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Ultrasound assisted extraction of polysaccharides from mushroom by-products

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ABSTRACT

The effect of ultrasound technology to extract the water soluble polysaccharides from dried and milled by-products generated from *Agaricus Bisporus* production was studied. Amounts of β-glucan 1.01 and 0.98 g/100 g dry mass were obtained in particle sizes of 355–250 μm and 150-125 μm from the mushroom by-products. Three parameters of extraction were studied; extraction time (0–15 min), ultrasonic amplitude (20–100 μm) and precipitation time (1 or 18 h). The application of ultrasounds enhanced the extraction polysaccharide yields compared to the untreated samples. The highest extraction yield of 4.7% was achieved with an extraction time of 15 min, maximum amplitude of 100 μm with 1 h of precipitation in 80% ethanol. The coefficient of determinations for predicted water soluble polysaccharides extraction yields showed good correlation with the experimental data at the 95% confidence level and indicated that the non-exponential Peleg's model could be employed to predict the extraction polysaccharide yields after ultrasound treatment.

1. Introduction

High quantities of by-products are generated during white mushroom (*Agaricus Bisporus*) cultivation. This material is made up mainly of stalks and cups that are misshaped or do not meet the specifications set by retailers, representing between 5 and 20% of fruiting bodies discarded (Wu & Sun, 2010). They offer significant economic potential as fungal cell walls containing chitin, other hemicelluloses, mannans and, among the most interesting functional components, β-glucans (Wong & Cheung, 2009). β-glucan is one of the soluble dietary fibres that has been shown to enhance immune function, lower blood cholesterol and potentially attenuate the glycaemic load of foods (Charlton et al., 2012; Shimizu et al., 2008; Wolever et al., 2011).

Hot water and alkali extraction followed by precipitation with alcohol are the most common techniques used for polysaccharides extraction, including β-glucans (Lewis, 1984; Zhong, Shahidi, & Naczk, 2013). However, these methods require long extraction times and high temperatures giving low extraction yields (Huang & Ning, 2010). This has led to use novel extraction techniques including ultrasound-assisted extraction (UAE), microwave-assisted extraction and supercritical fluid extraction. Among these, UAE offers an inexpensive, environmentally friendly, less time consuming and efficient alternative to conventional extraction techniques (Chavan & Singhal, 2013; Hossain et al., 2012). The enhancement in extraction caused by ultrasound is mainly attributed to the effect of acoustic cavitations produced in the solvent by the passage of an ultrasound wave (Ma et al., 2008; Veličković, Milenović, Ristić, & Veljković, 2008). Therefore, cell wall structure is disrupted and diffusion through membranes is accelerated (Benito-Román, Alonso, & Cocero, 2013).

The application of ultrasound as a technique for assisting extraction of bioactive plant origin metabolites (Knorr, 2003; Vilkhu, Mawson, Simons, & Bates, 2008), flavonoids from foods using a range of solvents (Zhang, Xu, & Shi, 2003) and bioactives from herbs (Vinatoru, 2001) has been widely reported. Previous studies have reported the feasibility of UAE to extract polysaccharides from *A. Bisporus* by optimizing parameter conditions by applying orthogonal or central composite design experiments

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resulting in extracting polyssacharides yields of around 5–6 g/100 g (Qiao, Zhao, Huang, Fan, & Han, 2012; Tian et al., 2012; Chen, Zhou, Li, Bi, & Yang, 2014). However, other paramaters to optimize the extraction polyssacharide yields could be taken into account such as ultrasonic amplitude or precipitation time. On the other hand, quadratic regression models have been used to describe UAE of polysaccharides from fungal material (Tian et al., 2012; Yan et al., 2016). The non-exponential Peleg's model has been successfully used to describe extraction kinetics of various bioactives from range of matrices such as UAE of anthocyanins from black chokeberry (Galván, Dimitrov, Vauchel, & Nikov, 2014), solid-liquid extraction kinetics of polyphenols from grape seeds (Bucić-Kojić, Planinić, Tomas, Bilić, & Velié, 2007), ultrasound assisted rehydration and dehydration processes (Clemente, Sanjuan, Carcel, & Mulet, 2014; Ghafoor, Misra, Mahadevan, & Tiwari, 2014; Hui-Xiao, Xi-Hua, Ming-Hui, Dong-Yang, & Yu-Jie, 2012) and for the intensification of ursolic acid ultrasound extraction from *Ocimum sanctum* (Vetal, Lade, & Rathod, 2013).

Therefore, the aim of this study was to study the application of ultrasound technology to extract water soluble polysaccharides (WSP) from dried by-products of *A. Bisporus* production. Optimization of extraction times and ultrasound amplitude on the extraction yield of WSP after precipitation was also determined. Moreover, the flexibility of Peleg model to provide key information about extraction kinetics will be evaluated to predict different extraction kinetic parameters. On the other hand, the impact of the particle size of the dried material on the β-glucan levels, as predominant soluble dietary fibre, was evaluated.

2. Materials and methods

2.1. Sample preparation

Mushroom by-product materials were kindly provided by G's Fresh at Chelbury mushroom farm (Cheltenham, UK). Around 15 kg of mushroom material was donated, including mushroom cups and stalks. Fresh mushrooms were also obtained from Sainsbury's Ltd (UK). As the mushroom by-products contained a large amount of soil residue, the material was washed in lukewarm water to remove the soil. They were then dried using a hot air method at 60 °C until they reached a constant weight. Dried mushroom material were then milled in ZM100 Mill (Retch, Dusselfdorf, Germany) in to a powder with a particle size of less than 0.25 mm, vacuum packed and stored at 4 °C.

2.1.1. Sample preparation for the study on β-glucan content

In order to determine the effect of particle size on the β-glucan content of the mushroom by-product powder, 100 g of sample was separated according to their particle size using a sieve shaker (Retch, Dusseldorf, Germany). The mesh size of the sieves were >425, 355, 250, 150, 125 and 90 μm. The samples were placed in the top sieve and shaken for 10 min at a setting of 60 rpm. The material was collected together and put through the sieve shaker an additional 2 times. The remaining residue was weighed and expressed as a percentage of the original sample weight. Each sample preparation was done in triplicate.

2.2. Nutritional evaluation

2.2.1. General composition analysis

The general composition of the mushroom by-product powder was determined using standard methods. Analysis of total moisture content as well as soluble, insoluble and total fibre content was done by following the Association of Official Analytical Chemists (AOAC) methods (AOAC, 1995). Ash contents were determined by burning in a muffle furnace at 550 °C for 3 h according to the AACC Approved Methods (AACC, 2000). Total fat content was determined using the Caviezel method (Pendl, Bauer, Caviezel, & Schulthess, 1998). Protein contents were determined using a Kjeldahl digestion system (KI 26, Gerhardt, Königswinter, Germany) based on the AOAC methods. As mushrooms contain non-protein nitrogen compounds, the conversion factor used to estimate protein content was 4.7 (Mattila, Salo-Vaananen, Konko, Aro, & Jalava, 2002). Dietary fibre (total, insoluble, and soluble fraction was analysed by the enzymatic-gravimetric AOAC method (Prosky, Asp, Furda, De Vries, & Schweizer, 1988). Total carbohydrate was calculated as follows (Eq. (1)):

$$\text{Total carbohydrate (g/100 g DM)} = 100 - \text{protein content (g/100 g DM)} - \text{lipid content (g/100 g DM)} - \text{ash (g/100 g DM)} \quad (1)$$

where DM was dry mass.

2.2.2. β-Glucan determinations

The content of β-glucan was determined enzymatically in the different mushroom by-product powder fractions according to their particle size (from >425 μm, 355-250 μm, 150-125 μm to <90 μm) using the commercial Yeast Beta Glucan Assay Kit (Megazyme International, Bray, Ireland). Total glucan was determined first by solubilising the α-glucan and β-glucan linkages in hydrochloric acid, then potassium hydroxide (KOH) and then filtered. Hydrolisation to D-glucose was then completed by the addition of a mixture of exo-1,3-β-glucanase and β-glucosidase. Free D–glucose was then measured spectrophotometrically at 510 nm α-Glucan was then determined by solubilisation with KOH then hydrolysis to D-glucose with amyloglucosidase plus invertase. The free D-glucose was then measured spectrophotometrically at 510 nm. The β-glucan content was calculated by inputting the data into Mega-Calc ™ excel sheet provided by Megazyme (Megaenzyme International Ltd., Bray, Ireland).

Each analysis was performed in triplicate.

2.3. Ultrasound-assisted extraction of water soluble polysaccharides

Ultrasound-assisted extraction (UAE) of WSP was carried out following the method proposed by Chen et al. (2012), with some modifications. An amount of 3 g of dried milled mushrooms were weighed in to centrifuge tubes and 30 mL of distilled water was added (1:10 *w/v*). Sonication was conducted by means of the UP400S Ultrasonic Processor (maximum nominal power of 400 W, 24 kHz; Hielscher, Teltow, Germany) at differing ultrasound amplitude levels (20, 60 and 100 μm amplitude) for varying periods of time (0, 3, 5, 10 and 15 min). After sonication, the extract was centrifuged at 12 000 *g* for 20 min at room temperature and the residue and supernatant separated. The supernatant for each sample was mixed with 80% ethanol (1:2 *v/v*) at room temperature. This treatment was carried out in two conditions, one set of samples were left for 1 h and the other set were left for 18 h at 4 °C. Separation of the crude WSP precipitate was done by centrifugation at 12 000 *g* for 20 min. The WSP was dried at 40 °C and then weighed. Yield was calculated using the following calculation (Eq. (2)):

$$Y(g/100g) = \frac{m_t}{m_i} \times 100 \quad (2)$$

where m_t was the crude extract and m_i was the mass of the