

PRESSURIZED HOT WATER EXTRACTION OF β -GLUCANS FROM BARLEY

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Abstract. Pressurized hot water (PHW) has been used for the extraction of β -glucans from waxy barley (6.0% β -glucan). The effect of operational parameters has been evaluated in terms of extraction yield, β -glucan molecular weight (MW), and formation of by-products during extraction. Temperature has been varied in the range 110-180°C, for extraction times in the range 15 to 45 min. The highest extraction yield was 54.0% (MW of 160kDa), achieved at 157.5°C and after 45 minutes. In order to maximize both extraction yield and MW, slightly different conditions were found: 155°C, 18 minutes at 50bar, leading to a 53.7% extraction yield and 200kDa.

The comparison between the optimal results obtained in the PHW process (155°C, 18min, 20 bar) and the conventional process (55°C, 3h, atmospheric pressure) revealed a lower extraction yield (53.7% vs. 73.2%) for the PHW; nevertheless MW of the extracted β -glucan resulted to be almost four times higher (200kDa vs. 55kDa). The presence of the sugar monomers degradation products, such as HMF, was only detected in the highest intensity conditions (calculated according to the severity factor) but not in the optimal conditions. This certified that PHW can be used to extract β -glucans from barley as they were not degraded under the conditions proposed as optimal. PHW presents extraction time reduction and increase of the β -glucan MW as major benefits.

Keywords: β -glucan, barley, extraction, pressurized hot water, polysaccharide

1. Introduction

β -glucans (mixed linkage (1-3),(1-4)- β -D-glucans are non-starchy, non-digestible and glucose based polysaccharides that can be found in the subaleurone layer and cell walls of different cereal grains, such as barley, oat rye or wheat [1]. The presence of the mixed linkages determines the final physical properties of the β -glucans, such as solubility and viscosity. Moreover, molecular weight and concentration affect the viscosity of the β -glucan solution [2]. β -glucans are known to be involved in process related to the reduction in postprandial blood glucose and cholesterol levels [3,4]. Recently FDA (United States Federal Drug Administration) and EFSA (European Food Safe Authority) have accepted a health claim in reduction of cholesterol levels, recommending an intake of 3 g of β -glucans per day to achieve this positive effect [5, 6]. In order to use the beneficial effects of cereal β -glucans for functional food products, beta-glucan preparations with high β -glucan content and high molecular weight have to be available for the food industry at a reasonable prize.

The extraction of β -glucans from a cereal matrix (such as barley) is complex since they are a minority component of the cereal grain, the used solvents (water at ambient pressure) are not selective and the solid/liquid extraction exhibits important mass transfer limitations [7]. Conventional extraction processes lead to a low molecular weight β -glucan product, due to the activity of endogenous β -glucanases responsible of the polysaccharide backbone rupture. When a pretreatment is implemented to increase the molecular weight (such

as enzymatic ethanol deactivation) [8], the extraction yield decreases dramatically. All the described reasons make desirable to develop an extraction procedure with a high extraction yield that allows preserving β -glucan molecular weight in a one single step (without pretreatment). In that sense, the use of Pressurized Hot Water (PHW) as extraction solvent can be a new, versatile and promising technology for this process, since it can provide the medium for β -glucanases inactivation together with an efficient extraction solvent. Considered by many authors as a “green solvent” [9], PHW has been widely used as solvent in the extraction of high added value compounds (bioactive and nutraceuticals, essential oils, lipids, carotenoids, proteins, polysaccharides) from plants or food matrixes [10-12]. PHW term refers to the water in liquid state in the range 100°C (boiling point) to 374°C (critical point) by the application of pressure. Density, surface tension, viscosity and diffusion of water change dramatically when changing pressure and, specially, temperature, having an effect on the mass transfer of the extraction process. PHW exhibits lower viscosity but higher diffusivity than water at room temperature, which favors the diffusion into the vegetal matrix and the release of compounds [11]. An increase in temperature also contributes to weaken the hydrogen bonds between the carbohydrates and the solid matrix, accelerating the compound desorption. Moreover, high temperatures can contribute either to initiate hydrolysis processes of the already dissolved compounds or to affect the structure of the natural matrix [10]. In the case of polysaccharides, they are broken into their monomers, and these units can then react to form their main degradation products, such as furfural (from pentoses) or 5-(Hydroxymethyl)furfural (HMF), from hexoses [13]. This phenomenon of monosaccharide degradation begins to be significant above 180°C [14]. The final performance in the β -glucan extraction will be a combination of time and temperature; in order to evaluate the simultaneous effect of time and temperature a Severity Factor (R_0) is introduced (1) to compare different treatments:

$$R_0 = t \cdot \exp\left(\frac{T - 100}{14.75}\right) \quad (1)$$

where t is time in minutes and T is temperature in °C. This severity factor was introduced by Overend et al. [15], and was used to evaluate hydrolysis reactions by Schacht et al. [16].

The effect of pressure on the physical properties of PHW is very limited compared to that of temperature. In general, liquids are highly incompressible in the subcritical region, and density and solvation power suffer slight increases with pressure at constant temperature. These smooth variations in the physical properties make the pressure to play a secondary role in the extraction process, limited to maintain the water in the liquid form [11], and in most extraction experiments of natural products in the literature, pressure is kept around 50 bar to ensure the liquid state. Moreover, given that the pressure is not critical for the extraction, it is not convenient to increase it very much, since the higher the pressure the more tighten the solid matrix, hindering the flow in fixed bed extraction processes [10].

PHW has been used in a few works to extract polysaccharides from natural matrix: Ho et al. [17] extracted lignans, proteins and carbohydrates from flaxseed meal; Buranov and Mazza [18] extracted hemicelluloses from flax shives; Askin et al. [19] extracted (1-3),(1-6)- β -D-glucans from *Ganoderma lucidum* fungus; Cacace and Mazza [20] used PHW to extract lignans from whole flaxseed; Hata et al. [21] focused their research in the extraction of total sugars, proteins and antioxidant activity from defatted rice bran and Song et al. [22] extracted hemicelluloses from spruce woods. When the raw material is rich in polysaccharides (cellulose or hemicelluloses) the purpose of the PHW treatment is not only the extraction of polysaccharides, but also their subsequent hydrolysis into their monomeric-sugars, as basis for the production of biofuels [22], and in those cases terms such as “autohydrolysis” or “hydrothermal treatment” are used in the literature.

The purpose of the present work was to study the effect of operational variables in the extraction of β -glucans from barley using pressurized hot water as solvent. The influence of temperature and time on extraction yield and molecular weight of the extracted β -glucans have been evaluated, together with the degradation products formed as a consequence of the extraction conditions.

2. Materials and methods

2.1. Raw Material

A waxy barley developed, grown and provided by ITACyL (Instituto Tecnológico Agrario de Castilla y León, Valladolid, Spain) has been used in this work as raw material. Complete composition of barley is shown in table 1, being the most significant features the content in β -glucan ($6.0\pm0.3\%$) and in starch ($50.3\pm3.2\%$). Barley was fine milled in the lab, resulting in an average particle size of the $240\pm4\ \mu\text{m}$ determined by DLS (Mastersizer2000, Malvern Instruments Ltd).

Table 1. Composition of barley used as raw material, (%) expressed in dry basis

Fats	Ash	Proteins	Starch	β -glucan	Dietary Fibre
2.8 ± 0.2	2.1 ± 0.3	20.0 ± 1.2	50.3 ± 3.2	6.0 ± 0.3	21.0 ± 2.1

2.2. Experimental Section

Extractions have been performed in a High-Pressure View Chamber Type HPVC 300-HT (Eurotechnica GmbH) made of inconel with an internal volume of 30mL and stirred via a magnetically coupled agitator. Maximum operating pressure and temperature of this equipment are 300 bar and 500°C , respectively. A picture of the experimental set-up is shown in figure 1. In every experiment, 1g of barley flour is located inside the high-pressure cell through one of the sapphire windows and after its closure, the chamber is filled up with distilled water and heated up. Once the desired values of pressure and temperature are reached, vigorous stirring is switched-on in order to keep barley flour in suspension. After the desired extraction time, liquid phase is sampled for analysis using the sampling line, and afterwards, the cell content is cooled down and decompressed. The PHW extraction process is compared with a “conventional extraction process” where the extraction is performed with water at 55°C and ambient pressure during 3h in a mechanically stirred tank. In this “conventional extraction process” the β -glucan extraction yield was 73.2 % and the average molecular weight of the extracted β -glucans was 55 kDa. A detailed description of this “conventional process” can be found at [7].



Figure 1. Picture of the high pressure view chamber

2.3. Chemical Analysis

The liquid extract sampled from the high-pressure chamber after the desired extraction time, has been analyzed to determine β -glucan concentration, β -glucan molecular weight and the presence of sugar degradation products.

The amount of β -glucans in the liquid extract has been determined using the “Mixed-linkage β -glucan” assay kit from Megazyme International Ltd. From these assays, the concentration of β -glucans is reported in g of β -glucan/100g of water.

Molecular weight (MW) of β -glucans has been determined by Size Exclusion Chromatography (HPLC-SEC) using a chromatographic system composed by an isocratic pump (Waters 1515), an automatic injector (Waters 717), and a differential refractive index detector (Waters 410). A combination of guard column (ShodexSB-G) and a GPC column (ShodexOHpak SB-804HQ) has been used for the SEC analysis. The column was kept at 35°C, and the flow rate of the mobile phase (0.1M NaNO₃ + 0.02% NaN₃) was set at 0.5 ml·min⁻¹. The β -glucan molecular weight standards were purchased from Megazyme International Ltd. (Ireland), and were in the range 40–359kDa.

HPLC has been used for the measurement of sugar degradation products (pyruvaldehyde and HMF) formed during the extraction process. Chromatographic system was basically the same as that described for the MW determination, but in this case a UV/Visible Detector (Waters 2489) has been used. Column and guard column were purchased to Shodex (model KS-802, guard column KS-G) and set at 60°C, and mobile phase (MilliQ water) was set at 0.8 ml·min⁻¹.

2.4. Experimental Design

In all the experiments presented in this work, pressure was set at 20bar and temperature and extraction time were the selected factors to study the PHW extraction process. A full factorial design approach was used to determine the influence of those two variables at three different levels. Temperature was studied in the range 135-180°C and time was varied from 15 to 75min. Extraction results have been evaluated in terms of β -glucan extraction yield, β -glucan Molecular Weight (MW) and degradation products. All the calculations and statistical analysis were done by means of the Statgraphics Statistical Software. A total of nine experiments were carried out plus three repetitions of the central experiment. The set of experiments and the main experimental results are presented in table 2.

An analysis of variance (ANOVA) with 95% confidence level was then carried out for the response variable in order to test the model significance and suitability. The significance of each coefficient was determined using the F-test. Data corresponding to the ANOVA test are not shown in this work.

3. Results and discussion

3.1. Effect of temperature and time on the extraction of β -glucans with PHW

Table 2 summarizes the operational conditions and the main results of the experiments performed to study the effect of temperature and time in the extraction of β -glucans from barley with PHW. Extraction yield, together with MW and degradation products concentration are shown in table 2.

Table 2. Experimental conditions and experimental results: extraction yield, β -glucan molecular weight and concentration of the main hydrolysis products. (The logarithm of the severity factor is also shown)

Extraction Conditions			β -glucan		Concentration (ppm)	
T (°C)	t (min)	Log R ₀	Ext. Yield (%)	MW (kDa)	Pyruvaldehyde	HMF
135.0	15	2.207	43.8	186	nd	nd
135.0	45	2.684	41.5	154	nd	nd
135.0	75	2.906	33.9	129	nd	nd
157.5	15	2.943	50.8	228	2.1	0.6
157.5	45	3.420	54.0	160	5.6	8.3
157.5	75	3.642	41.9	62	7.6	30.5
180.0	15	3.532	37.4	86	11.8	19.8
180.0	45	4.009	33.9	47	26.3	38.2
180.0	75	4.231	23.4	26	41.8	62.5

nd: not detected

In order to evaluate the effect of each variable, as well as the interaction between these two experimental variables, the analysis of variance test (ANOVA) was done by means of the Statistical Software Statgraphics. According to this ANOVA test, the effect of temperature and extraction time on the extraction yield was statistically significant, since F-ratio was higher than the critical value of 4.96, at a 95% confidence level.

Effect of temperature and time on β -glucan extraction yield. Temperature and extraction time have shown a dramatic effect on the β -glucan extraction yield. Temperature affects the extraction process in two different ways: for temperatures up to 155-160°C the extraction yield increases with temperature, however, above 160°C the amount of dissolved β -glucans in the liquid extract decreases. The change in the properties of the water when temperature is increased up to 160°C helps to weaken the bonds between the target polysaccharide and the barley, promoting β -glucan release from the cereal matrix. However, further increases in temperature mean a decrease in the concentration of β -glucan in the final extract and an increase in the concentration of sugar degradation compounds. Hydrolysis kinetics is dramatically accelerated, being this effect dominant over the increase in the solubility.

In addition to high temperatures, long times cause a rapid decrease in the β -glucan concentration in the liquid extract. The longer the β -glucan was exposed to the high temperatures the higher is the degradation. The effect of time and temperature on the extraction yield can be observed in figure 2.

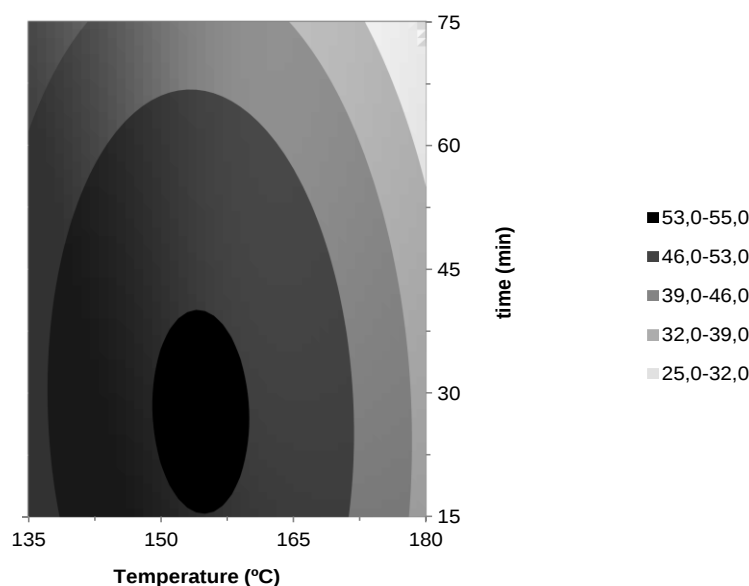


Figure 2. Contour plot for the effect of temperature and time on β -glucan extraction yield at 20bar.

In order to evaluate the simultaneous effect of the two studied factors on β -glucan extraction, the severity factor (equation (1)) has been introduced. In figure 3 extraction yield is plotted versus the logarithm of the severity factor. It can be observed that there is a critical combination of temperature and time that causes the concentration of β -glucans in the liquid extract begin to decline. This critical value of the logarithm of the severity factor is between 3 and 3.5 (that corresponds to temperatures of 157.5°C and times between 20 and 65 minutes).

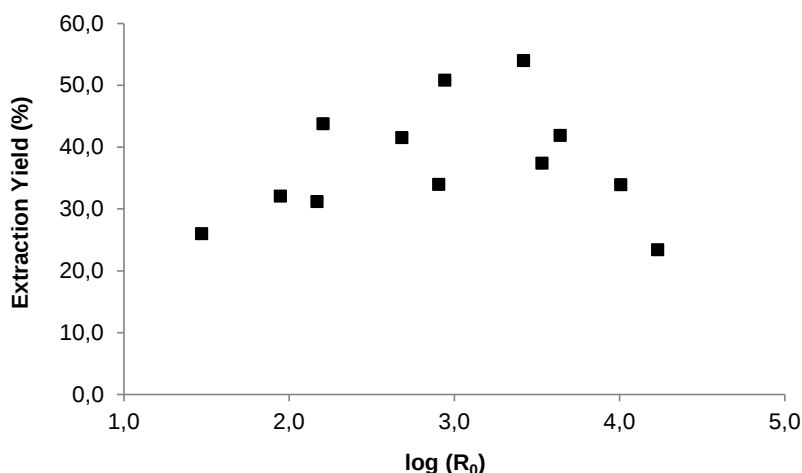


Figure 3. Simultaneous effect of temperature and extraction time in β -glucan extraction yield. P = 20 bar

Effect of temperature and time on β -glucan molecular weight. The molecular weight of the extracted β -glucans is clearly affected by the extraction conditions. The results presented in table 2 show that the extraction carried out at 157.5°C for 15 minutes led to the highest molecular weight (228kDa). An increase of severity of the extraction conditions causes a significant loss of molecular weight. These trends can be easily observed in figure 4. According to figure 4, the highest molecular weights are obtained after short extraction times with temperatures up to 157.5°C. However, when temperature is further increased, a rapid decrease in the MW is observed. This means that β -glucan molecules are temperature resistant up to temperatures in the range 155-160°C. On the other hand, the time that β -glucans are exposed to the PHW is crucial for the molecular weight. Once the β -glucan is dissolved, the depolymerization (evaluated in terms of MW) takes place. The longer the β -glucan is exposed to the hot water, the higher the decrease in the MW. This discussion is also supported with the analysis of the hydrolysis products presented in section 3.1.3.

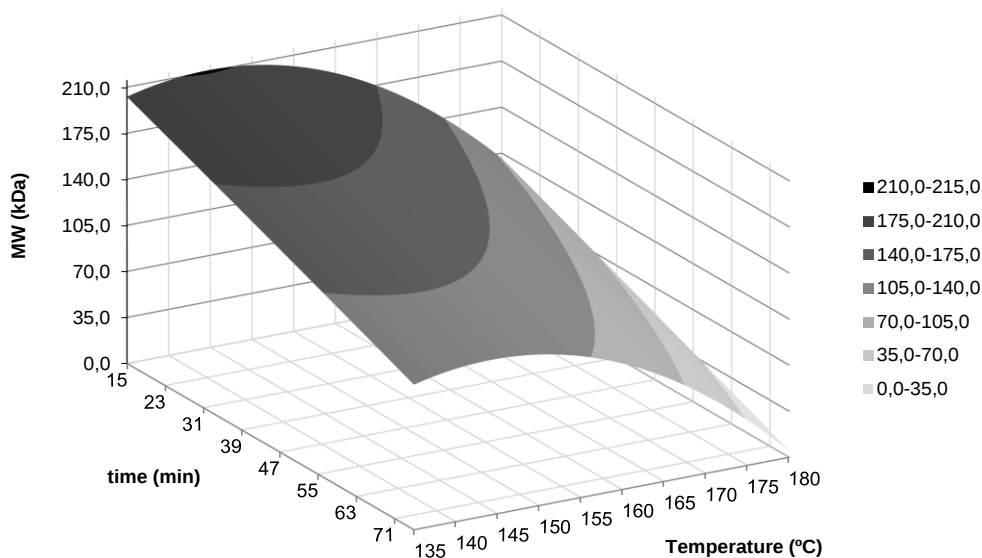


Figure 4. Surface plot for the MW of the extracted β -glucan as function of the extraction conditions

Effect of temperature and time on degradation products. In sections 3.1.1. and 3.1.2., the effect of temperature and time on β -glucan extraction yield and their molecular weight has been discussed. To complete this discussion it is necessary to evaluate the degradation products that appear during the extraction with PHW. This hydrolysis process is very complex, as there is a huge number of species taking part and different types of reactions (hydrolysis, isomerization, bond cleavage, dehydration) are involved [23]. So in this work only the final hexose degradation products (pyruvaldehyde and HMF) will be shown. The concentration of these two species is represented in figure 5:

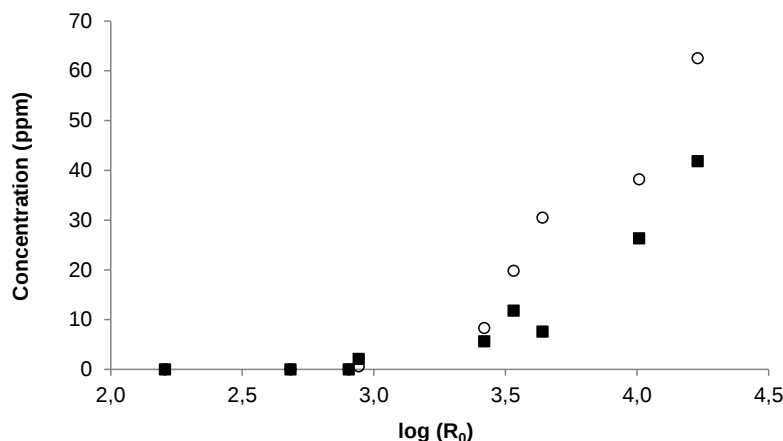


Figure 5. Degradation products determined during the extraction of β -glucans from barley using PHW as solvent. KEY: pyruvaldehyde (■) and HMF (○)

From figure 5 it can be concluded that the final sugar degradation products (pyruvaldehyde and HMF) only appear when severe extraction conditions are used ($\log R_0 > 3.4$). Conditions more severe than those, lead to an exponential increase in the concentration of those two compounds. This conditions of $\log(R_0)$ correspond to an extraction performed at 157.5°C and 45 min, indicating that higher temperatures not only do not help to extract β -glucans, but also accelerate their decomposition, as it was seen in figure 3 where the concentration of β -glucans decrease when $\log R_0$ is higher than 3.4.

3.3. Optimization of the PHW extraction process and comparison with “conventional extraction”

If the MW and the extraction yield results are simultaneously evaluated, it can be seen that the conditions in which extraction yield and molecular weight are maximum are close. At low temperatures, β -glucans require long times to be dissolved; but long extraction times affect the β -glucan MW, as it has been previously described. At high temperatures, β -glucans are dissolved in shorter times; but for temperatures higher than 160°C the dissolved β -glucans can be easily depolymerized, resulting in a low extraction yield and low molecular weight. According to these results the extraction has to be performed at medium temperatures and relatively short times. Both, molecular weight and extraction yield, are maximized for temperatures in the range 152-155°C and extraction times in the range 17-20 minutes, and pressure of 20 bar. The logarithm of the severity factor calculated for those conditions (155°C, 18 min) is 2.87. Under these conditions, a confirmatory experiment was run: the extraction yield was 53.7 % and average molecular weight resulted to be 200 kDa.

If these results are compared with that obtained with a “conventional extraction process” (described in section 2.2), it can be seen that the molecular weight of β -glucans extracted with PHW is very high compared to that obtained in the “conventional extraction process” (only 55 kDa), in a one single step process, and in a much shorter extraction time (18 min vs. 3 h). Therefore, the use of PHW as extraction solvent for β -glucans from barley represents a real intensification of the process. Water at high temperatures helps to destroy the interactions and bonds between polysaccharides and matrix, favoring the release of β -glucans to the solvent. Under the selected conditions, β -glucanases suffer thermal degradation losing their activity, and β -glucans are not broken down because of their activity. In the PHW extraction, molecular weight is decreased by the

combination of temperature and time once the β -glucans are in solution in the PHW. Therefore, at 155°C and 18 min, extraction is efficient enough and depolymerization and degradation are controlled.

4. Conclusions

It can be concluded from this study that PHW is a suitable solvent to extract β -glucans from barley, by reducing dramatically the extraction time and by increasing the molecular weight by more than three times compared to the conventional process. There is a practical limit in the extraction of β -glucan; around 155-160°C and 45 minutes. Above these values the degradation of β -glucans begins to be more important than the amount of extracted β -glucan, as probes the rapid increase in the sugar degradation products detected and the depletion of β -glucan concentration observed.

The optimal conditions to extract β -glucans, high extraction yield and high molecular weight, were 155°C, 18 minutes and 50 bar, with a extraction yield of 53,7%, a molecular weight of 200 kDa and degradation products such as HMF were not detected.

Acknowledgments

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Abbreviations

β -glucans: (1 $\rightarrow$ 3), (1 $\rightarrow$ 4)- β -D-glucan
HMF: 5-(Hydroxymethyl)furfural
MW: Molecular Weight
PHW: Pressurized hot water
 R_0 : Severity factor, defined by equation (1)

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