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Effect of processing on the content and biological activity of polysaccharides from *Pleurotus ostreatus* mushroom

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ABSTRACT

Water soluble polysaccharides (WSP) were isolated from processed and non-processed fruiting bodies of oyster mushroom (*Pleurotus ostreatus*). The processing methods involved: blanching, boiling and blanching followed by fermenting with a strain of lactic acid bacteria (*Lactobacillus plantarum*). The yields of WSP ranged from 78.7 ± 1.5 mg/g to 120.1 ± 4.9 mg/g dry weight of sample. Blanching did not affect the content of WSP. Boiling for 15 min, led to the substantial decrease in the amount of WSP (34.7% decline). The isolated samples differed in carbohydrate, protein and phenolics content. FTIR spectroscopy of the WSP samples confirmed the presence of both α- and β-glycosidic linkages. Gel permeation chromatography showed the presence of compounds having the molecular weight of 198.3, 11.9, 3.1 kDa. The samples possessed antioxidant capacity measured by ABTS method (14.14 ± 0.63 to 29.48 ± 1.12 μmoles of Trolox per 1 g dw) and FRAP assay (2.49 ± 0.54 to 16.52 ± 0.55 μmoles of Trolox equivalents per 1 g dw). The antioxidant potential was decreased by the processing. Similarly, antiproliferative activity of WSP towards human breast cell lines (MCF-7 and T-47D) was lower due to the processing.

1. Introduction

Mushrooms are abundant source of polysaccharides which are the part of their cell walls. They differ greatly in molecular weight, structure, conformation and physical properties (Wasser, 2002). Additionally, some of them are reported to exert beneficial effect on health and can be used in the treatment of some diseases. Biological activity of mushroom polysaccharides is mainly related to their immunomodulating and anticancer properties. Moreover, they are also known to exhibit antiviral effect, lower blood lipids or possess antioxidant and antiproliferative activity (Roupas, Keogh, Noakes, Margetts, & Taylor, 2010; Stachowiak & Reguła, 2012).

Pleurotus ostreatus (known as oyster mushroom) is a popular, widely cultivated edible fungus, known for its hypocholesteroloemic properties. A few different polysaccharides were isolated from fruiting bodies of this species, including pleuran, a high molecular weight β-(1 → 3) (1 → 6)-glucan. These polysaccharides are reported to demonstrate immunomodulating, antioxidant, antiproliferative or prebiotic activity. Number of studies revealed that they vary in their chemical structure in terms of molecular weight, glycosidic bond conformation, branching, tertiary conformation or sugar composition. Moreover, the chemical structure of mushroom polysaccharides affect their biological activity (Zhang, Cui, Cheung, & Wang, 2007). High molecular weight (2200–2900 kDa), β-(1 → 3) (1 → 6)-glucan possessing prebiotic activity was obtained by Synytsya, Míčková, Jablonský, Sluková, and Čopíková (2008). Other authors isolated proteoglycans of lower molecular weight (1–31 kDa) which were capable of inhibiting proliferation of various cancer cell lines (Lavi, Friesem, Geresh, Hadar, & Schwartz, 2006; Martin & Brophy, 2010; Tong et al., 2009). Antiproliferative effect results from the induction of apoptosis of cancer cells. Antioxidant activity of *P. ostreatus* polysaccharides was demonstrated by

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proteoglycans described by other researchers (Sun & Liu, 2009; Xia, Fan, Zhu, & Tong, 2011).

Mushrooms are rarely eaten raw and they require to be processed before the consumption. Most often thermal or hydrothermal treatments are applied. It is well known that processing of food may cause substantial changes in its chemical composition and thus affect nutritional and health properties. However, few studies describe the effect of processing on mushrooms-derived biologically active polysaccharides. Fan, Li, Deng, and Ai (2012) investigated how different drying techniques affect antioxidant activity of polysaccharides that were isolated from the medicinal mushroom *Ganoderma lucidum*. Thetsrimuang, Khammuang, Chiablaem, Srisomsap, and Sarnthima (2011) compared antioxidant and antiproliferative activities of polysaccharides obtained from fresh and dried *Lentinus polychrous* fungus. So far, however, no studies focused on the influence of hydrothermal processing on the biologically active mushrooms-derived polysaccharides. Therefore, the present paper aims to verify the impact of some processing methods on the content, chemical composition, antioxidant and antiproliferative activity of water soluble polysaccharides (WSP) obtained from *P. ostreatus* fruiting bodies. The applied processing used in this work included boiling, blanching and fermenting with lactic acid bacteria (*Lactobacillus plantarum*). Lactic acid fermentation is a process which allows to develop food probiotic products with improved nutritional quality (Beena Divya, Kulangara Varsha, Madhavan Nampoothiri, Ismail, & Pandey, 2012).

2. Materials and methods

2.1. Mushroom samples

Fresh fruiting bodies of *P. ostreatus* were purchased directly from a producer (in 2013) and belonged to the same crop. After a harvest they were kept at 5 °C and were subjected to processing within 5 h.

2.2. Processing of mushrooms

The mushrooms were divided into four groups (500 g per group) and were further submitted to processing technologies: blanching in water containing 0.5% (w/v) citric acid (5 min, 95 °C); boiling in water (15 min, 100 °C); blanching in water (as reported above) and fermenting with lactic acid bacteria (see below). The fourth group was not processed (control).

The fermented mushrooms were prepared as follows. Fruiting bodies after blanching were washed with cool water and put tightly into screw-capped plastic vessels (PET). The brine solution was added which consisted of sucrose (10 g/1 kg of blanched mushrooms) and 3% (w/v) NaCl. The mixture was then inoculated with 5 mL of bacterial suspension (10^6 cfu/mL). The mushrooms were fermented for 10 days at 21–22 °C and then stored 20 days at 5 °C. The final pH of the product was 3.7.

All the samples were then subjected to freeze-drying with an Alpha 1-2LD plus freeze dryer (Christ, Germany) and ground to fine powder.

2.3. Extraction of water soluble polysaccharides

Four grams of powdered mushrooms were suspended in 200 mL of 80% ethanol and extracted in a rotary shaker at 80 °C for 60 min. The ethanolic extract containing low molecular weight compounds was removed by centrifugation (3755× g, 20 min) and the solid residue was washed twice with 80% ethanol and centrifuged. The alcohol insoluble fraction was then re-suspended in deionised water (ratio 1:50 w/v) and autoclaved at 115 °C for 180 min. The obtained slurry was cooled and centrifuged (3755× g, 20 min) and the supernatant was concentrated with a rotary evaporator and precipitated with three volumes of 2-propanol (24 h, at 5 °C). The precipitate was then centrifuged, washed twice with 80% methanol, re-dissolved in hot deionised water, lyophilised and weighed. The extraction process was done in triplicate.

2.4. Chemical characteristics of polysaccharides

2.4.1. Determination of total carbohydrate, protein and phenolics content

The content of carbohydrates in water soluble polysaccharides was measured with phenol-sulphuric acid method, using glucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The amount of protein was determined according to the method developed by Bradford (1976), using bovine serum albumin as a standard. Total phenolics content was measured according to the method of Singleton and Rossi (1965) with gallic acid used as a standard.

2.4.2. FTIR spectroscopy

FTIR spectra of lyophilised water soluble polysaccharides were recorded on Nicolet NXR 9650 spectrometer (Thermo, USA). The data was collected in the range of 4000–600 cm^{-1} and ATR technique was applied.

2.4.3. Gel permeation chromatography

The molecular weight of the isolated polysaccharides was determined with gel permeation chromatography, according to the modified method described by other authors (Malinowska, Krzyczkowski, Łapienis, & Herold, 2009). The samples were dissolved in aqueous solution of NaN_3 (0.1%, w/v) and were applied to three TSK-GEL columns: G5000PWXL, G3000PWXL and G2500PWXL (7.8 × 300 mm, Tosoh, Japan). The chromatographic system was equipped with K-501 pump (Knauer, Germany) and Refracto Monitor IV refractive index detector (LDC Analytical, USA). The flow of the mobile phase (0.1% (w/v) NaN_3) was set at 1 mL/min. Pullulans of different molecular weight were used to construct a standard curve.

2.5. Antioxidant activity

2.5.1. ABTS radical scavenging activity

The assay was done according to the method described by Re et al. (1999). ABTS reagent was prepared by incubating 7 mM ABTS solution with 2.45 mM potassium persulfate solution for 16 h at room temperature. The ABTS+ solution was then brought to an absorbance of 0.7 (at 734 nm). The samples (25 μL, 1 mg/mL) were mixed with 975 μL of ABTS+ solution and left to stand for 15 min at room temperature. The absorbance was measured at 734 nm against a blank sample. The calibration curve was done with different concentrations of Trolox (20–200 μM) and the results were expressed as micromoles of Trolox equivalent (TE) per 1 g of mushroom dry weight.

2.5.2. Ferric reducing antioxidant power (FRAP)

The ability of polysaccharides to reduce ferric ions was analysed according to the method described by Benzie and Strain (1996). Fresh FRAP reagent was prepared daily by mixing 300 mM acetate buffer (pH 3.6) with 10 mM 2,4,6-tripyridyl-striazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ (10:1:1 ratio). The reagent was incubated at 37 °C for 90 min before an analysis. Aliquots of the samples (100 μL) were mixed with FRAP reagent (final volume, 2 mL) and were then incubated at 37 °C for 90 min. The change in the absorbance was measured at 593 nm. Trolox aqueous solutions (20–200 μM) were used to construct the calibration curve