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Nutritional and correlation study of fresh and dry *Agaricus bisporus* and *Pleurotus ostreatus*

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Abstract
Two edible mushrooms *Agaricus bisporus* and *Pleurotus ostreatus* species are the most acceptable species among the cultivated mushroom, and have been revered for their immense health benefits due to their specific biochemical compounds that are responsible for improving human health. The two edible mushrooms were studied to obtain basic information on biochemical properties that are helpful in studying their nutritional value. The total soluble sugar, protein, phenol, and starch content of dry and fresh of these two edible mushrooms were estimated, which ranged from 14.10 ± 0.21 to 50.53 ± 0.16 mg/g, 2.01 ± 0.00 to 12.86 ± 0.23 mg/g, 1.78 ± 0.02 to 5.75 ± 0.12 mg/g, 4.03 ± 0.24 to 29.70 ± 0.17 mg/g, respectively. Furthermore in this study protein showed a strong positive correlation with phenols (0.99) which influences the structural, functional and nutritional properties of mushrooms. The increasing awareness about high nutritional value of mushrooms can be an important ingredient in food industry and as an alternate to non-vegetarian foods.

Keywords: *Agaricus bisporus*, correlation, edible mushrooms, nutritional value, *Pleurotus ostreatus*

Introduction
Mushrooms are widely consumed by people seeking a healthier and more nutritional diet due to their high nutritional value. Mushrooms are considered as medicinal foods that are rich in nutrition which is recognized by medical professions through the world. Mushrooms have eight important amino acids, polyunsaturated fatty acids and small amounts of saturated fatty acids and have higher nutritional values than fish or beef (Alemu 2015) [2]. It is known mushrooms are known to be rich in essential nutrients such as carbohydrates, proteins, fibre, vitamins and minerals. In general, their sporocarps, on dry weight basis, contain about 17.5% protein, 2.9% fats and a considerable amount of minerals (Latiff *et al.*, 1996) [8]. Since mushrooms have carbohydrates, fiber, protein, essential amino acids, unsaturated fatty acids, vitamins, low calories, and minerals such as potassium, iron, copper, zinc, and manganese, which are high in their fruit bodies, they are recognized as a healthy food with nutritional benefits because of these qualities, Food and Agricultural Organization (FAO) has suggested their usage as food supplement for protein deficient population of developing and underdeveloped countries. During the early civilization, mushrooms were consumed mainly for their palatability but presently mushroom usage is undergoing a paradigm shift that is entirely different from traditional consumption as a lot of research has been focused on their biochemical characterization which has revealed that they can be used as a food supplement because of their high nutritive value (Khatua *et al*., 2013) [7]. Mushrooms possess great potential for both nutrition and therapeutic use. It has been found that edible and medicinal mushrooms contain many biologically active compounds with antiinflammatory, antitumor, antibacterial, antiviral, cardiovascular, antihypercholesterolemia and antioxidant activities (Wani *et al*., 2010; Patel *et al*., 2012; Wasser, 2014) [19, 14, 20]. Mushrooms constituent bioactive occurring in, phenolic compounds focus much attention due to their antioxidant activity (Bubueanu *et al*., 2017) [5]. Fruit bodies of the edible fungus are considered a food of high nutritious value of low caloric and lipid content and with a high content of protein, vitamins and minerals of importance for human nutrition. The mushrooms are widely consumed because of their flavour, nutritional benefits, and therapeutic characteristics (Gupta *et al.*, 2018) [6]. The objective of this study is to understand the nutritional properties of edible mushrooms.

Method and Material

The fresh fruiting bodies of two edible mushrooms *Pleurotus ostreatus* and *Agaricus bisporus* species were collected from the mushroom unit division of plant pathology, SKUAST Jammu. The mushrooms of two species were washed with tape water then sliced into thin pieces and kept in hot air oven at 35 ^{0}C for about 24 hours, then the dried mushrooms was crushed to make a fine powder for analysis, where as fresh bodies were directly crushed for biochemical analysis.

Determination of total soluble sugar

Total soluble sugar was estimated by anthrone method, samples were extracted with 2 mL of hot 80% ethanol. It was centrifuged at 10,000 rpm for 30 mins at 4 ^{0}C. The extraction process was repeated thrice with 80% hot ethanol and supernatant was collected in 25 mL volumetric flask. The supernatant was then evaporated out on a hot plate with a sand layer on the platform to evaporate the ethanol. Then 5 mL of double-distilled water was added to dissolve the sugar to make sample extract for further analysis.0.1 mL of sugar extract was made up to 1mL with doubled distilled water. 4 mL of 0.2% anthrone reagent prepared in 95% ice cold sulphuric acid was added and mixed thoroughly using vortex. All tubes were incubated for 8 min in a hot boiling water bath and the blank was maintained with the doubled distilled water and reagents. The reaction mixture was cooled to room temperature and the green to dark green colour was measured at 630 nm against blank using spectrometer. The total soluble sugar was calculated from the calibration curve using glucose as a standard (100 µg/mL) (Lee *et al*., 1992) [9].

Determination of total soluble protein

The mushroom samples were analysed for protein by the method described method described by Bradford (1976) [4]. Both fresh and dried mushroom (100mg) with 2 micro liter beta mercaptoethnol and 2 mL of potassium phosphate buffer 0.1 M pH 7.4 containing 0.5% polyvinylpyrrolidone (PVP) was homogenized in a pre-chilled pestle and mortar and centrifuged at 10,000 rpm for 20 min. After centrifugation supernatant was collected for the protein estimation. The reaction mixture for quantification of protein content contained 10 micro liter supernatant and 990 micro liter of phosphate buffer. It was mixed with 5mL dye and kept in dark for 5 minutes. After incubation absorbance of the solution was measured at 595nm using a UV-VIS spectrophotometer. The calculation was calculated from the standard curve prepared using bovine serum albumin BSA (100 µg/mL) curve.

Determination of total phenolic content

The extraction for phenolics was done same as described in the total soluble sugar. 0.1 mL of crude extract was made up to 3mL by adding distilled water and mixed thoroughly with 0.5 mL of 0.1 N Folin–Ciocalteu reagent and kept undisturbed for 3 min in dark followed by addition of 2 mL of sodium carbonate solution (20%). The mixture was kept in a water bath (80 ^{0}C) for 1 minute and then cooled. The absorbance of the blue coloured solution was taken spectrophotometrically at 650 nm. The total soluble phenolic was calculated from the calibration curve using catechol as a standard (25 µg/mL) (Peri and Pompei.1971) [15].

Determination of starch content

0.1g of the sample was homogenized in hot 80% ethanol to remove sugars and centrifuge to retain the residue and repeatedly washed with hot 80% ethanol till the washing did not give color with anthrone reagent. Residues were dried well in hot air oven at 50 ^{0}C. To the residue 5.0 mL of distilled water was added to dissolve the sugar extract for analysis. 0.01 mL of extract was taken to which distilled water was added to make 1 mL. 4 mL of anthrone reagent was added to sample and mixed well by using vortex. Samples were incubated for eight minutes in a boiling water bath, cooled rapidly and the intensity of green to dark green colour was read at 630 nm against blank using UV-VIS spectrophotometer. The starch content was calculated from the calibration curve using glucose as a standard (100 µg/mL) (Ludwig and Goldberg 1956) [10].

Statistical Analysis

All the experimental parameters were carried out in triplicate. One way ANOVA was used to identify differences between treatments performed using R software version 4.0.2 (2020) for the window. The pairwise treatments means was conducted using Fishers least significant difference (LSD) to assess the difference among the treatment means and Pearsons correlation coefficient was also analysed through R-studio for windows 8.1.

Result and Discussion

The total soluble sugar (TSS)

The total sugar content in dry and fresh *Agaricus bisporus* and *Pleurotus ostreatus* are shown in the table 1. In the present study, the highest TSS content was obtained from dry *Agaricus bisporus* (50.53 ± 0.16 mg/g), followed by dry *Pleurotus ostreatus* (19.52 ± 0.62 mg/g), fresh *Pleurotus ostreatus* (14.24 ± 0.31 mg/g) and fresh *Agaricus bisporus* (14.10 ± 0.21 mg/g). It is quite evident from the results that dry mushrooms contains more TSS than fresh mushrooms because because of high moisture content 90-95%. (Ajlouni *et al.*, 1995) [1] also conducted similar research on *A. bisporus* and reported somewhat similar results. Furthermore TSS showed a positive correlation with starch, phenolics and proteins (0.85, 0.80, and 0.77) respectively (Fig 1). Total sugars also play a major role in disease resistance since sugars are the precursors for the synthesis of phenolics and phytoalexins which suppress the pectolytic and cellulolytic enzymes that are essential for pathogenesis.

Total soluble protein

In presented result, the highest protein content was obtained in AGDP (12.86 ± 0.23 mg/g) followed by PLDP (11.20 ± 0.49 mg/g), AGFP (2.045 ± 0.04 mg/g) and PLFP (2.01 ± 0.00 mg/g). Edible mushrooms are a good source of proteins and is the most critical component contributing to the nutritional value of food and edible mushrooms or their products occupy an elite position vis-a-vis natural source of proteins. According to a study conducted by (Sadiq *et al*., 2008) [17], reported 11.01% protein contents in *A. bisporus*. In addition proteins showed a strong positive correction with phenolics (0.99) as shown in the Fig 1. (Quispe *et al*., 2021) [16] has showed a positive correlation between protein and phenols in their study which was conducted on *Chenopodium quinoa*. Protein–phenolic interactions influence the structure, functional and nutritional properties, and digestibility of proteins and have been widely studied for their health promoting and disease preventive activities in humans are well-known to have high affinity to bind proteins (Ozdal *et al*., 2013) [13].

Total soluble phenolics

As summarized in Table 1, the highest phenol was observed in AGDP (5.75 ± 0.12 mg/g) followed by PLDP (4.92 ± 0.13 mg/g), PLFP (2.37 ± 0.22 mg/mL) and PLFP (1.78 ± 0.02 mg/mL). The phenolic content of *A. bisporus* was found to be higher in the present investigation which is similar to the findings of (Alispahic *et al*., 2015) [3]. Phenols present in mushrooms are important mushroom constituents because of their scavenging ability due to the presence of hydroxyl groups they exhibit antioxidant properties thus neutralize harmful oxygen radicals by inhibiting oxidative reactions in the body (Nizamova *et al*., 2011) [11].

Total starch content

The edible mushrooms when investigated for the quantitative analysis of total starch AGDP showed highest starch content (29.70 ± 0.17 mg/g) followed by AGFP (15.92 ± 0.26 mg/g), PLDP (5.08 ± 0.71 mg/g) and PLFP (4.03 ± 0.24 mg/g). The amount of starch found in the *A. sylvaticus* mushroom was 36.21% (Shibata and Demiate 2013). Starch is considered as fuel for body system, when starchy foods get into your body system, different enzymes proceed to break them into the substance that the body can use and store glucose.

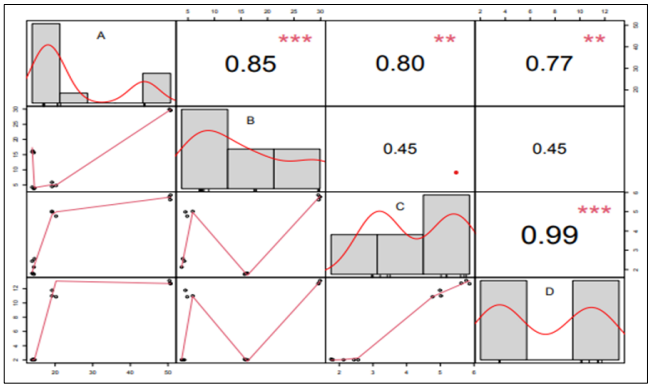

Fig 1: Correlation between Biomolecules. TSS, (B) Starch (C) Phenol and (D) Protein

Table 1: nutritional value of two edible mushrooms *Agaricus bisporus* and *Pleurotus ostreatus*

Samples	TSS	Protein	Phenolics	Starch
AGDP	50.53 ± 0.16^{a}	12.86 ± 0.23^{a}	5.75 ± 0.12^{a}	29.70 ± 0.17^{a}
AGFP	14.10 ± 0.21^{c}	2.045 ± 0.04^{c}	1.78 ± 0.02^{d}	15.92 ± 0.26^{b}
PLDP	19.52 ± 0.62^{b}	11.20 ± 0.49^{b}	4.92 ± 0.13^{b}	5.08 ± 0.71^{c}
PLFP	14.24 ± 0.31^{c}	2.01 ± 0.00^{c}	2.37 ± 0.22^{c}	4.03 ± 0.24^{d}

Agaricus dry powder (AGDP), *Agaricus* fresh powder (AGFB), *Pleurotus* dry powder (PLDP) and *Pleurotus* fresh powder (PLFB). All the values are expressed as mean ± SE

Conclusion

The proximate analysis dry and fresh of the two edible mushrooms species (*Agaricus bisporus* and *Pleurotus ostreatus*) showed the presence of appreciable amount of phenols, proteins, TSS and starch). Despite, quantitative differences in individual biochemical components was observed yet each of the samples exhibited richness in one or the other attributes. The reasonable amount of phenolic content in these mushrooms could make a significant contribution in constituting a healthy diet for patients with elevated blood pressure, diabetes and cholesterol related ailments as they exhibit high antioxidant activity. The protein content of mushrooms is as high as that of most vegetables, with the exception of green peas and pulses. Mushrooms have potential to serve the purpose of meat substitute, as now people are becoming health and vegetarian conscious. Thus it may be concluded that both the species of mushrooms are good source diet.

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