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Beta-Glucan Enriched Products obtained from different Barley Milling Fractions and their Mixtures

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The effect of the milling on the extraction of β-glucans has been studied with a hull-less waxy genotype barley cultivar (H13, commercially known as GALIS, with a β-glucan content of 4.64±0.16 % and a 58.5±3.6 % of starch). Barley was milled in a Chopin CD1 mill, obtaining three different milling fractions: brans (50 % w/w; 4.5 % β-glucan), reduction flour (20 % w/w; 6.6 % β-glucan) and break flour (30 % w/w; 2.4 % β-glucan). Differences between fractions were found in particle size, composition, as well as in the extraction yield and the molecular weight of the β-glucan isolated (up to 723 kDalton, according to the GPC results obtained). The best results were obtained when reduction flour was used as source of β-glucans: the product contained 28 % of β-glucan (643 kDa) and 39 % of starch, showing a total β-glucan recovery of 63.6 % and a gum yield of 16.6 %. Mixtures of brans and reduction flour contributed to increase the average β-glucan recovery up to 60 %, expressing the importance of having a homogeneous raw material with a lower particle size distribution as possible to perform successfully a β-glucan extraction process. According to these results, milling fraction, considered as a pretreatment of barley, contributes to make easy and more efficient the final β-glucan recovery, because leads to lower particle size flours and to a more quality product in terms of higher molecular weight.

1. Introduction

β-glucans are a kind of non-starchy polysaccharide that can be found in several kinds of cereals, such as barley, oat or rye in concentrations from 2 to 12 % in dry basis. They form part of the dietetic fiber, nevertheless β-glucans are soluble in water.

Research on β-glucan extraction has become more interesting in the last years due to the appeal of these polymers for the human health. The control of cholesterol and glucose concentration in blood is the major benefit β-glucan can offer (Brennan and Cleary, 2005). β-glucans responsible for these beneficial properties are those that have high molecular weight, leading to higher viscosity and special rheological behaviour solutions (Irakli *et al.*, 2004).

Although the amount of procedures reported in literature to obtain β-glucan enriched products is large, all of them can be grouped in two categories as it is suggested by

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Vasanthan and Temelli (2008): those experiments carried out in dry conditions, and those performed in wet conditions. Dry processing technologies include dry milling and sieving techniques (Kiryluk et al., 2000), achieving a final product that contains a 15.2% in β-glucan, as the best result, and dry milling and air classification (Vasanthan and Bhatty, 1995). Wet procedures are more complex, since they involve at least two or three stages (Brennan and Cleary, 2005): cereal bran or flour is used as raw material, which is put in contact with a solvent (usually water, basified water or semi-alcoholic solution), obtaining an aqueous extract as a result. This aqueous extract contains other species apart from β-glucan (such as starch, proteins and fats), making necessary a purification step. For this purpose, β-glucans are precipitated by addition of an alcohol as anti-solvent, or separated by other procedures. The precipitate obtained in this way, once isolated and dried, results in a product that contains from 20 to 70% of β-glucan.
The purpose of the present work is to combine dry processes (milling and fractionating of cereal grain) as a previous step with wet techniques (deactivation of β-glucanases with ethanol, extraction with hot water, β-glucan precipitation, filtration and drying) in order to obtain β-glucan enriched products. By using milling techniques it will be possible to obtain homogeneous flour (particle size) with a higher concentration of β-glucan, that facilities the further extraction stage and allow to obtain a final enriched β-glucan product with higher molecular weight.

2. Experimental section

2.1 Materials and methods

In this work a hull-less waxy genotype barley cultivar (H13), commercially known as GALIS, has been used. This barley, harvested in 2006 and with a β-glucan content of 4.64±0.16 % and a 58.5±3.6 % of starch, has been supplied by ITACYL (Instituto Tecnológico Agrario de Castilla y León; Valladolid, Spain). This barley was milled using a Chopin-CD1 mill (Chopin, France) placed at CETECE (Centro de Tecnología de Cereales; Palencia, Spain). Three different milling fractions were obtained: **bran** (particle size above 0.8 mm), including hulls when hulled barley was milled, very heterogeneous; **reduction flour** (particle size between 0.16-0.5mm) and **break flour** (particle size under 0.16 mm). After milling, brans yielded 50 % approximately, reduction flour 20% and break flour the remaining 30 %. When using Chopin CD1, barley was milled at constant moisture (15.5 %). Chemical composition of each milling fraction is presented in Table 1.

Table 1: Composition of the milling fractions, milled at constant moisture of 15.5%.

Barley Sample	Milling Fraction	β-Glucan (% wt)	Starch (% wt)	Protein (% wt)	Dietary Fibre (g/100g)
H13-Galis	Bran	4.54	47.8	15.59	17.1
	Reduction Flour	6.64	41.3	17.73	22.5
	Break flour	2.38	65.4	11.29	4.5

2.2 Chemical analysis

β-glucan and starch determination was done using the "Mixed linkage β-glucan assay kit" and the "Total Starch assay kit", respectively. Both of the kits were supplied by *Megazyme,* Ireland. Starch and β-glucan concentration was determined in the liquid

extract, being expressed in both cases in percentage of the total liquid extract obtained. Both species were analysed in the final gum product.

The extraction process efficiency was evaluated according the following definition:

$$\%\ \text{Extraction yield} = \frac{\text{wt.of β-glucan in the liquid extract}}{\text{wt.of β-glucan in the initial flour}} \times 100 \quad (1)$$

Moreover, two parameters were introduced in order to compare the results obtained with those reported by other researchers: *Gum yield* and *β-glucan recovery* (this parameter quantifies the efficiency of the overall β-glucan extraction process)

$$\%\ \text{Gum yield} = \frac{\text{wt.of solid extract}}{\text{wt.of initial flour}} \times 100 \quad (2)$$

$$\%\ \beta\text{-Glucan recovery} = \frac{\text{wt.of } \beta\text{-glucan in the solid extract}}{\text{wt.of } \beta\text{-glucan in the initial flour}} \times 100 \quad (3)$$

Molecular weight of β-glucans was determined by Gel Permeation Chromatography (GPC) according the method described in a previous work (Benito *et al*., 2009).

Extractions with each milling fractions were conducted in duplicate. β-glucan and starch analysis of each extract were performed in duplicate.

2.3 Experimental procedure

2.3.1 Endogenous enzyme deactivation by the use of ethanol

As previous step to the extraction, and after the milling process, 80 g of barley sample were suspended in 500 mL of ethanol (80% v/v) and boiled under reflux for two hours with continuous stirring. After the treatment, the barley and the ethanol were separated. Barley was dried at 90 ºC and milled again in order to get homogeneous flour. Subsequently, extraction process was carried out, according the procedure described in section 2.3.2.

2.3.2 Batch procedure for extraction of β-glucans

Extraction was carried out in a 1L jacked vessel. In each experiment, 25 g of barley were suspended vigorously (500 rpm, *Heidolph RZR 2021 stirrer*, Germany) in 200mL of deionized water for 3 h at 55ºC (*Ecoline Staredition E100*, Lauda, Germany). After the extraction, the mixture was centrifuged for 10 min at 5000 rpm (Kubota 5100, Japan). Solid material was discarded, while liquid extract was kept at 4 ºC. All the extraction experiments were carried out in duplicate. β-glucan precipitation was done by adding an equal volume of ethanol (96 %, v/v) to the liquid extract. The white solid product obtained was separated from liquid by vacuum filtration, and set at 60 ºC overnight. Once dried, gum was milled and kept in a sealed glass tube until the moment of being analysed.

2.3.3 Mixture of milling fractions

Different combinations of milling fractions were tried. Initially, brans and reduction flour were mixed together after milling the barley. Break flour was no considered, due its lower content in β-glucan. The resultant product contained 71 % of brans and 29 % of reduction flour. Three different mixtures were studied: mixture A, in which brans were milled in order to obtain more homogeneous fraction and subsequently mixed with the reduction flour. In mixture B, both milling fractions were mixed together and then re-milled. Finally, mixture C resulted from mixing the two fractions with no further milling. In the three cases, after having obtained the desired mixture, samples were

pretreated with ethanol, as described in paragraph 2.3.1, and extracted β-glucans according to 2.3.2.

3. Results and discussion

3.1 Milling fractions

Important differences were observed when extraction was performed from milling fractions, compared to those results got from unique milling fraction of barley ("flour") obtained with a grain mill without fractioning. These results are shown in Table 2.

Table 2: Composition of the products obtained from H13 barley milling fractions

Sample	Extraction yield %	β-glucan %	Starch %	MW Da	gum yield %	β-glucan recovery %
Brans	41.7	34.0	40.1	728000	6.7	44.1
Reduction Flour	57.5	27.7	39.1	643000	16.6	63.5
Break flour	71.5	23.8	48.2	720000	7.2	59.9
Flour[1]	43.7	36.7	29.5	496000	4.7	32.7
Flour[2]	62.8	33.7	4.4	98000	4.3	29.6

Flour: Unique milling fraction (dp = 0.5 mm, 4.43±0.13 % of β-glucan and 50.5±3.3 % of starch)
[1] flour with ethanol pre-treatment [2] flour without ethanol pre-treatment

Higher extraction yield was obtained when lower particle size and more homogeneous milling fraction was: this is explained by the fact that break flour, despite of having the lowest β-glucan content, gave the best extractability of β-glucans (71.5 %, which is an excellent result with a high MW, 720 kDa). Starch concentration in the final product was the highest: 48.2 %. However, β-glucan extraction from brans was more difficult, due to its wide particle size distribution. This represents a challenge because brans represent the 50 % of the milling fractions obtained, with a slightly higher content of β-glucans than barley as a whole (4.54 % vs 4.32 %). Reduction flour extraction yield was intermediate, as the particle size distribution is between brans and break flour. This fraction has the higher β-glucan content, but it represents only the 20 % of the three milling products. The yield of each milling fraction may be modifying by means of optimization process of milling parameters. Milling the barley at different moistures did not reveal important differences neither in yields nor in composition of each fraction.

H13 flour extraction yield was around 44% when was pretreated with ethanol, substantially lower than that achieved from barley not pretreated with ethanol (62 %). Ethanol pre-treatment increased the molecular weight of the β-glucan extracted by five times, and hence increased the viscosity leading to an important decrease of the stirring efficiency. When performing the extraction from milling fractions, important increases of the extraction yield were obtained as well as in final β-glucan recovery. Total β-glucan recovery was in the range 44 to 63.5 %, almost twice the recovery achieved

when using just flour. Gum yield obtained in all the cases was around 50 % higher than the obtained when working with the raw barley flour.

Despite of the important increase in the β-glucan recovery, amount of starch was increased dramatically. The use of ethanol pretreatment enhanced the molecular weight but allowed that starch co-extraction. In order to upgrade the final product, part of this co-extracted starch will have to be removed by following an enzymatic treatment.

Molecular weight of the polymers extracted was increased when the fractionating milling was performed. Barley flour pretreated with ethanol lead to a polymer of 500 Da, high compared to the obtained when barley was not pretreated with ethanol. But the further milling to obtained fractions with different compositions and particle size lead to another increase of the MW, up to 700 KDa.

3.2 Mixtures of milling fractions

Three different mixtures of brans (71 % w/w) and reduction flour (29 % w/w) were tested. Significant differences were observed when varying the pretreatment procedure and the way of executing the milling, as can be seen in Table 3.

Table 3: Characterization of products obtained from mixtures of barley milling fractions

Sample	Extraction yield	β-glucan	Starch	MW	gum yield	β-glucan recovery
	%	%	%	Da	%	%
Mixture A	60.3	22.8	49.6	650000	15.6	58.8
Mixture B	49.7	29.3	41.4	588000	7.9	39.6
Mixture C	45.0	35.8	32.6	741000	6.0	35.1

Best results in terms of β-glucan recovery were achieved when mixture A was used. 58.9 % of the initial β-glucans present in the raw material were recovered in the final product. Extraction yield was high (60.3 %), and higher than that achieved when brans and reduction flour were extracted separately: 41.7% and 57.5%, respectively (see Table 2). Also gum yield was extremely high: 15.6 %. Particle size of brans seems to be the most limiting factor for the extraction to be successful. However, this lower particle size facilitates the extraction of starch, and hence, there is a high concentration of starch in the final product (around 50 %). Mixtures B and C showed a similar behaviour when were extracted: similar extraction yields, β-glucan recovery and compositions of solid product obtained. Mixture B (brans and reduction flour were mixed together and subsequently milled) results were slightly better: extraction yield 50 %, with a total β-glucan recovery of 40 %.

The particle size and in the homogeneity of the raw material could explain this fact: in mixture A brans were milled separately and later mixed with the reduction flour. This sequence of operation allowed has a more accurate control of the particle size achieved when milling. This means the lower particle size improves the extractability of β-

glucans being this effect on β-glucan extraction more important than the initial concentration of β-glucans in the raw material.

4. Conclusions

In this work the effect of the milling on the extraction of β-glucans from a hull-less waxy genotype barley cultivar has been studied. Barley was milled in a Chopin CD1 mill, obtaining three different milling fractions: brans (50 % w/w; 4.5 % β-glucan, dp>0.8 mm), reduction flour (20% w/w; 6.6% β-glucan, 0.16<dp<0.8 mm) and break flour (30 % w/w; 2.4 % β-glucan, dp>0.8 mm). Important differences between fractions were found in the extraction yield and the molecular weight of the β-glucan isolated. Best results were obtained when reduction flour was used as source of β-glucans: the resulting product contained 28 % of β-glucan (643 kDa) and 39% of starch, showing a total β-glucan recovery of 63.6 %. Mixtures of brans and reduction flour contributed to increase the average β-glucan recovery up to 60%, expressing the importance of having a homogeneous raw material with a lower particle size distribution in order to perform successfully a β-glucan extraction process. According to these results, milling pretreatment of barley, contributes to make easy and more efficient the final β-glucan recovery, because leads to lower particle size flours and to a more quality product in terms of higher molecular weight.

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