09 <sup>a,b</sup>	0.46 ± 0.10 <sup>a</sup>	15.05 ± 0.06 <sup>c</sup>	12.56 ± 0.01 <sup>b</sup>	2.01 ± 0.01 <sup>b</sup>	0.40 ± 0.02 <sup>a</sup>
>150>180 µm	17.60 ± 0.07 <sup>c</sup>	13.87 ± 0.01 <sup>b</sup>	2.22 ± 0.15 <sup>b</sup>	0.59 ± 0.13 <sup>a,b</sup>	16.42 ± 0.03 <sup>d</sup>	12.75 ± 0.00 <sup>b</sup>	2.04 ± 0.10 <sup>b</sup>	0.58 ± 0.10 <sup>a,b</sup>
>180>106 µm	18.97 ± 0.19 <sup>d</sup>	13.69 ± 0.03 <sup>a,b</sup>	2.19 ± 0.09 <sup>a,b</sup>	0.85 ± 0.12 <sup>b</sup>	17.23 ± 0.02 <sup>e</sup>	12.94 ± 0.00 <sup>b</sup>	2.07 ± 0.00 <sup>b</sup>	0.69 ± 0.00 <sup>b</sup>
<106 µm	14.83 ± 0.08 <sup>a</sup>	11.50 ± 0.01 <sup>a</sup>	1.84 ± 0.01 <sup>a</sup>	0.53 ± 0.00 <sup>a,b</sup>	12.96 ± 0.00 <sup>a</sup>	10.06 ± 0.00 <sup>a</sup>	1.61 ± 0.08 <sup>a</sup>	0.46 ± 0.07 <sup>a,b</sup>
Fraction	IAC-8501/31				IAC-8501/12			
	CP	P	PN	NPN	CP	P	PN	NPN
< 1 mm	14.58 ± 0.04 <sup>a</sup>	12.19 ± 0.01 <sup>a</sup>	1.95 ± 0.10 <sup>a</sup>	0.38 ± 0.11 <sup>a</sup>	15.48 ± 0.08 <sup>b</sup>	12.94 ± 0.01 <sup>b</sup>	2.07 ± 0.02 <sup>b</sup>	0.40 ± 0.40 <sup>a</sup>
>180 µm	16.61 ± 0.11 <sup>b</sup>	14.06 ± 0.02 <sup>b</sup>	2.25 ± 0.02 <sup>b</sup>	0.40 ± 0.01 <sup>a</sup>	17.57 ± 0.31 <sup>c</sup>	14.75 ± 0.05 <sup>d</sup>	2.36 ± 0.02 <sup>d</sup>	0.45 ± 0.03 <sup>a</sup>
>150>180 µm	17.93 ± 0.07 <sup>c</sup>	14.19 ± 0.01 <sup>b</sup>	2.27 ± 0.02 <sup>b</sup>	0.59 ± 0.04 <sup>a,b</sup>	17.87 ± 0.06 <sup>c</sup>	14.00 ± 0.01 <sup>c</sup>	2.24 ± 0.00 <sup>c</sup>	0.62 ± 0.01 <sup>b</sup>
>180>106 µm	20.25 ± 0.17 <sup>d</sup>	15.25 ± 0.03 <sup>b</sup>	2.44 ± 0.00 <sup>b</sup>	0.80 ± 0.03 <sup>b</sup>	20.17 ± 0.06 <sup>d</sup>	14.94 ± 0.01 <sup>d</sup>	2.39 ± 0.05 <sup>d</sup>	0.83 ± 0.06 <sup>c</sup>
<106 µm	14.31 ± 0.10 <sup>a</sup>	11.06 ± 0.02 <sup>a</sup>	1.77 ± 0.01 <sup>a</sup>	0.52 ± 0.02 <sup>a</sup>	14.54 ± 0.10 <sup>a</sup>	11.50 ± 0.01 <sup>a</sup>	1.84 ± 0.02 <sup>a</sup>	0.49 ± 0.01 <sup>a,b</sup>
Fraction	IAPAR-39-ACUMAI				IAC-8501/22			
	CP	P	PN	NPN	CP	P	PN	NPN
< 1 mm	12.65 ± 0.06 <sup>b</sup>	11.06 ± 0.01 <sup>a,b</sup>	1.77 ± 0.03 <sup>a,b</sup>	0.26 ± 0.02 <sup>a</sup>	15.26 ± 0.09 <sup>b</sup>	12.81 ± 0.01 <sup>a,b</sup>	2.05 ± 0.00 <sup>a,b</sup>	0.40 ± 0.01 <sup>a</sup>
>180 µm	14.66 ± 0.04 <sup>c</sup>	12.94 ± 0.00 <sup>c</sup>	2.07 ± 0.00 <sup>c</sup>	0.28 ± 0.01 <sup>a</sup>	17.61 ± 0.08 <sup>c</sup>	14.94 ± 0.01 <sup>c</sup>	2.39 ± 0.13 <sup>c</sup>	0.43 ± 0.12 <sup>a</sup>
>150>180 µm	15.06 ± 0.07 <sup>c</sup>	12.69 ± 0.01 <sup>b,c</sup>	2.03 ± 0.14 <sup>b,c</sup>	0.38 ± 0.12 <sup>a</sup>	18.02 ± 0.23 <sup>c</sup>	14.56 ± 0.04 <sup>b,c</sup>	2.33 ± 0.67 <sup>b,c</sup>	0.55 ± 0.10 <sup>a</sup>
>180>106 µm	17.37 ± 0.01 <sup>d</sup>	13.06 ± 0.00 <sup>c</sup>	2.09 ± 0.04 <sup>c</sup>	0.68 ± 0.04 <sup>b</sup>	19.84 ± 0.01 <sup>d</sup>	15.69 ± 0.00 <sup>c</sup>	2.51 ± 0.05 <sup>c</sup>	0.66 ± 0.05 <sup>a</sup>
<106 µm	11.84 ± 0.24 <sup>a</sup>	10.62 ± 0.04 <sup>a</sup>	1.70 ± 0.01 <sup>a</sup>	0.20 ± 0.03 <sup>a</sup>	14.55 ± 0.12 <sup>a</sup>	11.62 ± 0.02 <sup>a</sup>	1.86 ± 0.13 <sup>a</sup>	0.46 ± 0.11 <sup>a</sup>

<sup>1</sup>CP=Crude Protein (Total Nitrogen × 6.25)

<sup>2</sup>P=Protein (Protein Nitrogen × 6.25)

<sup>3</sup>PN=Protein Nitrogen; <sup>4</sup>NPN=Non Protein Nitrogen

<sup>5</sup>Means of three trials followed by standard deviations

<sup>6</sup>Tukey test

Brazil with those grown in others countries. The flour fractionation method might be a good alternative for protein concentration in the food industry to produce HB ingredients with high added value, and also to produce enriched fractions at low cost. The chemical composition of hulless barley of the Brazilian varieties studied were found to be similar to those reported for Canadian and Swedish cultivars. We have identified a fraction of hulless barley flour with the highest protein content of about 2% extra protein.

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