ANTIOXIDANT AND FUNCTIONAL PROPERTIES OF β–GLUCAN EXTRACTED FROM EDIBLE MUSHROOMS *AGARICUS BISPORUS, PLEUROTUS OSTREATUS* AND *COPRINUS ATRAMENTARIUS*

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ABSTRACT

β-glucan was extracted from three edible varieties of mushroom namely *Agaricus bisporus, Pleurotus ostreatus* and *Coprinus atramentarius*, using hot water extraction method and studies were carried out to investigate their structural, antioxidant and functional properties. The antioxidant activities were determined using different assays viz., DPPH (2,2-diphenyl-1-picryl-hydrazyl), reducing power, metal chelating ability and ABTS (2,2-Azino-bis,3- ethylbenzothiazoline-6-sulfonic acid). The FTIR was used to elicit the structural conformations of various β-glucans. The antioxidant activities varied significantly among all the sources of beta glucan, however, the beta glucan from *Coprinus atramentarius* showed highest values for DPPH (EC50=5.12±0.205), reducing power (3.75±0.195), chelating ability (2.89±0.256) and ABTS (3.5±0.503), where as β-glucan from *Pleurotus* showed the strongest lipid peroxidation inhibition (EC50 4.15±0.503) as compared to others. As far as the functional properties are concerned, *Coprinus* β-glucan also showed the highest swelling power, fat binding, foaming and emulsifying properties, however, all the functional properties of beta glucan varied significantly among all its sources. It was concluded that the β-glucan from *C. atramentarius* showed better antioxidant and functional properties as compared to β-glucan from *A. bisporus* and *P. ostreatus*.

Keywords: mushrooms, β-glucan, antioxidant, structure, functional properties

INTRODUCTION

Human kind has been valued fungal sources, particularly mushrooms as an important edible and medical resource since times immemorial, and they are used as an attractive source for the development of drugs and nutraceuticals [1]. Mushroom belongs to a special group of macroscopic fungi. The antioxidant activity of mushroom extracts was found to be due to their polysaccharide content as well as to their total phenolic contents [2]. The free radical species produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes are causing a wide variety of pathological effects, such as DNA damage, carcinogenesis and cellular degeneration related to aging [3] and they also contribute to progressive decline of the immune system [4]. β-glucans obtained from mushrooms are recognized as biological response modifier (BRM) which are used for the treatment of cancer and various infectious diseases both in modern medicine and traditional chemotherapeutic drug [5]. Polysaccharides are potentially useful biologically active ingredients for pharmaceutical use, such as for immune regulation, for anti-radiation, anti-blood coagulation, anti-cancer, anti-HIV and hypoglycemic activities [6-7]. The mushroom derived polysaccharides lentinan, schizophyllan, and krestin have been accepted as immunoceuticals in Japan, Korea and China [8].

MATERIALS AND METHODS

Wild and cultivated varieties of mushroom were procured from the Kashmir Valley. The cultivated varieties, namely *A. bisporus* and *P. ostreatus* were collected from Mushroom Research and Training Centre, Division of Plant Pathology, SKUAST-K, and the wild variety, namely *C. atramentarius* sample was randomly collected from the outskirts of Srinagar.

Extraction of β-glucan from various mushrooms

β-glucan was extracted from the various mushrooms following the method of Smiderle *et al*. [9]. Fresh fruiting bodies of *A. bisporus, P. ostreatus* and *C. atramentarius* were collected washed with distilled water, sliced and then dried in an oven at 30 ºC for 24 hours. The dried mushrooms were milled and submitted to successive cold and hot aqueous extraction, successively for 6h (×3:1,000 ml each).The cold extraction was performed aiming to separate other compounds, such as phenols, heteropolysaccharides, and glycogen. The hot aqueous extracts from each mushroom were evaporated to a small volume and the polysaccharides were precipitated by addition to excess ethanol (3:1;v/v) and centrifuged at 10,000 rpm, at 10 ºC, for 20 min. The sediment was dialyzed against distilled water for 24 (12-14 kDa cut off), concentrated under reduced pressure and freeze dried. The purification was performed by freeze thawing process [10]. The recovered fraction were dissolved in water and the solutions were submitted to freeze and thaw slowly untill complete separation of soluble and insoluble polysaccharides. The precipitates, obtained after centrifugation (10,000 rpm at 4 ºC, for 20 min), were treated with dimethyl sulfoxide (50 ml), for 2h, at 60 ºC, dialyzed against tap water for 24 and then resubmitted to the freeze-thawing process, giving rise to soluble fractions of β-D-glucans.

Antioxidant activity assays

Assay for DPPH radical scavenging activity: The radical scavenging activity of β-glucan was conducted by using the method previously carried out by Fu *et al*. [11]. The 0.1 mM solution of DPPH radical in ethanol was prepared and 2 ml of this solution was added to 2 ml of water solution containing different content of IOPS (1, 2, 4, 8, 10 mg). Briefly, the absorbance of solutions at 517 nm was measured using UV–vis spectrophotometer (UV-2450, Shmadzu, Japan). Vitamin C was used as the positive control. The DPPH radicals scavenging rate of sample was calculated as the following equation.

$$\text{Inhibition } \% = (A_{blank} - A_{sample})/A_{blank} \times 100\%$$

where A_{sample} was the absorbance with sample and A_{blank} was the absorbance without sample. Ascorbic acid was used as positive control.

Ferric-reducing antioxidant power assay: Reducing power was determined according to Oyaizu [12]. Each polysaccharide powder (0.1 to 20 mg/ml, 2.5 ml) in Milli-Q water was mixed with 2.5 ml, 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was vortexed and incubated at 50 ºC for 20 min. Then 2.5 ml of 10% trichloroacetic acid (w/v) was added and the mixture was centrifuged at 2000g for 10 min. The upper layer (5 ml) was mixed with 5 ml of Milli-Q water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm against a blank using a UV/Vis spectrophotometer (Shimadzu UV-1650 PC, Japan). The blank was the solution with all reagents but without extract. A higher absorbance indicates a higher reducing power. Ascorbic acid was used as the positive control.

Chelating ability on ferrous ions: Chelating ability was determined according to the method of Dinis, Madeira, and Almeida [13]. Each polysaccharide powder (0.1 to 20 mg/ml, 1 ml) in Milli-Q water was mixed with 3.7 ml of methanol and 0.1 ml of 2 mM ferrous chloride. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine. After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm against the blank. Blank was the solution with all reagents but without extract. A lower absorbance indicates a higher chelating ability. The EC50 value (mg extract/ml) is the effective concentration at which ferrous ions were chelated by 50%. Citric acid and EDTA were used for comparison.

ABTS assay : For ABTS assay the procedure followed the method of Arnao *et al*. [14] with some modifications. The stock solutions included 7.4mM ABTSd+ solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS*+ solution with 60 ml methanol to obtain an absorbance of 1.170.02 units at 734 nm using the spectrophotometer. Fresh ABTSd+ solution was prepared for each assay. Fruit extracts (150 ml) were allowed to react with 2850 ml of the ABTS*+ solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer over the linear range of the standard curve.

Functional properties of various fungal β-glucan

Various functional properties of β-glucan are studied such as swelling power, fat binding capacity, emulsifying capacity and stability, foaming capacity and stability, bile acid binding capacity were studied.

Swelling power: The swelling power was determined according to the method described by Bae *et al.*, [15]. A mixture of 0.3 g of sample and 10 ml of distilled water was placed in a shaking water bath at 70 °C for 10 min, then transferred to a boiling water bath. After boiling for 10 min, the tubes were cooled with tap water for 5 min and centrifuged at 1700g for 4 min. Swelling power was expressed as the ratio of wet sediment weight to dry sample weight.

Fat Binding Capacity: *In-vitro* fat-binding capacity was determined according to the method reported by Lin and Humbert [16]. β-Glucan samples (0.2 g) were dispersed in soy oil (10 ml), and the mixtures were placed at room temperature ambient conditions for 1 h and agitated on a vortex mixer every 15 min. After centrifugation at 1600g for 20 min, the supernatant was decanted and the residue was weighed. The fat absorption was obtained from the amount of soy oil bound to 1 g of dry sample.

Emulsifying properties: 1% sample was homogenized with 5 ml of refined oil. The emulsions were then centrifuged at 1100 g for 5 min (5810, Eppendorf, Hamburg, Germany). Subsequently the height of the emulsified layer and the total contents in the tube were determined. The emulsion capacity was obtained through the following calculation.

Emulsion capacity (%) = (Height of the emulsified layer/ Total height of tube contents)× 100

Emulsion stability was evaluated by heating the emulsion for 30 min at 80 °C and centrifuging for 5 min at 1100 g.

Emulsion Stability (%) = (height of the emulsified layer after heating/ height of emulsified layer before heating) ×100

Foaming capacity and Stability: Aqueous dispersions (2% w/v db) of the sample were homogenized in a high speed homogenizer (Remi Instruments Division, Vasai, India) at 10,000 rpm for 1 min.

Foaming capacity was calculated as the per cent increase in volume of the sample dispersion. The foam stability was determined by measuring the foam volume with time and computing half-life.

$$\text{Foaming capacity (\%)} = \frac{(\text{volume after whipping} - \text{volume before whipping})}{(\text{Volume before whipping})} \times 100$$

$$\text{Foam stability (\%)} = \frac{\text{foam stability after standing time (60 min)}}{\text{Initial foam volume}} \times 100$$

RESULTS

Antioxidant assays

It was concluded from Fig. 1 and Table 1 that *Coprinus* β-glucan has the highest DPPH scavenging activity and reducing power as compared to *Agaricus* β-glucan and pleurotus β-glucan. The chelating ability and ABTS of *Pleurotus* β-glucan was more as compared to *Agaricus* β-glucan and *Coprinus* β-glucan. So far as the functional properties are concerned, *Coprinus* β-glucan has the highest swelling power, highest fat binding capacity, emulsifying properties and foaming properties.

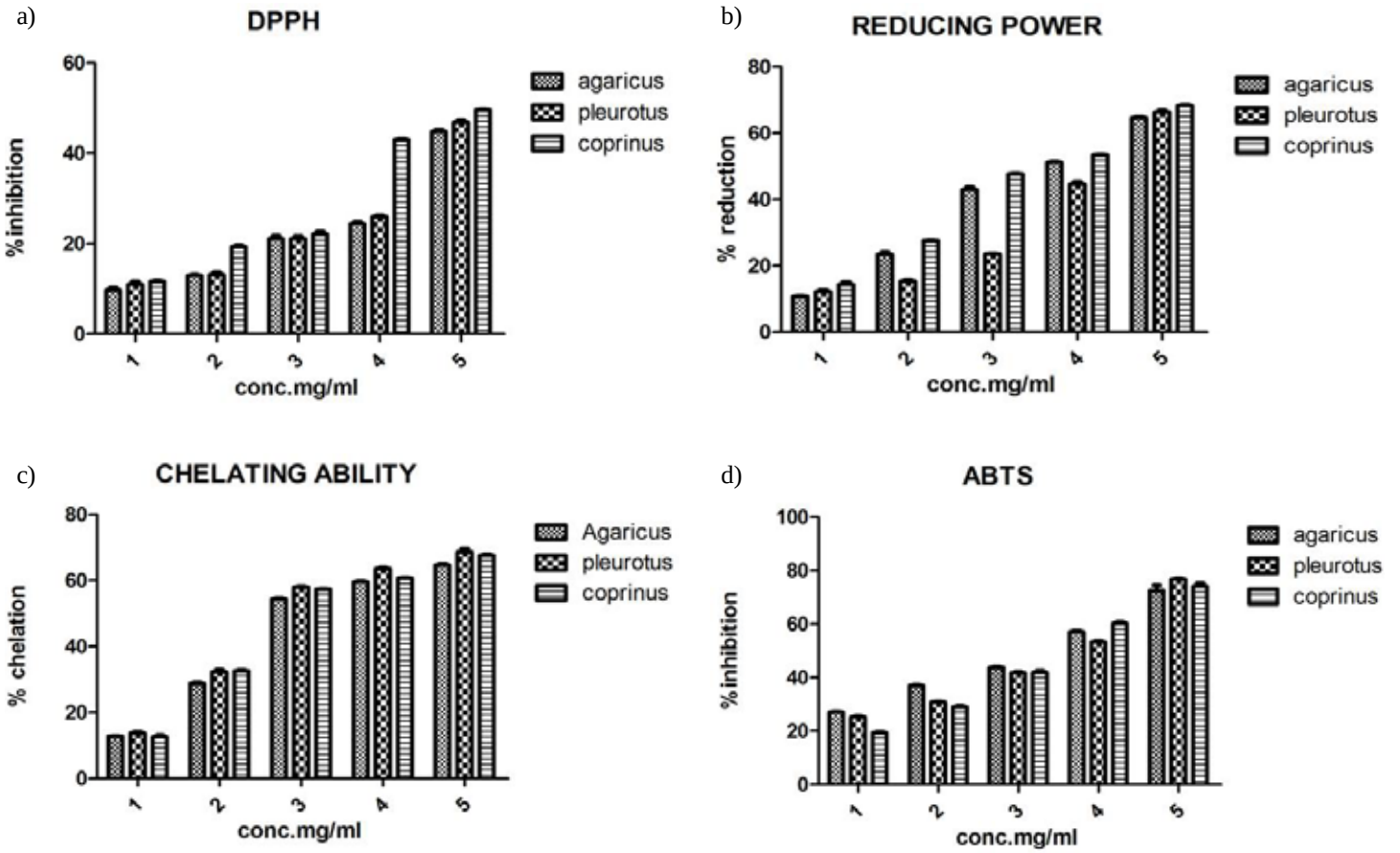

Figure 1. a) DPPH inhibition, b) reducing power, c) chelating ability and d) ABTS inhibition of *Agaricus*, *Pleurotus* and *Coprinus* sp.

Table 1. Functional properties of mushroom beta glucans

Source	Swelling power. (g/g sample)	Fat binding capacity. (g oil/g sample)	Emulsion capacity (%)	Emulsion stability. (%)	Foaming capacity. (%)	Foaming stability. (%)
Agaricus	3.45 ±0.19	5.38±0.27	64.26±0.01	94.64±0.96	9.80±0.04	6.06±1.27
Pleurotus	3.74±0.21	5.53±0.32	65.35±0.03	96.73±0.02	10.20±0.36	9.16±1.44
Coprinus	4.59±0.10	6.65±0.35	65.47±0.08	97.68±0.20	9.93±1.46	8.33±1.44

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