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A comprehensive analysis of the nutritional quality of edible mushroom *Pleurotus sajor-caju* grown in deproteinized whey medium



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ABSTRACT

Use of edible mushroom as nutritious food or feed supplementation is widespread. Here, we evaluated the nutritional quality of edible mushroom *Pleurotus sajor-caju* as a single cell protein (SCP) grown in supplemented whey medium. Crude protein content of the SCP was found to be $39.25 \pm 3.89\%$ of the dried biomass. Essential amino acids (EAAs) including high amount of lysine, leucine, threonine and phenylalanine were identified in the *P. sajor-caju* SCP. Three independent 'scores' were calculated based on EAA content to understand nutritive value of the SCP. The ash content of the SCP was high $(16.2 \pm 0.83\%)$ with presence of major and trace minerals. The SCP contained both saturated and unsaturated fatty acids in its lipid and is an excellent source of polyunsaturated fatty acid, linoleic acid. High amounts of arabinose, mannose and N-acetylglucosamine were measured in the SCP while glucose and galactose contents were low. The SCP was found to be a rich source of B-vitamins. These results suggested *P. sajor-caju* SCP is a rich source of nutrition.

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1. Introduction

Studies on production of non-conventional proteins as an alternative source of dietary supplement have increased with growing global demand for quality food (Rajoka, Ahmed, Hashmi, & Athar, 2012). Mushrooms are an attractive source of food and feed protein (Kim et al., 2002). Though mushrooms have important role as biopharmaceuticals for human diseases, they are largely used as delicious and nutritious food (Kim, Shim, Kim, & Jang, 1999; Lee et al., 1994; Preuss, Echard, Bagchi, & Perricone, 2010). Traditionally, cultivation of mushrooms in India was not up to expectation, though production has been growing in steady rate in last two decades. In 1990, India produced about 5000 tons of mushrooms which increased up to 100,000 tons in 2010 (Singh, 2011). Increased production as mushroom is profitable and can play an important role in country's stride towards ensuring food security. Alternative ways of production and estimation of nutritive values for quality assurance are two important steps towards improvement of mushroom cultivation. Earlier, we have reported the optimization of fermentation conditions of edible mushroom *Pleurotus sajor-caju* using deproteinzed whey medium (Mukhopadhyay, Chatterjee, Chetterjee & Guha, 2005).

Whey, the liquid by-product of dairy industry, causes environmental pollution due to its high biochemical oxygen demand (BOD) (Mukhopadhyay, Chatterjee, & Guha, 1999). In contrast, it is a valuable source of lactose, milk proteins and micronutrients and dumping of whey leads to loss of these potential nutrients (Panesar & Kennedy, 2012). Production of single cell protein (SCP) through fermentation of whey is an exciting area of whey utilization. Exploitation of such waste for the production of microbial biomass is an excellent way to generate alternative source of food grade protein and to reduce the pollution load of whey (Mukhopadhyay et al., 1999). Production of different food grade yeasts such as Kluyveromyces fragilis (Giec & Kosikowski, 1982; Paul, Mukhopadhyay, Chatterjee, & Guha, 2002), Kluyveromyces marxianus (Belem & Lee, 1998), Saccharomyces fragilis (El-Samragy, Chen, & Zall, 1988) and Candida intermedia (Moulin & Galzy, 1984) in whey permeate have been reported.

To understand the role of a SCP as a source of dietary supplement, nutritional parameters should be studied in details. In the

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present study, we report the comprehensive nutritional profile of edible mushroom *P. sajor-caju* grown under submerged condition in whey medium.

2. Materials and methods

2.1. Microorganism and fermentation

P. sajor-caju (MTCC 141) used in this study was obtained from The Institute of Microbial Technology, Chandigarh, India and maintained in Potato destrose agar medium as described earlier (Mukhopadhyay et al., 1999). At the end of fermentation, mycelia were separated from the medium by centrifugation at 5000 rpm for 20 min at $4~^{\circ}\text{C}$ and dried by freeze-drying process.

2.2. Fractionation of the dried mycelia and nitrogen content

Dried mycelia (2 g) were suspended in 200 ml of 5% tricholoroacetic acid (TCA) at 20 °C and incubated for 30 min. The supernatant obtained after centrifugation was termed as cold TCA soluble fraction. The residue was suspended in 200 ml of 75% ethanol at 50 $^{\circ}\text{C}$ and centrifuged. The supernatant fluid was termed as alcohol soluble fraction. The residue from this step was suspended in 200 ml of 1: 1 solution of ether and 75% ethanol for 15 mm at 50 °C. The supernatant obtained after centrifugation was termed as alcohol-ether soluble fraction. The residue was then suspended in 200 ml of 5% TCA and placed in a boiling water bath for 30 mm. After centrifugation, the supernatant fluid termed as hot TCA soluble fraction. Finally, the residue from the last step, termed as alcohol insoluble protein fraction, was washed with 300 ml of acidified ethanol and then with 300 ml ether. Nitrogen content of all the above five fractions and dried biomass were determined by microkjeldahl procedure. 90 mg of biomass or 5 ml of fractions from above experiments were added to 5 ml of conc. H₂SO₄ and 1 g of catalyst and heated. The digests were cooled and distilled with 10 ml of water. Then 20 ml of 40% NaOH was added and steam distilled for 45 min. Liberated ammonia was collected on 25 ml 4% boric acid and was titrated with 0.04 N HCl. The nitrogen content was calculated from the amount of ammonia liberated.

2.3. RNA and DNA

RNA content was calculated from hot TCA soluble fraction using spectrophotometric method after comparing with a yeast RNA standard (Baker yeast RNA type III soluble, Sigma, USA) (Gottlieb & Van Etten, 1964). DNA in hot TCA soluble fraction was determined by diphenylamine method using standard DNA from calf-thymus (Sigma, USA). (Dische, 1955).

2.4. Amino acid

Amino acid analysis was performed by hydrolyzing 200 g dried mycelia using 6 N HCl at 100 °C for 24 h. The hydrolyzed samples were analyzed in HPLC (Waters, USA) using Pico. Tag Amino Acid system. Tryptophan was determined separately using spectrophotometric method after extraction of the mycelia with 0.1 N NaOH.

2.5. Ash and minerals

Ash content was determined by the procedure described in AOAC using 5 g of the dried mycelia (AOAC, 1984). The metal ions were determined by atomic absorption spectrophotometer (Perkin Elmer model 2380) using air-acetylene flame and specific hollow cathode lamp after refluxing 2 mg of the sample with conc. HNO_3 and 70% $HClO_4$ (1:1) for 30 min.

2.6. Fat and fatty acid

Fat content was determined by Soxhlet extraction procedure (AOAC, 1984). 1 g of dried mycelia was homogenized first with 10 ml methanol and followed by 20 ml chloroform in presence of butylated hydroxytoluene (BHT) as antioxidant. After filtration, the solid residue was re-suspended in 30 ml chloroform; methanol (2:1) and homogenized. The procedure was repeated thrice. To the total filtrate 30 ml water with 0.88% KI was added. The extracted lipid was washed thrice with 1:1 methanol and water and nitrogen gas was passed over till the solvent evaporated out. Anhydrous Na₂SO₄ was added to remove water. Samples were kept sealed in a tube in nitrogen atmosphere until further study. Methyl esters of lipid extract were prepared by using acid based transesterification (Wollenweber, Seydel, Lindner, Lüderitz, & Rietschel, 1984). Solvents used contained BHT as an antioxidant and C17:0 fatty acid as internal standard. Methyl esters were analyzed on a Hewlett-Packard Model 6890 Gas Chromatograph using a DB 23 column. Chromatograms were analyzed using HP Chemstation software.

2.7. Crude fiber

Fiber content of the dried biomass was determined by removing fat from it as described above and then by boiling consecutively with 1.25% (w/v) H_2SO_4 and 1.25% (w/v) NaOH solution (AOAC, 1984). The residue was washed thrice with water followed by ethyl alcohol and dried to a constant weight. The value was corrected for ash content of the sample.

2.8. Carbohydrate

The monosaccharides of the dried mycelia were measured as described earlier (Paul et al., 2002). The liberated monosaccharides were reduced with sodium borohydride followed by conversion to alditols by addition of 0.1 N HCl in methanol. After drying, residues were suspended in water and extracted with freshly distilled chloroform. Identification and estimation of neutral and amino monosaccharides as their alditol acetates were performed by Hewlett-Packard 5890 Gas-Liquid Chromatograph using SP 2340 and HP-5 columns with nitrogen as carrier gas. The monosaccharide distribution is expressed as percent of total sugars present.

2.9. Vitamins

Thiamine, riboflavin, niacin, pyridoxine, p-pantothenic acid, folic acid and cyanocobalamine were measured using standard procedures of spectrophotometric or microbiological estimation (Paul et al., 2002).

3. Results and discussion

3.1. General composition

The composition of *P. sajor-caju* biomass after fermentation in deproteinized whey is presented in Table 1. Crude protein content

Table 1General composition of *P. sajor-caju* dried mycelia grown on whey.

Composition	g/100 g
Crude protein	39.25 ± 3.89
Ash	16.2 ± 0.83
Fat	5.8 ± 1.06
Crude fiber	5.5 ± 0.48
Carbohydrates (by difference)	33.25 ± 3.68

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