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Study of the effect of different pretreatments on the performance of the extraction of β-glucan from barley

Oscar Benito Román, Susana Lucas Yagüe*, Esther Alonso Sánchez
Departamento de Ingeniería Química y Tecnología del Medio Ambiente.
Prado de la Magdalena s/n. Facultad de Ciencias. Universidad de Valladolid (Spain)
e-mail: susana@iq.uva.es Tfno: +34 983 42 31 75 Fax: +34 983 42 30 13

The aim of this work is to study the effect of different pretreatments on the extraction of β-glucan from barley in order to optimize the overall process. Two different treatments were studied separately: ultrasounds, to enhance the final extraction yield, and ethanol to increase the molecular weight of those β-glucans extracted. Barley flour was boiled with ethanol (80%, v/v), prior to extraction stage, for two hours. According to the results obtained by size exclusion chromatography, this treatment increased the molecular weight of the polymers from 32000 Dalton to 717000 Dalton, leading to much higher viscosity solutions. When the use of ultrasounds as pretreatment was tried, extraction yield was increased dramatically in all the tests carried out (9 different ultrasound conditions were tested). In all cases β-glucan extraction yield was over 80% (compared to 47,2±2,4% obtained when barley was not sonicated). The best result was obtained when barley was sonicated for 30 seconds, at 78% of the total power of the ultrasound equipment (yield 87,7±2,5%).

1. Introduction

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, 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, 2004*). The amount of nourishment that contains β-glucan as an additive has risen significantly, so the FDA (*Food and Drug Administration*, USA), apart from including β-glucan in its list of products that contribute to lowering the cholesterol level in blood, has recently indicated how to label those products that contain β-glucan.
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 fibre, nevertheless β-glucans are soluble in water.

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Extraction of β-glucans from cereals presents several difficulties. A typical extraction process involves, at least, three stages (*Laroche, 2007*): (1) pretreatment of raw cereal, (2) extraction of β-glucans with suitable solvent and conditions, and finally, (3) a purification-isolation step. Each stage, in turn, involves different steps, increasing both the complexity and the economical costs of the whole process. This is the most limiting factor for the common β-glucan extraction processes.

Pretreatment stage can pursue different objectives: first, to deactivate the endogenous enzymes (β-glucanases) causing the depolymerisation of β-glucans. A second objective is to facilitate the later extraction stage, increasing the overall yield of the process: physical treatments, such as sonicating the barley flour suspended in water prior to the extraction stage. This treatment can contribute to free easily β-glucans into the solvent. Finally, a special milling of the barley can be done in order to produce milling fractions enriched in β-glucans (*Kiryluk, 2000*), increasing the amount of β-glucans extracted.

All these pretreatments can lead to enhance the performance of the β-glucan extraction process. In this work two different pretreatments were studied. On one hand the effect that ethanol treatment has on the molecular weight of the polymer extracted and, on the other hand, the consequences of sonicating the barley on the final extraction yield.

2. Experimental section

2.1 Materials and methods

A waxy hull-less barley (*H13*, harvested in 2006) was used in this work. It was supplied by ITACYL (*Instituto Tecnológico Agrario de Castilla y León*; Valladolid, Spain) and milled to pass through a 500μm sieve. Chemical composition of this variety can be observed in table 1. Ethanol used for the pretreatment was 96% (v/v; analytical grade) supplied by *Panreac* (Spain).

Table 1. Chemical composition (%) of barley

Variety	Moisture	Starch	β-glucan	Fats	Dietetic Fibre	Protein	Ash
H13-06	11,07	52	4,13	2,26	13,85	16,7	1,78

2.2 Chemical analysis

β-glucan determination was done using the "Mixed linkage β-glucan assay kit" (*Megazyme,* Ireland), and starch dissolved was determine using a modification of the "Total Stach assay kit" (*Megazyme,* Ireland) suggested by Megazyme.

Molecular weight of β-glucans was determined by Size Exclusion Chromatography (HPLC-SEC). The chromatography system consisted of an isocratic pump (*Waters 1515*), an automatic injector (*Waters 717*), a guard column (*Waters Ultrahydrogel Guard Colum*n) a SEC column (*Waters Ultrahydrogel 1000*: 7,8x300mm; pore size of 1000Å; exclusion limit $1x10^6$ Dalton) and finally a differential refractive index detector (*Waters 410*). The column was kept at 35ºC, and flow rate of the mobile phase (0,1M $NaNO_3$+0,02% NaN_3) was set at 0,6ml·min^{-1}. The β-glucan molecular weight standards were purchased from *Megazyme* (Ireland).

2.3 Experimental procedure

2.3.1 Batch procedure for extraction of β-glucans

In each experiment, 15 grams of barley were suspended vigorously (multistirrer plate *Fisher Scientific*, USA) in 150ml of deionized water for 3 hours at 55ºC in a water bath (*Ecoline Staredition E100,* Lauda, Germany). After the extraction, the mixture was centrifuged for 10 minutes at 5500rpm (*Kubota 5100*, Japan). Solid material was discarded, while liquid extract was kept at 4ºC. All the extraction experiments were carried out in duplicate.

2.3.2 Endogenous enzyme deactivation by the use of ethanol

As previous step to the extraction, 75g of barley flour (H13-06) were suspended in 750ml of ethanol (80% v/v) and boiled under reflux for two hours. After the treatment, the barley and ethanol were separated. The barley was dried at 50ºC and milled again in order to get homogeneous flour. Subsequently, extraction process was carried out, according the procedure described in section 2.c.(1). When this treatment was done, experiment was completed with the purification and isolation of β-glucans. Liquid extract was incubated at 96ºC with α-amylase thermostable (BIALFA T, kindly provided by *Biocon Española,* Spain) for one hour. After centrifugation, an equal volume of ethanol (96% v/v) was added to supernatants in order to precipitate β-glucans. After having stirred the solution for two hours, the white solid precipitate obtained was separated from the liquid by vacuum filtration. The gum obtained was dried overnight at 60ºC. Once dried, gum was milled, accurately weighted and kept in a sealed glass tube until the moment of being analysed.

2.3.3 Sonication of barley prior to extraction

The second part of the pretreatment experimentation consisted of sonicating the barley prior to extraction. Firstly, barley flour (H13-06) not pretreated with ethanol, was weight (15g) and suspended in water (150ml). Then it was sonicated by means of 130W Ultrasonic Processor (*Bioblock Science*, France). Nine different combinations of time of sonication and power were tested. The sonication times tried out were: 15, 30 and 60 seconds. The power of sonication studied was 78, 85 and 92% of the total power output of the equipment. After the sonication step, extraction was carried out in the conditions described in section 2.c.(1). When sonication was the selected pretreatment, no further purification or isolation step was performed. In all the samples the concentration of β-glucan and starch in the liquid extract, as well as the energy supplied by the ultrasound equipment to the suspended barley, was measured.

3. Results and discussion

3.1 Deactivation of endogenous enzymes by action of ethanol

The use of ethanol deactivated most of the β-glucanases present in the raw cereal. The barley refluxed with ethanol was extracted, leading to a β-glucan extraction yield of 37,7%, slightly lower than that achieved when barley was not boiled with ethanol (47,2%). Starch concentration present in the liquid extract was much higher in the case of barley treated with ethanol (0,313g/l vs 0,123g/l when barley was not). Viscosity of

the liquid extract was very high, being difficult to keep the barley in suspension. A purification-isolation step was performed, leading to a gum rich in β-glucan. The composition and molecular weight of the polymers extracted from this gum can be observed in table 2.

Table 2. Composition of liquid and solid extract obtained from barley pretreated and not pretreated with ethanol

	Liquid Extract			Solid Extract[1]		
	Extraction (%)	**Starch (g/l)**	**MW[2] (Da)**	**β-glucan (%)**	**Starch (%)**	**MW (Da)**
Barley NOT pretreated	47,2	0,123	<20000	38,9	2,1	32000
Barley pretreated	37,7	0,313	596000	39,9	3,3	717000

1. *Solid extract obtained after purification-isolation step from liquid extract, by addition of ethanol, immediately after the extraction stage.*

2. *Molecular weight measured after having let the liquid extract for 8 days at 4ºC.*

According to table 2, this treatment decreased almost completely the activity of the β-glucanases during the extraction stage, leading to a polymer 30 times higher quality in terms of molecular weight. However, in spite of the treatment of barley with ethanol, some residual activity of the β-glucanases was still present in the liquid extract, causing as a result, a partial depolymerisation of the β-glucans dissolved (MW decreased from 717000 to 596000 Dalton in eight days). These results make necessary to perform a purification-isolation step immediately after the extraction stage to prevent the action of residual β-glucanases present in the liquid.

3.2 Effect of ultrasounds on extractability of β-glucans

The use of ultrasounds as pretreatment had a pronounced effect on the amount of β-glucans extracted. It was observed that short sonication times (15 seconds) led to extraction yields strongly influenced by the power of sonication selected. For instance, a power of 78% gave an extraction yield of 87,7±2,5%, while at 85% the result was 75,2±4,2%. A further increase in the treatment length, up to 30 seconds, showed a decrease in the extraction yield differences, compared to those observed at 15 seconds. Finally when the treatment lasted 60 seconds, no difference was observed in extraction yield, becoming independent of the power selected.

Thirty seconds was seen to be the optimal length for this pretreatment, in spite of the power selected: all the sonication powers tried gave the maximum extractability when they were applied for 30 seconds. In addition, 60 seconds treatment produced a decrease in the amount of β-glucan extracted: in these conditions an irreversible damage of the polymers could be produced as a consequence of both the temperatures achieved during the sonication process and the high energy ultrasounds.

This phenomenon was also observed in the case of starch, even more accentuated. Starch concentration was measured in each liquid extract after the extraction. A higher

decrease in starch concentration was observed when the treatment was more aggressive (the lowest concentration of starch was measured when the treatment was applied in the most extreme conditions: 92% for 60 seconds, in this case the energy involved in the treatment was 320 joules). However, at 78% not very remarkable difference in the amount of starch dissolved was observed: starch dissolved remained constant.
All these results are summarized in table 3.

Table 3. Results of the application of ultrasounds as pretreatment in the extraction yield of β-glucans when barley H13 was used as raw material

Power (%)	Length (s)	Extraction Yield (%)	Starch (g/L)	Energy[1] (Joule)
78	15	81,6±0,0	0,151	61
	30	87,7±2,5	0,146	126
	60	82,9±5,2	0,147	231
85	15	75,2±4,2	0,128	75
	30	83,8±3,0	0,061	152
	60	83,3±0,2	0,077	296
92	15	84,1±3,7	0,143	80
	30	85,2±2,2	0,081	155
	60	83,4±5,9	0,041	320

[1.] *Energy supplied by the ultrasounds processor to the barley suspended in water.*

4. Conclusions

Two different treatments were tried in order to improve the performance of the β-glucan extraction process. Boiling barley with ethanol, previously to the extraction, deactivate most of the β-glucanases, responsible for the depolymerisation of β-glucans. The result was a polymer extracted with MW higher than 700000 Dalton. Nevertheless, barley pretreated with ethanol showed to have lower β-glucan extractability: extraction yield was reduced for almost 20%, from 47,2%. This was probably due to the difficulty for keeping the barley in suspension into the solvent: viscosity was much higher because of the presence in the liquid of very high molecular weight β-glucan and greater starch concentration. In further work the stirring system will have to be improved.
The physical treatment used, ultrasounds, led to very high extraction yields in all the conditions tried. In all cases extraction yield was above 80%, which represents a very significant improvement of the overall extraction process. The best conditions were established as: 78% of the total power for 30 seconds, with 126 joules of energy applied to the barley. It was also seen that ultrasounds can break other polymers present in the media, such as starch: the higher energy barley receives, the lower concentration of starch dissolved.

In the future the effect that ultrasounds have on the MW of the β-glucan extracted will have to be tested. To do this, an experiment combining the two pretreatments tried in this work will be carried out: barley refluxed with ethanol will be sonicated in one experiment and not in the other. Then extraction will be performed, including the final purification-isolation step. The molecular weight of the polymers present in the final purified solid extract will be measured, and conclusions will be made.

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