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Barley β-glucans extraction and partial characterization

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ABSTRACT

Barley is rarely used in the food industry, even though it is a main source of β-glucans, which have important health benefits and a technological role in food. This work evaluated the humid extraction of barley β-glucans and partially characterized them. The extraction was studied using surface response methodology with both temperature and pH as variables. The extracted β-glucans were characterized by chemical and rheological analysis, infrared spectroscopy and scanning electron microscopy. The effect on extraction of linear and quadratic terms of pH and temperature corresponding to the regression model was significant, and we obtained a maximum concentration of 53.4% at pH 7.56 and temperature 45.5 °C, with protein and mainly starch contamination. The extracted β-glucans presented a higher apparent viscosity than the commercial ones, the behavior of the commercial and extracted samples can be described as Newtonian and pseudoplastic, respectively. The results of infrared spectroscopy and scanning electron microscopy were characteristic of commercial β-glucans, indicating that this method is efficient for extracting β-glucans.

1. Introduction

Barley (*Hordeum vulgare* L.) is the fourth cereal crop in worldwide production and it is underutilized in the food industry, with just two applications: feed or malt (Arngren, Hansen, Eriksen, Larsen, & Larsen, 2011). Flavor and appearance are the limiting factors when using barley for human food. Nevertheless, in the last decade, an increasing interest in barley research as a food source has occurred, mainly because barley flour contains a large amount of soluble dietary fibers, especially β-glucans (Bhatty, 1999; Bilgi & Celik, 2004). This fact makes barley an important cereal because of its nutritional and functional properties. β-glucans is the common name given to the glucose polymer (1 → 3) (1 → 4)-β-D-glucans, the most abundant component of the soluble dietary fiber fraction both in barley and oats (Johansson, Tuomainen, Ylinen, Ekholm, & Virkki, 2004). It has been studied because of its structure and properties (Izydorczyk, Macri, & Macgregor, 1998; Wood, Weisz, Fedec, & Burrows, 1989).

A number of nutritional studies have shown a link between cereal consumption with the recommended β-glucan content and a reduction in the risks of chronic health problems, such as those associated with cardiovascular diseases because of the reduction in blood cholesterol and those associated to diabetes because of the regulation of blood glucose levels (Li, Kaneko, Qin, Wang, & Wang, 2003).

The main attribute of β-glucans that renders them beneficial is the fact that they can form very high viscose solutions and, therefore, increase intraluminal viscosity (Jenkins et al., 1978).

Apart from being nutritionally important, β-glucans show an important technological role in processed foods, where they can be used for the elaboration of products with high dietary fiber content as non-caloric thickening and stabilizing agents, as an aid in the production of cheese and ice-cream, as a fat substitute in dairy products and as a gel-forming component (Reed & Nagodawithana, 1991).

Food and supplement industries are increasingly interested in concentrating this bioactive grain component at a commercial level to incorporate β-glucans as an ingredient in product formulation (Vasanthan & Temelli, 2008).

Isolation techniques of β-glucans from barley and oats have been evaluated by several authors. Among these, we can mention the milling, sifting (Sundberg & Aman, 1994; Wu, Stringfellow, & Inglett, 1994) and solvent extraction techniques (Bhatty, 1995; Temelli, 1997; Vasanthan & Temelli, 2008; Wood et al., 1989). Processes that involve α-amylase for starch hydrolysis have been researched for the preparation of β-glucan concentrates from

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barley and oats (Ghotra, Vasanthan, Wettasinghe, & Temelli, 2007; Saulnier, Gévaudan, & Thibault, 1994).

Results from several studies suggest that temperature and pH have a significant effect on the extraction and properties of the β-glucans. The extraction conditions may also affect the physical, chemical and functional properties of β-glucans (Ahmad, Anjumb, Zahoorb, Nawazc, & Ahmedd, 2010), while high native viscosity may be sometimes degraded during extraction and additional processing (Burkus & Temelli, 2005).

There is a need to explore the possibilities of increasing the consumption of barley related products for humans through the development of products with an added value. Therefore, the objective of this work was to evaluate the humid extraction of β-glucans and partially characterize it.

2. Materials and methods

2.1. Material

For this study, we used whole barley flour from the BRS 195 cultivar, furnished by SL Alimentos Ltd., Mauá da Serra, Brazil.

The extracted β-glucan was compared with a sample of the commercial product from Cargill, Barliv®. All of the analyses for the extracted β-glucans were also performed for the commercial sample.

2.2. Extraction of β-glucans

For barley β-glucan extraction, the method described by Wood, Siddiqui, and Paton (1978) and adapted by Temelli (1997) was used with a few modifications. For starch precipitation, 50 g of the barley flour sample was added to 500 mL of distilled water at an adjusted pH in accordance to the surface response plan with calcium carbonate (20% p/v) and warmed at temperatures defined by the surface response plan in a water bath with agitation for 30 min. The solution was centrifuged at 4940*xg*/30 min and 4 °C. The residue was removed, and the pH of the supernatant was adjusted to 4.5 with HCl 2 mol L^{-1}, centrifuged at 4940×*g*/30 min 4 °C to separate precipitated proteins, which were discarded. The pH was adjusted to 4.5 because according to Yalçın and Çelik (2007), protein solubility of barley flour at acidic pH values decreases around pH 4 and 6 and increases with basic pH values. An equal volume of ethanol was added to the supernatant to precipitate the β-glucans. After 12 h at 4 °C, the solution was centrifuged at 3780×*g*/10 min. The precipitate was re-suspended in ethanol, filtered, rinsed with ethanol and dried in an oven (model 400/D 200 °C, Nova Ética®) at 25 °C with forced air circulation for 1.5 h. The dried β-glucan extract was stored in a desiccator for 24 h and then ground with the aid of liquid nitrogen.

2.3. Chemical analysis

The β-glucan fraction was chemically characterized by the determination of protein (Kjeldahl N × 6.25), total starch and β-glucan content. For the last two, enzyme kits from Megazyme International Ireland Ltd. (Bray, Co. Wicklow, Ireland) were used. In addition to these components, the barley flour was also analyzed to determine moisture, soluble and insoluble dietary fiber, lipids and ash contents. All of the determinations were conducted following the official methods of the American Association of Cereal Chemists (AACC. American Association of Cereal Chemists., 2000).

For apparent amylose in the flour, the *iodine sorption: Blue Value* (Gilbert & Spragg, 1964) was used.

2.4. Rheological tests

The rheological behavior was characterized by preparing aqueous dispersions of 1% (p/v) of the extracted and commercial β-glucans (Barliv® – Cargill) at 80 °C (1 h), followed by continuous agitation for 2 h at room temperature. This procedure guaranteed complete hydration and dispersion without forming clumps. The rheological measurements were obtained with a rotational rheometer (*Brookfield Engineering Laboratories model* RVDV-III *Ultra*, Stoughton, MA, USA) with concentric cylinders *spindle* ULA, and the obtained results were processed through the Rheocalc® 32 (version 2.5) software. Four temperatures were used (25, 45, 65 and 85 °C) to evaluate the rheological behavior of the β-glucan dispersions. For the extracted β-glucans, the interval of shear rate was 24–96 s^{-1}, and for the commercial β-glucans, an interval of shear rate from 61 to 157 s^{-1} was applied.

2.5. Infrared transmission spectroscopy

Infrared spectroscopy was performed with equipment from ABB Bomem brand, model 2000-100 FTLA.

The method used was a FT-IR in KBr solid. The KBr was pulverized in an agate mortar and pestle, and the sample, which was also sprayed together with KBr, was added. With the resulting powder, a tablet was made by compression with a pressure of 10 tons. The infrared transmittance of the resulting pellet was analyzed by FTIR; 12 scans were performed with a resolution of 2 cm^{-1}.

2.6. Scanning electron microscopy

The samples were fixed to stubs with carbon tape and covered with two layers of gold of 200 A° each in a Baltec sputter coater, model SCD 005.

The visualization and photography of the samples were performed using a JEOL JSM-6390LV electron microscope at an acceleration voltage of 10 kV.

2.7. Experimental design and statistical analysis

For the extraction variable, an experimental project of response surface methodology known as central composite design was used and adjusted to a second order regression model. Two factors were used for extraction, extraction pH of the starch and the water bath temperature. The variation levels of the factors are presented in Table 1. The response surface was developed using the Statistica program (Statistica version 7.0, Statsoft Inc., Tulsa, OK, USA).

A central composite design was adopted, including a total of 14 treatments: four treatments corresponding to a complete factorial set 2^2, where the two factors were pH = Starch extraction pH and T = extraction temperature (°C), each with two codified levels, treatments −1 and +1; four including the axial points minimum and maximum levels of each of the two factors codified as $-\alpha$ e $+\alpha$, where $\alpha = (2^2)^{1/4} = 1.414$; six repetitions of the central point treatment were performed where all of the factors were at an average level coded as zero (Table 1).

The second order regression model was represented by the Eq. (1):

$$z = b_o + b_1^* x + b_{11}^* (x)^2 + b_2^* y + b_{22}^* (y)^2 + b_{12}^* x^* y + \text{error} \quad (1)$$

where z is the response, x the pH, y the temperature and b_o, b_1, b_{11}, b_2, b_{22} is the regression coefficients estimates.

Table 1
Levels of variation and variables of the extraction process.

Independent variables	Variation levels				
Codified values	$-\alpha$[a]	−1	0	+1	$+\alpha$[a]
pH	6.6	7	8	9	9.4
Temperature (°C)	35	38	45	52	55

[a] $\alpha = 1.414$.

3. Results and discussion

3.1. Barley flour characterization

The barley flour used in the study had a composition of 10.56% moisture, 10.69% protein, 2.44% lipids, 1.35% ash, 10.19% insoluble dietary fiber and 5.06% soluble dietary fiber. The β-glucan content was 4.75%, and the obtained residual starch was 58.07%. Similar results were described by Fujita and Figueroa (2003) for Brazilian barley cultivars.

The apparent amylose level (18.38%) characterizes a percentage of regular starch, because according to Blazek and Copeland (2008), the starch can be characterized according to its amylose content so that waxy starch has up to 2%, normal starch has up to 34% and starch with high amylose has up to 43%.

Barley flour composition indicates that barley is a good source for the extraction of β-glucans and starch because of the large amounts of these components and to the reduced lipid concentration that facilitates the extraction.

3.2. Evaluation of barley β-glucan extraction

The general equation with the regression coefficients is represented by the Eq. (2):

$$\hat{z} = 52{,}8627 - 0{,}83452^{*}x - 1{,}0147^{*}x^{*}2 + 0{,}13156^{*}y - 3{,}43837^{*}y^{2} - 0{,}80147^{*}x^{*}y \quad (2)$$

where $\hat{z}$ is the estimated result, x the pH and y is the temperature

The determinant coefficient of the model was $R^2 = 0.77$.

The degree of significance (p value) of the coefficients and lack of fit of the model was: pH (Linear) = 0.010525, pH (Quadratic) = 0.00674, Temperature (Linear) = 0.503724, temperature (Quadratic) = 0.000017, pH Linear by temperature Linear = 0.041935 and lack of fit = 0.001489.

The pH showed a linear and quadratic linear effect on extraction, and the temperature showed a quadratic effect on extraction. However, to maintain the hierarchy of the model used, the linear effect of temperature was used in the equation, even though it was not significant. The yield of extracted β-glucan was higher (maximum 53.38%) for pH 7.56 and temperature 45.5 °C (Fig. 1).

The β-glucan extraction temperature is related to its solubility. As the temperature increases, the solubility increases too, allowing the β-glucan to remain soluble in the supernatant. However, at higher temperatures, the starch may also solubilize. According to Hoseney (1991), the gelatinization temperature of starch from barley varies from 51 to 60 °C. However, its partial solubilization starts below these temperatures. Wood et al. (1978) described an increase in oat β-glucan extraction at 45–63 °C, which, according to the authors, is the optimum temperature range to avoid starch contamination.

Mälkki, Myllymäki, Autio, and Suortti (1992) developed a combination of dry and wet milling processes with ethanol and produced an oat-bran concentrate with 15–20% β-glucans, which are lower values than those obtained in this study.

Burkus and Temelli (2005) obtained 71.1% β-glucans using pH 9.4 and temperature of 53–55 °C from a mixture of two experimental cultivars of waxy barley. Temelli (1997) used a similar method and obtained higher β-glucan values with a content of 86.5% at 55 °C at pH 7 and 8. The highest yield obtained for these authors could be related to the use of barley type and to the use of higher centrifugation speed.

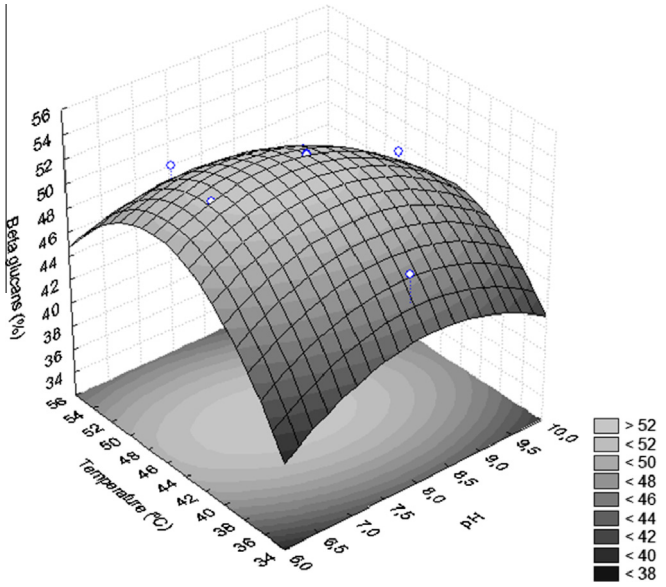

Fig. 1. pH and temperature effects on the extracted barley β-glucans.

3.3. Characterization of the extracted β-glucans

3.3.1. Chemical analysis

The extracted fraction of β-glucans used in this study contained 53% of this component, while the commercial β-glucans (71%) and both samples showed the presence of starch and protein. The extracted sample showed 13.83% starch and 4.15% protein, while the commercial sample had 1.53% starch and 2.59% protein. The extracted β-glucans contained higher amounts of starch. This fact could be explained by the partial solubilization of this component during the extraction procedure used. Other researchers have also found extracted starch, protein and other components together with the β-glucans (Wood, 2002; Burkus & Temelli, 2005). Ahmad et al. (2010) obtained similar extraction values of protein during oat β-glucan extraction using the same extraction principle.

Mikkelsen, Jespersen, Moller, Laerke, Larsen and Engelsen (2010) suggested enzymatic treatments to remove impurities such as starch and proteins from extracted β-glucans.

3.3.2. Rheological measurements

The apparent viscosity of the extracted sample at 45 °C and pH 8.0 (Fig. 2A) obtained with the different tested temperatures was superior to the viscosity of the commercial sample (Fig. 2B). The commercial sample maintained the viscosity with increasing shear rate ranges, while the extracted sample exhibited a decrease in viscosity. The behavior of the commercial and extracted samples can be described as Newtonian and pseudoplastic, respectively. Wood et al. (1989) and Bhatty (1995) also described the solutions' β-glucans that were extracted from barley and oats as having pseudoplastic behavior.

The presence of other components than β-glucans in greater quantity in the extracted fraction is another important factor responsible for viscosity change, which explains differences in behavior (Faraj, Vasanthan & Hoover, 2006). For rheological measurements, all samples were treated at 80 °C, a temperature that promotes gelatinization of starch. The extracted sample has a

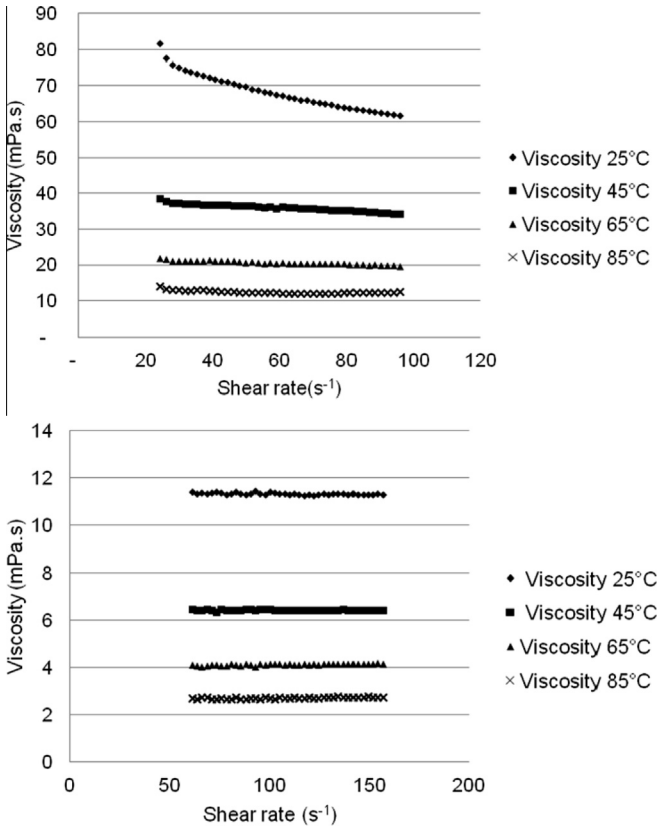

Fig. 2. Relationship between apparent viscosity x interval of shear rate of the extracted sample at 45 °C and pH 8.0 (A) and commercial (B) β-glucans at different temperatures.

higher contamination of starch (13.83%) compared with the commercial sample (1.53%); therefore, the different amounts present of this component may be responsible for the different behavior displayed by the extracted β-glucans and commercial dispersion. Bhatty (1995) made that suggestion in a study, where four β-glucans preparations with variable chemical composition showed a different behavior in relation to viscosity. According to the same author, the differences in the viscosity of β-glucans could also be explained in relation to the molecular size and fine structure that influence physical properties.

When increasing the shear rate, the viscosity exhibited maintenance for commercial β-glucans in all treatment temperatures; for the extracted β-glucans, there was a decrease in viscosity with increasing temperature (Fig. 2A and B). Wood et al. (1989) reported a viscosity fall during extraction of oat β-glucan of <100 s^{-1}, but there was no loss of permanent viscosity during extraction at shear rates up to 1.500 s^{-1}.

Another important factor that affects the viscosity of β-glucan extracts is the activity level of the endogenous β-glucanase during extraction. Oat reflux with ethanol 70–80% has been adopted by some researchers (Saulnier et al., 1994; Wood et al., 1978) to inactivate the glucanases before the extraction. However, this treatment was not used in this study.

Burkus and Temelli (2005) found higher viscosity values in extracted barley β-glucans using α-amylase for purification. According to Ahmad et al. (2010), acid and alkali extraction methods reduce the viscosity of the extracted β-glucans.

The flow properties were expressed by the Power Law equation, where k (consistency index) and *n* (flow behavior index) are constants, and when the value of *n* decreases, the shear sensibility increases. The results varied from 0.836 to 0.903 for the extracted β-glucans and from 0.980 to 1.027 for the commercial sample (Table 2) according to the temperature increase.

Bhatty (1995) related a flow behavior index of >0.7 for barley and for oat β-glucans. The pseudoplasticity of the β-glucans is an established fact, having a high consistency index (*k*) and a low flow behavior index (*n*) (Burkus & Temelli, 2005).

Generally, viscosity diminishes with temperature, which is a reversible alteration of β-glucans. As expected, the viscosity of extracted and commercial β-glucans decreased with increasing temperature at the studied range (Table 2 and Fig. 2). Elleuch et al. (2011) observed a viscosity increase with increasing concentrations and a decrease in viscosity when increasing temperatures for a soluble fiber solution (β-glucans).

In general, the β-glucan viscosity profiles at different temperatures are important functional and process parameters for the food industry (Mikkelsen et al., 2010). Considering that the viscosity of β-glucans is highly responsible for glucose regulation and blood cholesterol reduction in hypercholesterolemia individuals (Wood, 2002), it is possible to suggest that this sample of extracted β-glucans has viscosity features for this function.

A reversible drop in viscosity with higher temperatures could be of use in the industrial process of β-glucans because by allowing easier pumping and agitation, it leads to lower energy consumption (Burkus & Temelli, 2005).

3.3.3. Infrared Transmission Spectroscopy (IR)

Infrared spectroscopy allows the measurement of molecular vibrations of covalent bonds. The IR region 4000–200 cm^{-1} provides information on the fundamental vibrations. The infrared spectra of extracted and commercial β-glucans are shown in Fig. 3.

In the region of 4000–3000 cm^{-1}, the extracted and commercial barley β-glucans spectra showed a wide band with maximum absorption (minimum transmittance) at 3424 cm^{-1} and 3434 cm^{-1}, respectively. This can be attributed to normal vibrational modes of asymmetric and symmetric stretching of OH groups because polysaccharides contain a significant number of

Table 2
Rheological parameters obtained using the model of the Power Law ($\eta = \kappa(\gamma)^{n-1}$) and the apparent viscosity of the extracted and commercial β-glucans at different temperatures.

Sample (°C)	Consistency index (k, Pa s^{-1})	Flow behavior index (*n*)	Apparent viscosity (Pa s^{-1})[a]	*R*
Extracted				
25	0.137	0.836	67	0.9997
45	0.051	0.922	37	0.9994
65	0.026	0.934	19	0.9995
85	0.016	0.903	11	0.9988
Commercial				
25	0.015	0.980	11	0.9995
45	0.006	1.003	7	0.9995
65	0.004	1.024	4	0.9995
85	0.002	1.027	4	0.9981

[a] Apparent viscosity at a shear rate of 76 s^{-1}.

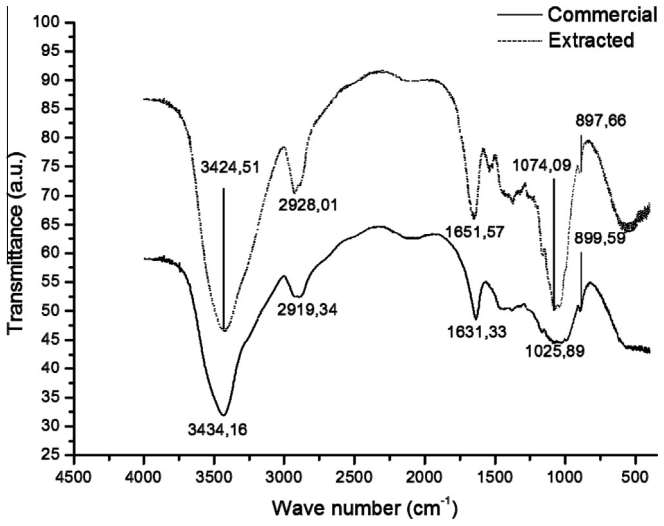

Fig. 3. Infrared spectra of extracted and commercial β-glucans.

OH groups, which exhibit an absorption band above 3000 cm^{-1} (Wang, Ahmed, Feng, Li, & Song, 2008).

The maximum absorption peaks occurring at 2928 cm^{-1} (extracted) and 2919 cm^{-1} (commercial) in the region of 3000–2840 cm^{-1} could be attributed to the relative values of the vibrational modes of asymmetric and symmetric stretches of CH groups (Vieira, 1998).

The strong absorption at 1651 cm^{-1} for extracted β-glucans and 1631 cm^{-1} for commercial β-glucans were due to the stretching of CN groups and NH groups of the proteins indicating the presence of amide linkages and the presence of protein in the sample (Ahmad et al., 2010; Wang et al., 2008). These results are in accordance with the chemical evaluation, indicating the presence of protein in both samples.

The region 1285–242 cm^{-1}, which showed peaks with maximum absorption at 1074 cm^{-1} (extracted) and 1025 cm^{-1} (commercial), corresponds to COC and CO bonds (Wang et al., 2008) of a ring of D-glucose, which are network vibrations in which all of the atoms of the macromolecular chain vibrate in phase and normal modes resulting from coupling of the CC and CO stretches (Vieira, 1998). Therefore, the peaks at 1074 cm^{-1} for the extracted sample and at 1025 cm^{-1} for the commercial one indicate the presence of glycosidic bonds and cyclic structures of monosaccharides.

According to Mikkelsen et al. (2010), starch is associated to bonds at 930 cm^{-1} and at 1078 cm^{-1}; the peak at 1074 cm^{-1} of the extracted β-glucans indicates the presence of starch, which was confirmed by chemical analysis.

Carbohydrates can be recognized by peaks at wave numbers of 1040 cm^{-1} (CO bond of the alcohol group), 2940 cm^{-1} (CH stretch) and 3400 cm^{-1} (OH stretch) (Wang et al., 2008).

It is also important to note that the spectra showed absorption peaks at 987 cm^{-1} for the extracted sample and 899 cm^{-1} for the commercial sample, which is indicative of a β-glycosidic anomeric bonds (Mikkelsen et al., 2010).

Johansson and et al. (2004) reported similar spectra (4000–650 cm^{-1}) for barley β-glucans samples.

The parameters evaluated in the partial characterization of extracted β-glucans are favorably compared with the commercial β-glucans in their high degree of purity.

3.3.4. Scanning electron microscopy

In general, the morphology of the two analyzed samples, extracted β-glucans and commercial β-glucans is similar (Fig. 4) with a porous and spongy appearance with no visible traces of cell wall structure. There were observed differences in the size distribution and shape of the particles.

A panoramic view of the samples revealed that extracted β-glucans (Fig. 4A and B) had an irregular and smaller particle size distribution. A few clusters of rounded structure and small loose particles with geometric shapes characterized the extracted sample. The commercial β-glucans (Fig. 4C and D) presented larger, rounder and homogeneous clusters.

A higher magnification of a cluster of the extracted sample (Fig. 4 B) showed a more porous and spongy structure than that found in the commercial sample (Fig. 4D). The structural differ-

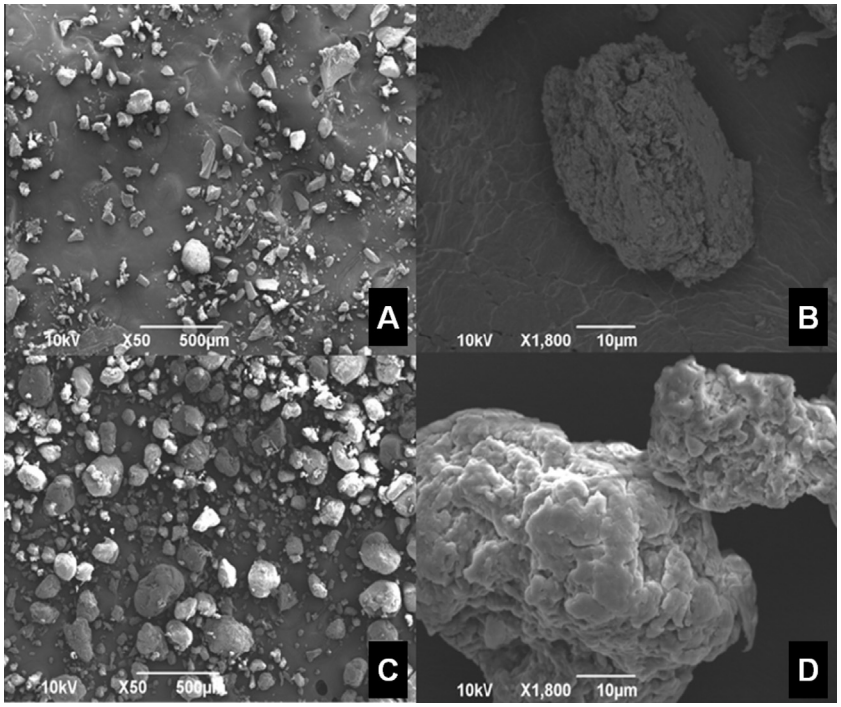

Fig. 4. Scanning electron micrographs of extracted (A and B) and commercial (C and D) β-glucans from barley.

ences between the samples are probably a consequence of the used extraction method, mainly in the drying and milling steps. In this study, the drying process was performed using an oven with forced air circulation, while the subsequent freezing was performed in liquid nitrogen and the manual grinding in a mortar and pistil, which may have reflected directly in the microstructure. This extraction stage may be replaced by another method such as lyophilization using a product with similar microstructure to the commercial β-glucans.

Vasanthan and Temelli (2008) also showed microstructure with similar characteristics to the ones obtained in this study for β-glucans extracted by an alkaline aqueous method.

4. Conclusions

The sample of extracted β-glucans showed high levels of this component, but it also contained starch and protein. The flow properties, the infrared spectroscopy and scanning electron microscopy results were properties of the commercial β-glucans. Therefore, it is possible to suggest that the barley β-glucans extracted in this study are potential thickeners or stabilizers of industrial food products because of the similarity between their characteristics and the ones from commercial β-glucans, expanding the use of barley in the food industry.

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