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β-Glucan recovery from *Ganoderma lucidum* by means of pressurized hot water and supercritical CO_2

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ABSTRACT

(1,3)-(1,6)-β-D-Glucans were extracted from *Ganoderma lucidum* (34.2%, w/w) using pressurized hot water as solvent ($P = 5$ MPa) in a fixed bed laboratory scale unit. A RSM Box–Behnken experimental design was used to evaluate the effect of the temperature (135–175 °C), flow rate (0.7–1.3 mL/min) and solvent to biomass ratio (20–60 mL/g) on the extraction yield of β-glucans and the content in β-glucans of the final product. A dramatic effect of the temperature was observed: the higher the temperature, the higher the extraction yield; however, at temperatures above 158 °C the β-glucan content in the final product began to decrease. It was also seen that experiments longer than 80 min are required to get the β-glucans dissolved. Finally, the effect of the flow rate on the extraction yield was not significant, indicating that external mass transport was not controlling the extraction process. The extraction conditions that maximize both extraction yield of β-glucan (64.9 ± 0.8%) and led to the highest content in β-glucans in the final product (61.7 ± 1.0%) are 158 °C, 1.3 mL/min and 60 mL/g.

The addition of supercritical CO_2 to the pressurized hot water throughout the extraction (done at 155 °C, 1 mL/min of PHW and 40 mL/g) produced a significant improvement of the extraction yield up to 72.5% in the best tested conditions; effect primarily attributed to the acidification of the media obtained by the supercritical CO_2.

1. Introduction

(1,3)-(1,6)-β-D-Glucans are non-starchy polysaccharides that exhibit good properties regarding to inmunomodulatory activity or the control of diseases (Askin et al., 2010). In this work, (1,3)-(1,6)-β-D-glucans have been extracted from *Ganoderma lucidum* (34.2%, w/w), an extensively grown mushroom in Asia due to its reported potent bioactive properties, mainly attributed to the β-glucans. Askin et al. (2010) reported several attempts to extract these polysaccharides from *G. Lucidum* by the use of organic solvents. The use of organic solvents presents some disadvantages which are related to the need of performing a purification step to remove the organic solvent, so the polysaccharides can be used in the food industry. As an alternative solvent, pressurized hot water (PHW) can be used. PHW term refers to the water in liquid state in the range 100–374 °C (critical point) by the application of pressure higher than the vapor pressure (Kronholm et al., 2007). PHW has been used by several authors to extract polysaccharides from natural matrix (Benito-Román et al., 2013), among other

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high added value compounds such as bioactive and nutraceuticals, essential oils, lipids, carotenoids or proteins. Density, surface tension, viscosity and diffusion coefficient 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 (Teo et al., 2010). 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 (Kronholm et al., 2007), so a careful selection of the extraction conditions must be done in order to preserve the structure of the extracted compounds. In the literature it is not possible to find works that deal with the extraction of β-glucans from *G. lucidum* using the excellent properties of the pressurized hot water. Therefore it is necessary to develop a complete and systematic study in which the effect of the different process parameters on the extraction yield and the composition of the final extract (evaluated in terms of richness in β-glucans) are evaluated.

The properties of the extraction media can be changed by the addition of supercritical CO_2 to the pressurized hot water. Despite of the low solubility of CO_2 in H_2O and vice versa, the resulting mixture can be highly reactive (Springer et al., 2012): CO_2 dissolved in water increases the availability of protons, catalyzing the hydrolysis reactions (Brunner, 2009). Thus these conditions tend to weaken the covalent bonds between the β-glucan and the matrix, so the β-glucans are easily released and the extraction improved. It is hard to find in the literature data about the solubility of CO_2 in H_2O at high temperatures and pressures. Most of the studies are done at low temperatures and pressures, related to the geological CO_2 sequestration and the carbonate precipitation. Springer et al. (2012) reported a solubility of 0.8% of CO_2 in H_2O at 150 °C and 5 MPa. Solubility at other different temperatures and pressures are also reported: Duan and Sun (2003) evaluated the solubility at temperatures up to 260 °C and pressures as high as 200 MPa and Crovetto (1991) reported the solubility of CO_2 in H_2O at different conditions up to the critical point. Other researchers have measured the solubility at temperatures up to 100 °C, and pressures up to 100 MPa (Diamond and Akinfiev, 2003) or 60 MPa (Spycher et al., 2003).

The low solubility of the supercritical CO_2 in the pressurized hot water is a challenge, which will make difficult the performance of the extraction using that mixture of solvents. It is important to evaluate the flow pattern of the mixture pressurized hot water-supercritical CO_2 in the reactor. For instance, if a pulsed flow is detected, the changes in the extraction yield can be attributed to the turbulence generated in the reactor, contrary to the acidification effect primarily considered.

The purpose of the present work was to study the effect of operational variables in the extraction of β-glucans from *G. lucidum* using pressurized hot water as solvent. The influence of temperature, extraction time and the flow rate on extraction yield and content in β-glucans of the final product was evaluated. In a second step of the work, CO_2 was added to the PHW at different conditions, and the effect of this addition on both the extraction yield and the richness was evaluated.

2. Materials and methods

2.1. Raw material

Finely milled *G. lucidum* mushroom (CMT-Vibrating Sample Mill TI-100, CMT Co Ltd., Tokyo, Japan) was used in this work. *G. lucidum* contained 34.2% in β-glucan (moisture 11.0%) and average particle size after milling was 26.5 ± 1.6 μm (Malvern Mastersizer 2000, Malvern Ltd.).

2.2. Experimental set-up

Extraction was performed in a 10 mL fixed bed extractor, placed in an oven in order to keep the extraction at the desired temperature. In each experiment, 2 g of the mushroom were loaded in the reactor. A total of 3 g of glass beads were incorporated in the extractor (half of them in the bottom and the rest on the top, in order to prevent the compression of the biomass over the sinter plate). Water was pumped by means of a HPLC pump at a constant flow rate and the pressure in the reactor (5 MPa in all the experiments) was controlled by means of a back pressure regulator. The liquid extract obtained was cooled down in an ice bath in order to prevent degradation after the extraction. After the operation time, pump was stopped and the reactor was suddenly depressurized and cooled down. The solid residue was weighted and dried. The liquid extracts obtained throughout the experiments were kept at 4 °C and subsequently analyzed to determine the content in both β-glucans and total solid material. This allowed to determine the total amount of biomass dissolved during the extraction and to calculate the content in β-glucans of the solid product (a measure of the richness in β-glucans of the dried extract).

When the extraction was done using a mixture of PHW and supercritical CO_2, CO_2 was pumped by means of a syringe pump model 260D (Teledyne ISCO, 266 mL). PHW and CO_2 were mixed at the entrance of the extractor. Pressure used in these experiments was in the range 5–10 MPa. A scheme of the experimental set-up used in this work is shown in Fig. 1.

2.3. Chemical analysis

β-Glucan content in the *G. lucidum* (expressed in grams of β-glucan per 100 g of mushroom) and in the liquid after the extraction was determined by means of the assay kit provided by Megazyme Ltd. (Ireland) "Mushroom and Yeast β-glucan". This assay kit allows to know the amount of β-glucan in the liquid extract, expressed in grams of β-glucan per 100 g of liquid extract. Then, once it is known the grams of β-glucans extracted under a given conditions, the extraction yield was calculated according to Eq. (1):

$$\text{Extraction yield (\%)} = \frac{\text{β-Glucan content in liquid extract } \left(\frac{\text{g}}{100\,\text{g}}\right) \cdot \text{liquid extarct (g)}}{\text{β-Glucan content in } G.\ lucidum \left(\frac{\text{g}}{100\,\text{g}}\right) \cdot G.\ lucidum\ \text{(g)}} \times 100 \quad (1)$$

The effect of the process parameters on the content in β-glucans of the extract was also evaluated according to Eq. (2). This equation provides the richness in β-glucans that has the final extract, expressed in dry basis. This value relates the amount of β-glucans extracted with the total co-extracted