

OPTIMIZATION OF THE EXTRACTION PROCESS OF BETA-GLUCANS FROM BARLEY

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Abstract

β -glucans are non-starchy polysaccharides composed of (1-3), (1-4) mixed linked glucose polymers. They are located mainly in the cell walls of the endosperm and the aleurone layer of different cereal grains, specially oat and barleys. In general, β -glucans are a major component of the soluble dietary fibre and they influence the nutritional values and functional properties of food. Recently, several studies have revealed the benefits of including β -glucans into the diet to the human health, such as cholesterol diminution in blood, and reduction of risk of cancer, coronary heart disease, and diabetes. Although there are several papers related to the extraction and purification of β -glucans from cereals, most of them describe extraction and purification methods at a laboratory scale, which involve a significant number and time-consuming steps. Moreover, since the β -glucan content in barley grains is low (<5%) it is necessary to develop and optimise an extraction/purification method that allow reducing operation times and reactive consumption, to make competitive this product at a big scale.

The aim of our research is focused on three different steps: (1) Raw material pre-treatment, (2) Extraction step, and (3) Purification step. Discussion of experimental results is made in terms of β -glucan and starch content in the extracts, and molecular weight of the extracted β -glucans.

- 1) Several alternatives have been studied for the pre-treatment of the barley flour: ultrasonic treatment with different energy powers, enzyme deactivation and microwaves.
- 2) A systematic study of the extraction variables such as: temperature, pH, extraction time, solvent ratio and solvent polarity have been performed in order to optimize the operational extraction conditions. Extraction with subcritical water has also been studied.
- 3) Two different techniques have been studied and compared for the precipitation step: precipitation with alcoholic antisolvents, and precipitation with supercritical CO₂.

In this work the effect of operating parameters on the extraction of β -glucans from different varieties of barley is analyzed. Several experiences have been carried out in batch extractors, with a reference experiment, in order to check the influence of temperature, pH, time, solvent and flour:solvent ratio. Time and temperature showed to have a strongly marked influence: the higher temperature, the higher extraction rate. Higher temperatures led to improve the extraction yield, increasing the amount of starch co-extracted. Extraction time was a critical factor up to 3 hours. Longer times did not increase the amount extracted. The influence of pH was not very remarkable, just generating a slight increase in extraction yield up to 8; beginning to decrease from that value. The use of alcohols (methanol and ethanol) as solvents, did not improve the extraction rate. On the other hand, it was observed an important decrease on the β -glucan solubility, greater the higher concentration of alcohol employed and the longer carbon chain. Finally, a extraction yield of 40-50% was obtained when operating at solvent:flour ratio from 7 to 12, at 55°C, in almost all range of pH, when lasting the extraction process more than 2,5 hours.

1. Introduction

β -glucans are a kind of non-starchy polysaccharides (formed by the linkage of glucose units via β -(1 \rightarrow 3) (approximately 30%) and β -(1 \rightarrow 4)-glucosidic linkages) that can be found in several kinds of cereals, such as barley, oat or rye in concentrations from 2 to 12% in dry basis. In barley, β -glucans are minor components (up to 6-7%), located majoritary in the aleurone and endosperm cellwalls of the grain [1]. They form part of the dietetic fibre (the same as other structural compounds of vegetals, such as cellulose and hemicelluloses, nevertheless β -glucans are soluble in water.

It is known that they have good properties for human health, if eaten. Among all these properties it is worth mentioning their implication in process of control of cholesterol and glucose concentration in blood [2,3,4]. These effects have been associated with the molecular weight of the polymers. Recently, FDA (Food and Drug Administration, USA) has included β -glucan in its list of products that contribute to lower the cholesterol level in blood, pointing out how to label the products that contain barley to remark its positive effect on health.

Extraction of β -glucans form cereals has to deal with several difficulties. A typical extraction process involves, at least, three stages [5,6]: inactivation of endogenous enzymes, extraction of β -glucans with a suitable solvent, and finally a purification-isolation stage, because other chemicals present in cereals (proteins, saccharides, or starch) can be co-extracted. All these stages increase the complexity of the overall process, increasing the economical costs, therefore. This is the most limiting factor for the extraction process.

When a solid-liquid extraction process is carried out, temperature, pH, extraction time and polarity of the solvent are some of the operating parameters that are to be taken into account. It is important to characterise the behaviour of the target compound when changing the operating variables, in order to improve the extraction process.

In this work a simple solid-liquid discontinuous extraction process from different waxy-type barley (H13-06 (hull-less) and D24-06) with hot water was proposed. The effect of most important operating parameters such as temperature, pH, extraction time and polarity of the solvent was checked in order to optimize the continuos extraction process. Moreover, a reference experience based on literature revision carried out at 55°C, 3 hours, water as solvent, solid:liquid ratio 1:10 was chosen as a comparative tool [7].

2. Experimental section

Two different kinds of barley were used, both of them grown and harvested in Castilla y León (Spain): H13-06 and D24-06. These varieties correspond to the waxy-type barley, a specie of barley which has higher content in β -glucan and a lower in starch than common species. The main difference between those two kinds of barley is that grains of H13 are hullless, not presenting external hull covering the kernel. Barley was supplied by Instituto Tecnológico Agrario de Castilla y León (ITACYL). Table 1 summarizes the composition of both varieties:

Table 1. Chemical composition of the barley used as raw material

	H13-2006 (%)	D24-2006 (%)
Moisture	11,07	10,98
Fats	2,26	2,48
Ash	1,78	1,86
Proteines	4,9	4,3
Starch	52	44,8
Amilose/Amilopectine	10,22/89,78	9,05/90,95
β -Glucan	4,13	5,33
Dietic Fibre	13,85	18,7

Barley employed was milled and sieved through a 0,5mm sieve.

Initially, temperature, pH, time, solvent, flour:solvent ratio were defined as the factors that had to be tested. According to the literature, basic conditions (of temperature) for the initial experiments were selected: 55°C, 3 hours, water as solvent, solid:liquid ratio 1:10 (these conditions constitute the basic experiment). In this reference experiment, pH of water was not modified, being around 6.

Barley flour was weighted and added to an erlenmeyer flask. Then water was added, being the flour suspended at high stirring rate. Temperature was kept constant in a water bath (Lauda Ecoline Staredition E100, Lauda, Germany). Stirring was obtained with a multistirrer plate (Fisher Scientific, USA), as shown in figure 1:

Once the extraction process was finished, solid:liquid separation was done by centrifugation (5500rpm, 10 minutes). Solid material was discarded and liquid (supernatant) was stored at 4°C until the moment of being analysed.

All the experiments were conducted in duplicate.



Figure 1. Experimental set-up used for the extraction of β -glucans from barley

β -glucan determination was made at ITACYL, using the Megazyme Assay Kit for mixed linkage β -glucan analysis (Megazyme, Ireland). β -glucan concentration is expressed as % (weight) of the total sample.

Yield results shown in the following figures was calculated by dividing the amount of β -glucan in the extract generated and the total amount of β -glucans in the barley flour used in the extraction process.

3. Results

β -glucan extraction yield was $41,5\pm 1,0\%$ for D24 and $47,2\pm 2,4\%$ for H13 for the reference operating conditions. This result, contrary to that expected, can be explained for the presence of interferences. H13 is a hullless barley, with a lower content in β -glucans. D24, despite of its higher content in this compound, is more difficult to extract them, probably due to the presence of the hull of the kernel. This problem can be solved by milling the barley in a industrial mill, that generates three milling fractions, with different compositions of β -glucans.

When the extraction process was divided in three stages (of one hour each), the amount of β -glucan extracted was around 60%, a 20% higher than when developing the process in one single stage.

Several experiments were carried out in order to check the influence of temperature, pH, time, solvent and flour:solvent ratio on β -glucans extraction, changing the study variable and keeping constant the remaining ones. The reference experiment was achieved at 55°C , pH around neutrality (6), 3 hours of extraction time and with 1:10 solid:solvent ratio.

Temperature effect (35-75°C): It was observed a constant increase in the extraction yield when increasing the temperature from 29% at 35°C up to 56% at 75°C . However when temperature is higher than 55°C , operational problems connected with the higher suspension viscosity and the co-extraction of starch in the extract were observed (the amount of starch in the extract was increased dramatically, from 0,12% to 0,71%, which makes more difficult the subsequent purification [8]). For these reasons, the optimal extraction temperature was fixed in 55°C (see Figure 2).

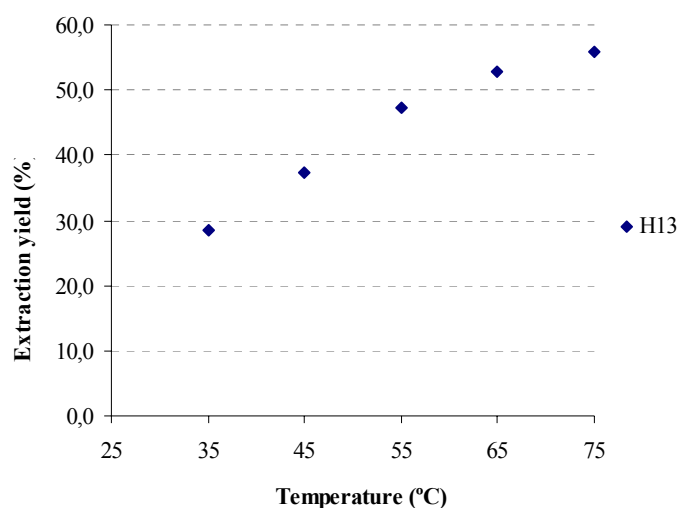


Figure 2. Temperature effect on the β -glucan extraction yield (H13 barley).

Extraction time effect (0.5-5 h). Times under 2,5 hours were found to be too short (yield fell down under 40%), but an extraction time of 5 hours did not entail a dramatic increase in the amount of β -glucan extracted, rather it kept constant (45-47%) after three hours of extraction. Extraction time of 3 hours is enough to ensure the equilibrium was reached and to get a high extraction yield (see Figure 3).

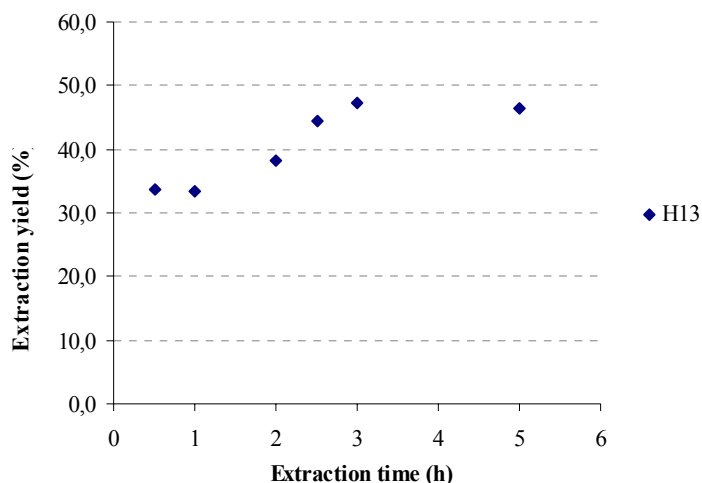


Figure 3. Effect of extraction time on the β -glucan extraction yield (H13 barley).

When the influence of pH (in the range 6-11) was studied (see Figure 4), a slight increase in extraction yield was produced. Water was weakly basified (pH 8) by addition of some drops of NaOH (6N). However, a further increase (pH 11), decreased the amount of β -glucans extracted. This decrease with pH was more marked in the case of H13 type. This could be due to the depolymerisation of our targeted compounds. This result reveals that it is not necessary to increase the pH of the original water used as solvent (close to neutral pH).

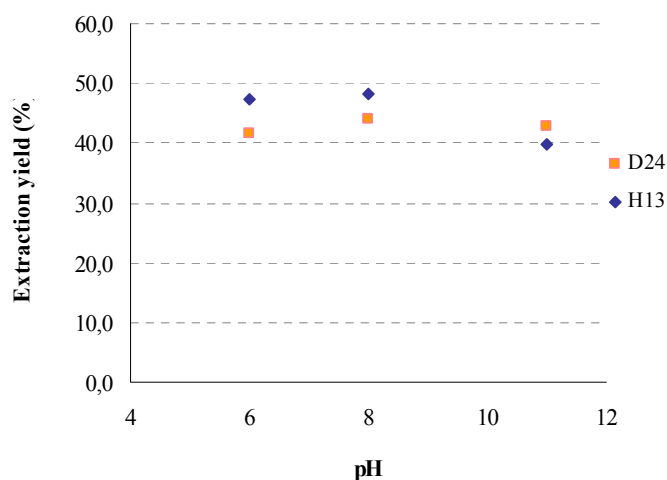


Figure 4. pH effect on β -glucan extraction yield (H13 and D24 varieties of barley).

Solid (flour):liquid ratio (in the range 5-15) was also tested for H13 variety. As it can be observed in Figure 5, an important change in extraction yield (over 50%) could be noticed when this ratio was shift from 5 to 10. The increase of this ratio to 15, did not produce a positive effect in the extraction of β -glucans. When using ratios of 8, 10 or even 12, it was observed a constant yield, around 47%. This result showed that it is possible to operate at lower solid:liquid rate (at 8), which is important taking into account a further purification step. Using less amount of solvent means simplifying the concentration of β -glucans.

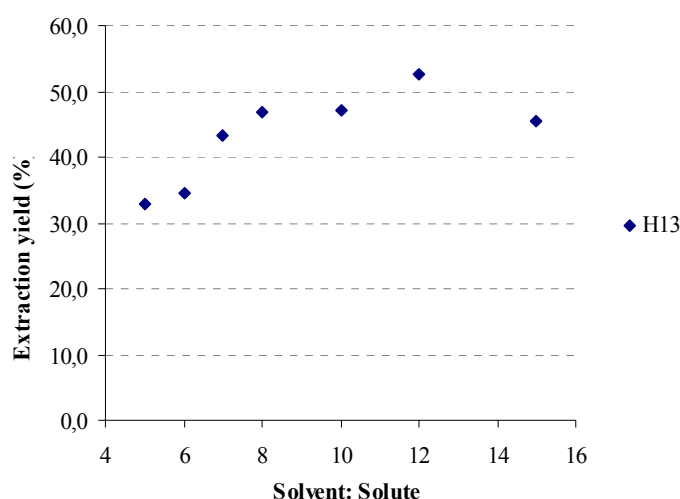


Figure 5. Effect of solvent:flour ratio on β -glucan extraction yield (H13 barley).

Polarity of the solvent can be classified as a critical parameter. This part of the study was developed using the D24 variety. The better results were obtained with water as solvent (yield was 41,5%). When an alcohol was used as solvent (5% in volume), the extraction yield decrease slightly to 41,4% in the case of the use of methanol and to 34,1% when ethanol was used. When the concentration of alcohol was increased (50% or 100%), it was not possible to extract β -glucans. The amount of polymer extracted in those cases was under the detection limit of the analysis method. According to this result, alcohols can not be used as co-solvents. This property would allow to the alcohols to be used to precipitate β -glucans, using the alcohols as anti-solvent.

4. Conclusions

Initial conditions fixed for discontinuous extraction of β -glucans from barley with water (55°C, 3h and flour:solvent ratio of 8) are suitable to get extraction yields in the range 40-50% for both varieties of barley tested. Time and temperature showed to have a strongly marked influence: the higher temperature, the higher extraction rate. Longer times than 3 hours didn't increase the amount of β -glucans extracted. The influence of pH was not very remarkable, just generating a slight increase in extraction yield up to 8; beginning to decrease from that value. The use of alcohols (methanol and ethanol) as solvents, did not improve the extraction rate. The operation in 3 steps of one hour each one, allowed to increase the maximum amount of β -glucan up to 60%. This result shows that β -glucan extraction can be enhanced when increasing the number of extraction steps, which justifies the need to develop a continuous extraction process.

5. References

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