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FUNCTIONAL AND MICROSTRUCTURAL PROPERTIES OF *PLEUROTUS FLORIDA* AND *CALOCYBE INDICA* FLOUR AND PROTEIN CONCENTRATE

T. R. Thirumuruga Ponbhagavathi[1*], S. Kanchana[2], C. Hemalatha[1], S. Vellaikumar[3], and K. Kalpana[4]

[1]Dept. of Food Science and Nutrition, Community Science College and Research Institute, TNAU, Madurai-India
[2]Dept. of Human Development and Family Studies, Community Science College and Research Institute, TNAU, Madurai; [3]Dept. of Agricultural Biotechnology, AC&RI, Madurai; [4]Dept. of Plant Pathology, AC&RI, Madurai-India
Corresponding author'se-mail:ponbhagavathi2015@gmail.com

ABSTRACT

Pleurotus florida(Oyster mushroom) and *Calocybe indica*(Milky Mushroom) are edible mushrooms commercially cultivated in India next to button mushroom has significant nutritional values and functional protein concentrates (PCs)and this can be obtained from nutrient dense powdered mushroom. Proximate composition and functional properties of flours and PCs derived from *P.florida and C.indica* were evaluated in this study. Fruiting bodies of mushroom were dried at 60-65°C in a cabinet drier and ground to produce flour.PCs were recovered from mushroom flours through isoelectric precipitation. Oyster mushroom (OM) and Milky Mushroom (MM) flour had protein content as 27.81% and 22.2% respectively. The protein content in the mushrooms increased to two-fold in PCs ie.from 27.81 to 50.47% in OM and 22.2 to 48.23% in MM.Flours were brighter contributing higher L* value than their corresponding concentrates, mainly due to pale yellow colour of mushroom flours and brownish colour of PCs.PCs presented significantly ($p<0.05$) higher Water Absorption Capacity, foaming capacity and emulsion capacity with lower bulk density and oil absorption capacity than their corresponding flours. Flours and protein concentrate had minimum gelation concentration of 6 and 8% for OM and MM respectively. The above results indicate that the flour and protein concentrates from *P.florida and C.indica* have remarkable functional properties, valuable in food industry with improved textural properties.

Key words:*Pleurotus florida, Calocybe indica,* protein concentrates, functional properties, isoelctric precipitation.

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INTRODUCTION

Increase in population and urbanization, with concomitant reduction in cultivation land results in shortage of food and unhealthy human life. Converting the residues from agricultural cultivation containing lignocellulosic matter into protein rich mushroom is economically suitable to address protein malnutrition in developing countries (Mane *et al*.2007).Mushrooms rich in nutrients and flavors are well-known popular healthy foods that contain large quantity of proteins, almost all essential amino acids, especially threonine, tyrosine, and arginine as compared with common vegetables such as potatoes and carrots(Zhao*et al*.2017).Compared with medicinal mushroom species, knowledge of the composition and nutritional value of culinary mushrooms was limited (Kalač 2009)

According to recent production data of ICAR-DMR, Solan (2018-2019) the total mushroom production in India is 136077.7 metric tonnes. *Agaricus bisporus* (button mushroom), *Pleurotus spp* (oyster mushroom), *Calocybe indica* (milky mushroom) are commercially grown in Tamil Nadu for culinary purpose. Mushroom industry in India is more focused on button mushroom for auspicious occasional foods which is a highly sophisticated and capital-intensive activity. Among the other abundant number of edible mushrooms Pleurotus genus is prolific because they are cultivated commercially due to their short life cycle, reproducibility in the agricultural wastes and low demand on resources and technology (Yildiz *et al*.2002). Proteins of Pleurotus sp mushroom have superior quality of higher digestibility and well distribution of essential amino acids, as well as non-essential amino acids particularly gamma aminobutyric acid, which act as neurotransmitter and ornithine which is a precursor in the synthesis of arginine (Deepalakshmi and Sankaran 2014; Del Toro *et al*.2006). Milky mushroom is morphologically similar to button mushroom and it has gained popularity in south Indian states due to adaptability to tropical climate (Krishnamoorthy *et al*.2000).

Usually vegetable proteins have been preferred due to increasing demand for low cost and good quality protein sources for human foods (Akintayo *et al*.1999). However, fungal proteins has also been prompted the interest for fortification in foods due to their high protein content. Mushrooms can be represented as an alternative source of high-quality proteins to animal origin food proteins for human nutrition. Some researchers reported

that the amino acid compositions of mushrooms are comparable to animal proteins (Mattila *et al*. 2001)

Protein concentrates have become important in food industries since it can be incorporated into foods to increase nutritional value and to improve specific functional properties. The edible mushroom *Pleurotus tuberregium* (Alobo 2003) and *Pleurotus ostreatus* (Cruz solorio *et al*. 2014) has been proposed as a source of protein concentrates. Therefore the objective of the present study was aimed for the production of protein concentrates from commercially grown *Pleurotus florida* and *Calocybe indica* by isoelectric precipitation method and to study the functional properties of mushroom flour and protein concentrate.

MATERIALS AND METHODS

Collection and preparation of mushroom flour: *Pleurotus florida* (oyster)and *Calocybe indica* (milky mushroom) were purchased from local mushroom growing farmers in Madurai, Tamil Nadu, India. Fresh mushrooms were blanched at 70°C for 3 minutes and then dried at 50-60°C in a cabinet drier for 8 hours. The dried mushroom slices were made to powder form, and then sieved through BS 60 sieve. The flour was sealed in air tight plastic containers and stored in freezer (-18°C) until used.

Preparation of Protein Concentrates: The method of Gupta *et al*. (2008) and Cruz solorio *et al*. (2014) was adapted with some modifications, to produce protein concentrates. Defatted mushroom flour was dispersed in distilled water (1:8) and pH of the suspension was adjusted to pH 10.0 with 1N NaOH. The samples were incubated at 50°C for 1 hour with continuous mixing and left overnight at 4°C. Thereafter, the supernatant was adjusted to pH 4.5 using 50% citric acid and left undisturbed for 3-4 hours at 4°C to collect the pellets. The pellets were neutralized using 0.1 N NaOH and spray dried at 120-150°C. The concentrate was packed in dry, airtight containers and stored in freezer before analysis. This method was separately followed for oyster and milky mushroom flours.

Proximate chemical analysis: Moisture, ash, protein (N x 6.25), fat and fiber content were estimated by AOAC (2000) methods.

Moisture: Moisture content was determined by the gravimetric method. The clean aluminum dish for moisture estimation was dried at 105°C in an oven for 1 h. Two grams of the sample was weighed in it and transferred to the hot air oven and dried at 105±1°C for 3 h. It was cooled in desiccator prior to weighing. Moisture content was calculated by using the following formula:

Moisture (% wt.basis) = W3-W1/W2-W1 X100

Where,

W_1= weight of empty dish, g

W_2= weight of dish with sample before drying, g

W_3= weight of dish with sample after drying, g

Crude fat: Five grams of sample was weighed in the extraction thimble. Sample was extracted with petroleum ether (boiling point: 40-60°C) for 45 minutes using SOCS PLUS extractor (Pelican Equipments, Chennai, Tamil Nadu, India). The beaker with extracted fat was dried in oven at 40°C, allowed to cool in desiccator and weighed.

Crude fat % = 100* W2-W1/S

Where,

W_1=Weight of beaker, g

W_2=Weight of beaker and extracted fat after drying, g

S= Weight of sample, g

Crude Protein: The protein content of mushroom flour and protein concentrates was determined by micro-Kjeldahl method. The sample (0.2 grams) was accurately weighed and transferred to a kjeldahl digestion tube followed by addition of 1 g digestion mixture ($CuSo_4$:K_2So_4 in the ratio of 1:9) and 10 mL of concentrated H_2SO_4. The contents were digested till the transparent clear fluid.

The digested content was transferred to a 100 mL volumetric flask and the volume made up to 100 mL by using distilled water. An aliquot (10 mL) of digested sample was distilled along with 10 mL of 50% sodium hydroxide and the liberated ammonia was collected in 10 mL saturated boric acid containing 2-3 drops mixed indicator (1 part of 0.2% alcoholic methyl red and 5 part of 0.2% alcoholic bromocresol green solution). Approximately 50-60 mL of distillate was collected in a 100 ml conical flask.

The content of the flask was immediately titrated against 0.02N HCL. Blank was determined by using distilled water in place of sample. The total nitrogen present and the percent protein were calculated as follows:

Nitrogen (%)= 14.007 (S-B)x Normality of HCL x dilution factor x100/ 1000x weight of sample.

Where,

S= mL of HCL required for sample

B= mL of HCL required for blank

N= Normality of HCL used, and

W= Weight of the sample in grams

Percent total protein= percent total nitrogen x conversion factor (6.25)

Total ash: Total ash content in the sample was estimated by the method as described. Three grams of sample was weighed and transferred to a pre-weighed porcelain crucible. The weighed sample was charred and crucible was transferred to a muffle furnace maintained at 550±5°C for 5 h. It was incinerated until light gray ash

was obtained. The crucibles were taken out from the muffle furnace and cooled in a desiccator and weighed.

Ash (%) = (W1-W2)/ (W1-W)

Where,

W_1= Weight in grams of dish with material before ashing, g

W_2= Weight in grams of dish with material after ashing, g

W=Weight in grams of empty dish, g

Crude fiber: The dried sample was taken in the pre weighed glass crucible (W_1) it was placed in crucible holder with the glass extractor. 150 ml of pre heated 1.25% H_2SO_4 was added in the extractor and the contents are boiled for 30 mins at 500°C and 30 mins for 400°C. The acid residue was drained out from the extractor through fibra flow system. The residue was washed with distilled water. Then 150 ml of pre heated 1.25% NaOH added and digested for 30 mins at 500°C and 30 mins at 400°C. Then the residue was washed with distilled water and dried for two to four hours at 100°C, cooled and weighted.

Crude fiber (%) = $(W_3 – W_2)/W_1$ x 100

W_1= Weight of sample used

W_2 = Weight of crucible

W_3 = Weight of residue with crucible

Carbohydrates: They were assessed by difference method (carbohydrate (%)=100%-(ashes(%)+fat(%)+crude fiber (%)+protein(%)).

Physical and functional properties

Colour value: Hunter lab Colormeter (Lovi bond tinto meter) was used to measure the colour of the flour and protein concentrates. Data were received through the software in terms of L^* (lightness), ranging from zero (black) to 100 (White), a^* (Redness) +60 (Red) to -60 (Green) and b* (Yellowness) ranging from +60 (Yellow) to -60 (Blue) values of international (CIE) colour system.

Bulk density: Bulk density was determined by the method described by (Alobo 2003). Twenty gram sample was weighed into a 100 ml graduated cylinder. The cylinder was tapped ten times against the bench top and the final volume was used to calculate the bulk density as g/cm^3

Water and oil absorption capacity: Water and oil absorption capacity of flours and protein concentrates were measured according to the method proposed by (Cruz solorio *et al.* 2018), with some modifications. Two grams of flour or protein concentrate were taken into centrifuge tubes and 10 ml of distilled water or groundnut oil were added. Samples were thoroughly mixed using vortex mixer and left undisturbed for 30 min, then centrifuged for 30 min at 2000 rpm. Excess water or oil was decanted by inverting the tubes. The weights of water and oil absorbed by the samples were determined by using the following equation.

Water /Oil absorption capacity (g/g of sample) = ml of absorbed water (or) oil / weight of initial sample (g)

Foam capacity and stability: Foam capacity and stability was determined based on the method given by (Alobo 2003). Two gram of sample was dissolved in 50 ml distilled water and the pH of the flour and PC suspensions were adjusted to 6.8. The content were transferred into a domestic blender and blended for 5 min. The contents were poured into a 100 ml graduated cylinder. The foam volume changes were recorded at 10 min intervals from 0–120 min. Foam capacity was calculated as percent volume increase immediately after blending (0 min); volume of foam at 1h after blending represented foam stability.

Emulsifying capacity and stability: Emulsifying capacity and stability was determined by methods reported by Chau *et al.* 1997. Emulsions were prepared with 1 g of each sample and 10 ml each of distilled water and refined peanut oil in calibrated centrifuge tubes. The tubes were centrifuged at 1600 rpm for 10 min. The height of the emulsified layer as a percentage of the total height of the material in the tubes was calculated as the emulsifying activity. The emulsion stability expressed in percentage was calculated as the ratio of the height of the emulsified layer to the total height of the material after heating the tubes at 80°C for 30 min, cooling for 15 min and centrifuging at 1600 rpm for 15 min.

Least Gelation concentration: The least gelation concentrations of the samples were determined by the method of Siddiq *et al.* 2010. Sample dispersions of 2–10% (w/v) were prepared and aliquots of 5 ml of each concentration were transferred into test tubes, then incubated in a boiling water bath for 1 h followed by immediate cooling under running tap water and stored at 4°C. After 30 minutes, the tubes were kept in inverted position to determine the point at which the sample did not slide down from it. The flow behavior and appearance of the sample dispersions have been described using (+) and (–) indices.

Microstructure analysis: Morphological properties were analyzed by Scanning Electron Microscope (SEM) in order to study the influence of extraction process on the structure of the mushroom protein concentrates as compared to flour. The samples were mounted on aluminium stub using a double backed cellophane tape, coated in auto fine coater, JEOL-JFC-1600, with gold palladium (60:40,g/g).

Statistical analysis: Statistical analysis was carried out using a SPSS Program version 20.0. Statistical significance of the terms was done by analysis of variance (ANOVA) and significant differences were

defined at $P<0.05$. XLSTAT 2019 was used for Principal Component Analysis (PCA) for multivariate analysis. Each sample was analysed in triplicates and average values are reported.

RESULTS AND DISCUSSION

Proximate composition of mushroom flour and protein concentrates (PCs): The proximate composition of mushroom flour and PC are presented in the Table 1. Mushrooms have 1.97 times as much nutrients in its dry matter (Mattila Pirjo *et al.*2002). Moisture content in mushroom flour ranged between 9.21- 9.45% and these values were comparable to dried edible mushrooms moisture content of 4.88- 11% (Salehi 2019).Oyster mushroom and milky mushroom flour had protein content of 27.81% and 22.2% respectively. Teklit (2015) also reported that *Pleurotus florida* contained 32.08% total carbohydrate, 1.54% fat, 27.1% protein, 23.18 % fibre and 9.41 % ash and both mushrooms showed no difference in proximate compositions. According to Alam (2008) 100 g of dried *C. indica* contained 20-23 g of proteins, 4.6-5.3 g of lipids, 11-15 g of fiber and 46-51 g of carbohydrates and our values obtained were almost similar to this study. Therefore, oyster and milky mushroom could be source of proteins.

The protein content in PCs was 50.47 and 48.23 % for oyster and milky mushroom respectively. These values are significantly higher ($p<0.05$) than flours in concentrates. This was higher compared with the average of 48.56-49.94% obtained from *P.osteratus* strains (Cruz solorio *et al.* 2014). This difference may probably be due to differences in extraction temperature (Gupta *et al.* 2008). Fat, ash and fiber contents of 0.85-0.91%, 4.94-5.12% and 4.65-7.5%, respectively, were lower than their corresponding flour. These results were in accordance with reports of gardencress seed flour and protein concentrates (Ali, 2013).

Colour Values: Colour is one of the important quality parameters of food as its changes during processing imply modification in organoleptic properties of food. The hunter colour values (L*,a* and b*) of flours and protein concentrates showed significant ($p<0.05$) differences and Duncan test indicated that flours were brighter than their corresponding concentrates, i.e. 49.92 for OMF versus 35.04 for OPC, 51.02 for MMF versus 27.06 for MPC. These values were mainly because of pale yellow colour of mushroom flours and brownish colour of protein concentrates. This colour is influenced by brown pigments generated through maillard reaction due to the presence of carbohydrates and temperature during extraction of protein concentrates. This study was supported by results from rice bran protein concentrates (Kaewka *et al.* 2009) and *P.ostreatus* protein concentrates (Cruz solorio *et al.* 2018). The increase in a* and b* values showed a shift towards redness and yellowness of the protein concentrates as compared to flours. The observed result was positively correlated with earlier reported results for oat flour and concentrates (Singh *et al.* 2018).

Table 1: Proximate composition (g/100g) of flour and protein concentrate (PC).

Component	Flour		Protein concentrate	
	Oyster mushroom (OMF)	Milky mushroom (MMF)	Oyster mushroom (OPC)	Milky mushroom(MPC)
Moisture (%)	9.21±0.3^{b}	9.45±0.8^{b}	6.85±0.12^{a}	5.36±0.24^{a}
Ash (%)	7.6±0.2^{b}	5.94±0.14^{ab}	4.94±0.23^{a}	5.12±0.29^{a}
Fat (%)	2.49±0.05^{b}	2.53±0.04^{b}	0.91±0.45^{a}	0.85±0.14^{a}
Fiber (%)	12.56±0.1^{c}	16.80±0.1^{d}	4.65±0.87^{a}	7.5±0.24^{b}
Protein (%)	27.81±0.1^{b}	22.2±0.54^{a}	50.47±0.14^{d}	48.23±0.85^{c}
Carbohydrate (%)	40.33±0.1^{b}	41.5±0.5^{b}	32.18±0.45^{a}	32.94±0.94^{a}

^{a,b,c,d} *Means with the different letter within the row are significantly different (P< 0.05)*
Values are Mean ± S.D (from 3 determinations)

Table 2: Colour values of mushroom flour and protein concentrates.

Hunter colour values	Flour		Protein concentrate	
	Oyster mushroom (OMF)	Milky mushroom (MMF)	Oyster mushroom (OPC)	Milky mushroom (MPC)
L*	49.92±1.52^{c}	51.02±0.86^{c}	35.04±1.25^{b}	27.06±0.76^{a}
a*	17.26±2.02^{c}	7.44±1.45^{a}	18.04±1.33^{c}	15.02±1.51^{b}
b*	25.01±0.95^{b}	17.39±0.15^{a}	38.76±3.65^{c}	36.15±0.76^{c}

^{a,b,c,d} *Means with the different letter within row are significantly different (P< 0.05)*
Values are Mean ± S.D (from 3 determinations)

Physical and functional properties: The functional properties of a food material determine its application and end use. Therefore, food items with good functional properties can be easily incorporated in other foods and it will yield good quality and acceptable end products. Functional properties of mushroom flour and protein concentrate are presented in Table 3.

Bulk density: The density of flours and protein concentrates is important as it affects mixing, packaging, and transportation. The bulk density of the protein concentrates (0.33-0.39 g/ml) was lower than that of mushroom flour (0.37-0.39 g/ml). Bulk density decreased significantly ($p < 0.05$) upon protein concentration. Lower bulk density in the protein concentrates compared with their respective mushroom flours could be due to reduction of carbohydrate content (Gernah, Ariahu, and Ingbian 2011). Nutritionally, low bulk density is advantageous because it gives rise to consumption of more quantity of the lighter food item and this will translate into more nutrients for the consumer (Ocheme *et al.*2018).

Table 3 Functional properties of mushroom flour and protein concentrates.

Properties	Flour		Protein concentrate	
	Oyster mushroom (OMF)	Milky mushroom (MMF)	Oyster mushroom (OPC)	Milky mushroom (MPC)
Bulk density(g/ml)	0.39 ± 0.01^{c}	0.37 ± 0.02^{ab}	0.33 ± 0.02^{a}	0.36 ± 0.01^{ab}
WAC (g H_2O/g of sample)	2.48 ± 0.27^{b}	2.21 ± 0.17^{a}	2.98 ± 0.09^{d}	2.80 ± 0.04^{c}
OAC (g / g of sample)	0.94 ± 0.08^{a}	0.95 ± 0.07^{ab}	0.96 ± 0.01^{bc}	0.97 ± 0.06^{c}
Foaming capacity (%)	58.24 ± 0.11^{b}	49.53 ± 0.58^{a}	85.64 ± 0.08^{d}	69.42 ± 0.04^{c}
Foaming stability (%)	33.05 ± 0.07^{b}	24.86 ± 0.46^{a}	63.58 ± 0.20^{d}	58.12 ± 0.05^{c}
Emulsion capacity (%)	42.22 ± 1.60^{a}	40.88 ± 0.80^{a}	58.22 ± 2.25^{b}	52.44 ± 2.73^{b}
Emulsion stability (%)	27.11 ± 1.17^{b}	24.22 ± 0.96^{a}	54.88 ± 0.22^{d}	47.55 ± 0.58^{c}

^{a,b,c,d} Means with the different letter within row are significantly different ($P< 0.05$)
Values are Mean ± S.D (from 3 determinations)

Water and oil absorption capacity: The ability of proteins present in the flour/protein concentrates which hold water against gravity determines the WAC (Shevkani *et al.*2014). In our study, significant differences ($p<0.05$) were found in WAC and four groups were established by Duncan test. Milky mushroom flour showed the lowest value (2.21 g H_2O/g of sample), OMF show intermediate value, in group b with value 2.48 g H_2O/g of sample while OPC and MPC are in groups "c" and "d", thus resulting significantly higher WAC values than their corresponding flours, i.e. 2.98 for OMF in contrast to 2.48 for OPC, 2.80 for MPC in contrast to 2.21 for MMF. Aletor et al (2002) reported average WAC of 2.66 (g/g) for leaf protein concentrates while Chandi and Sogi (2007) reported water absorption range of 2.48-3.77 g/g for rice bran protein concentrates which were in accordance with the values obtained in the present study. It was also compared well with average WAC of oat flour and protein concentrates (Singh *et al.*2018)

Oil absorption capacity of OPC and MPC showed non- significantly($p >0.05$) higher values than their corresponding flours, i.e. 0.96 (g/g) for OPC against 0.94 (g/g) for OMF, 0.97 (g/g) for MMF while 0.975(g/g) for MPC. Protein extraction at 50°C increased the oil absorbing that could be attributed to denaturation of proteins exposing the hydrophobic sites resulting in increased binding capacity. Similar observations have been reported for *P.tuberregium* sclerotia flour (Alobo 2003) and gardencress seed flour and protein concentrates (Ali 2013)

Foaming properties: The foaming capacity increased significantly ($p <0.05$) in protein concentrates as compared to flours i.e MMF (49.53%) < OMF (58.24%) < MPC (69.42%) < OPC (85.64%) and this increasing order continues after one hour and also establishing good foaming stability, probably due to the increased protein content of the protein concentrates. Kouakou *et al.*(2013) reported that foaming capacity was positively correlated with protein content. Our results are comparable to the data previously reported for *P.tuberregium sclerotia* (Alobo 2003) and *P.ostreatus* (Cruz-Solorio *et al.* 2018) flour and protein concentrates.

Emulsifying properties: Flour and protein concentrates possess higher emulsion capacity and emulsion stability and the data are presented in Table 3.Theemulsion capacity and stability ranges from 40.88-42.22%, 52.44-58.22% for *P.florida and C.indica* flour in our study, which is lower than the values for *P.sajor-caju* powder having emulsion capacity 51.67% and stability 95.37% (Han *et al.* 2016) but higher than *P.tuberregium sclerotia* flour of emulsion activity 30% and stability 18.1%. *P.tuberregium sclerotia* protein concentrates results are inline with our results in terms of comparison with flour

and protein concentrates by increased emulsion capacity and stability of concentrates than their respective flours (Alobo 2003).According to Garba and Kaur (2014), soluble proteins enhance the emulsifying capacity of foods. Increased emulsifying capacity and emulsifying stability of protein concentrates indicated that inclusion of concentrates as a food component will enhance the suitability of purpose for incorporation into food systems (Ocheme *et al.*2018).

Least gelation concentration (LGC): LGC is a qualitative attribute that determines least protein concentration required to form gel. The gel forming ability of protein gives structural matrix that helps in water binding (Nicole *et al.* 2010). It relies mainly on certain characteristics like viscosity, elasticity and plasticity. *P. florida* protein concentrates and flours showed a good gelification property with a lower minimum concentration for gelification 6%, than *C. indica* protein concentrates and flours of 8% are presented in table 4. Our study results were higher than *Pleurotus ostreatus* protein concentrates and flours with LGC 2% (Cruz solorio *et al.*2014), whereas LGC of *P. florida* protein concentrate was similar to *P. tuberregium* protein concentrates(Alobo 2003). The variations in gelling ability of different samples were due to the differences in their protein, lipid and carbohydrate contents (Sibt-e-abbas2020). Moreover, LGC plays imperative role in food system by contributing towards texture and rheology of end product.

Table 4: Least Gelation Concentration (LGC) of mushroom flour and protein concentrates.

Sample concentration (% w/v)	Flour		Protein concentrate	
	Oyster mushroom	Milky mushroom	Oyster mushroom	Milky mushroom
2	-	-	-	-
4	±	-	±	-
6	+	±	+	±
8	+	+	+	+
10	+	+	+	+

Gelling	Gel structure
-Not gelled	Liquid
± Gelled slightly with some floccules	Pourable
+ Complete gelation	Fixed and not pourable

Microstructure: Structural morphology of the mushroom flour and protein concentrates was studied with the aid of scanning electron microscope (SEM). SEM pictures of mushroom flour and their protein concentrates (Figure 1) showed distinct surface structures. The OMFand MMF contains all food components including carbohydrate, protein, fat and fibre, their morphological structure showed the mixture of β-glucan in fibrous form and protein particles in flaky plate shapes. The OPC appeared more compact in structure; whereas MPC showed big flaky plate like structure. The results indicated that protein extraction technique changed the microstructure of mushroom protein. It was suggested that the big flaky plate like structures of MPC contributes to improve the solubility (Mao and Hua, 2012). Similar flaky structure of protein concentrate was also reported for groundnut protein isolate (Kaptso *et al.,* 2015) and apricot protein isolates (Thakur *et al.,* 2019). The different microstructure of OMF, MMF, OPC and MPC may contribute to the overall physiochemical and functional properties of mushroom protein.

Principal component analysis (PCA): PCA is a useful multivariate analysis procedure to describe interrelationships between multiple variables, which are achieved by reducing dimensions through lineal combinations of the original variables (Meena *et al.* 2005). In this study, PCA was used to analyze the data in order to make a correlation of mushroom flour and protein concentrates physical properties. PCA of the 8 variables from different treatments resulted in two principal components (Table 5) with eigenvalues greater than 1.0, a common statistical cut-off point (Chapman *et al.*2001). The biplot made from PCA of flour and protein concentrates physical parameters defined by the first and second PCA dimensions is presented in Fig.1 (a). The sum of principal components PC1 and PC2 contributed to 96.23% of variance among the physical properties. The first principal component (PC1) accounted for 78.08% of the total variation and the second principle component (PC2) contributed to 18.15% of the total variation. PC1 was positively correlated with OAC,WAC, foaming and emulsifying properties.PC2 was positively correlated with least gelation concentration (LCG) and negatively correlated with bulk density.Our results were in line with principal component (87.69%) of total variance of shrimp shell waste protein concentrates(Yuan *et al.* 2019). In the present study, a loading with an absolute value greater than 0.600 (shown in bold type in Table 5) represents

strong influence. The absolute correlation or loading of WAC, foaming and emulsifying properties was found to be more than 0.900, while OAC loading was found to be more than 0.700 in PC1 indicating the strong influence on the quality of mushroom flour and protein concentrates. From the Fig 1(b), PCA results strongly evidence the functional properties of oyster mushroom flour as well as production and use of oyster mushroom concentrate can be a great alternative and may be of commercial interest due to the characteristics of the protein.

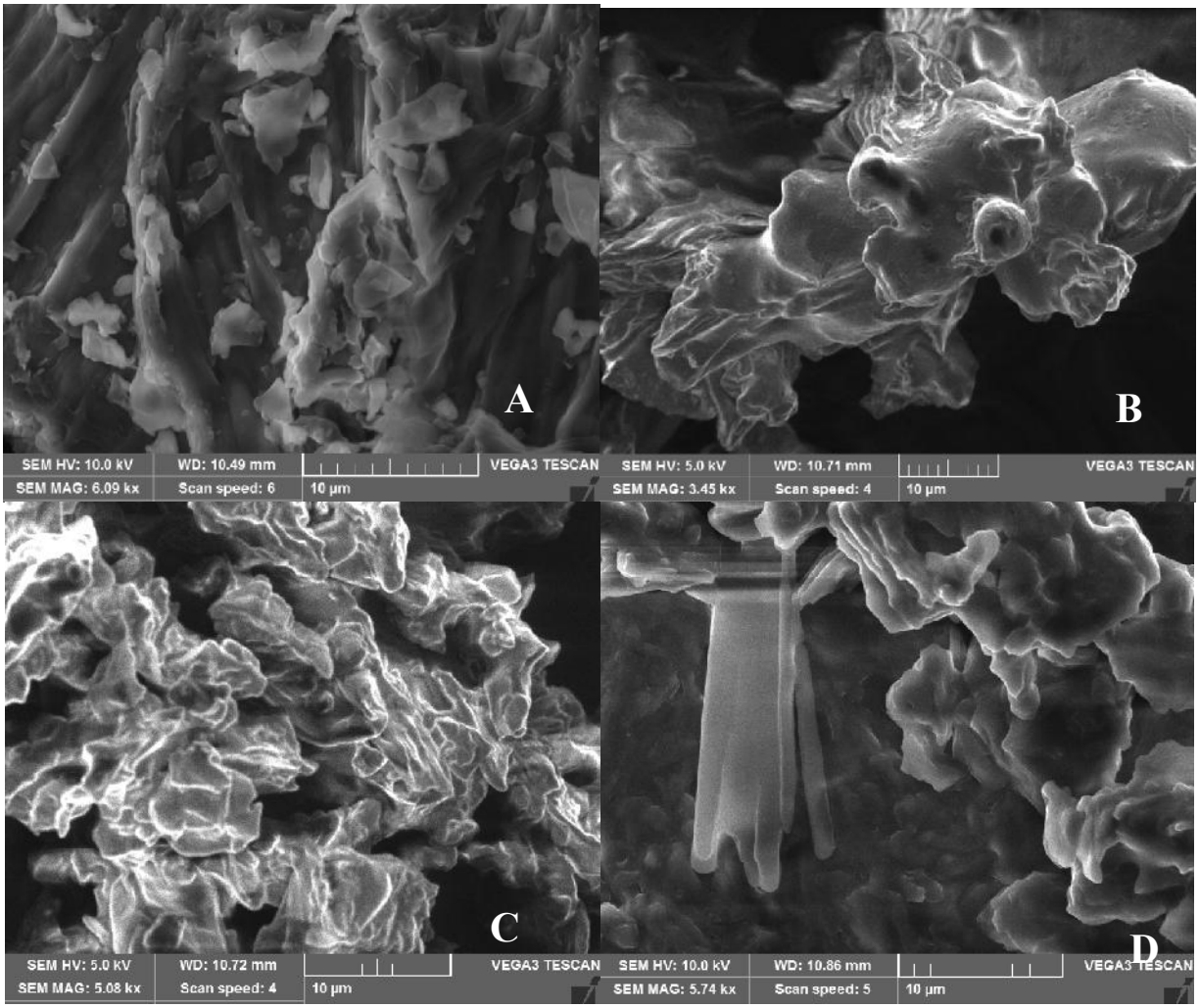

Figure 1) Scanning electron microscope pictures of OMF (A), OPC (B), MMF (C), MPC (D).

Table 5:Eigen values, percentage variances and variable loadings for the two principal components.

Parameter	PC1	PC2
Eigen values	6.24	1.45
Variance (%)	78.07	18.15
Cumulative variance (%)	78.07	96.23
	Loadings	
Bulk density	-0.877	-0.087
WAC	**0.968***	-0.156
OAC	**0.758**	**0.643**
Foaming capacity	**0.969***	-0.241
Foaming stability	**0.986***	0.023
Emulsion capacity	**0.999***	-0.014
LCG	-0.233	**0.972***
Emulsion stability	**0.998***	0.039

** Loadings with an absolute value greater than 0.600 are shown in bold type*

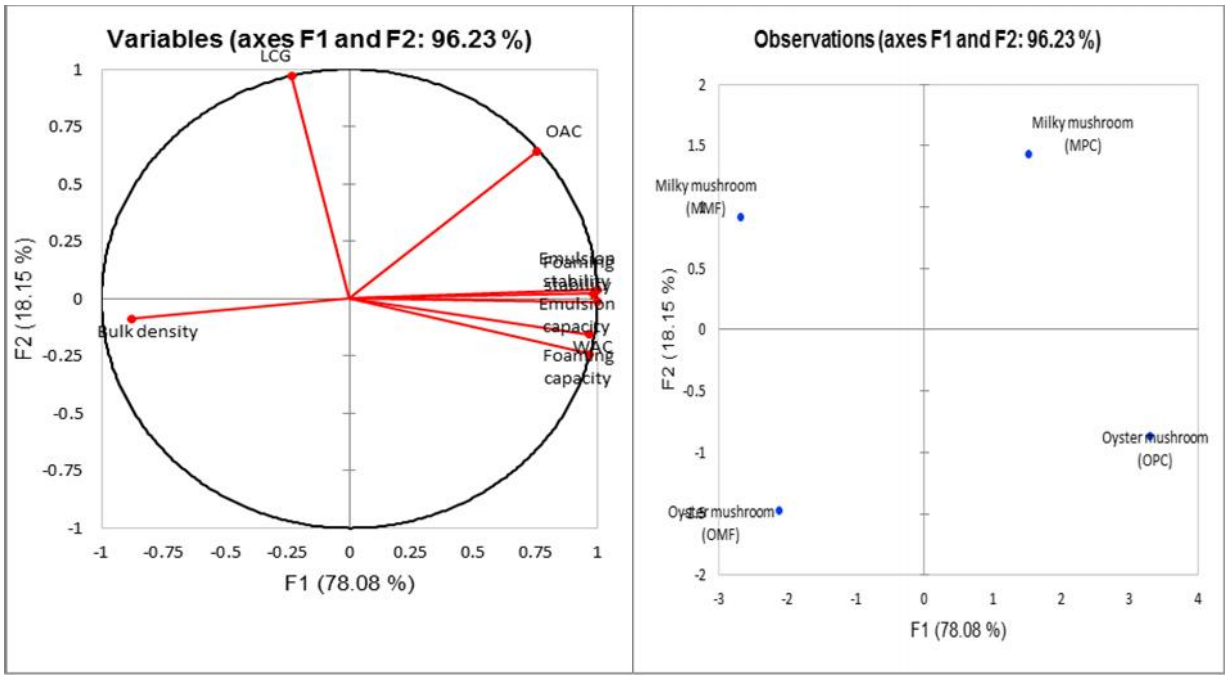

Figure 2) Principal component analysis (PCA) of mushroom flour and protein concentrates (a) Loading plot of variables; (b) loading plot of observations

Conclusion: In the present study, *Pleurotus florida* and *Calocybe indica* were used for analyzing and exploring the proximate and functional properties of mushroom flours and protein concentrates. This result has shown that both the flour and protein concentrate were good sources of protein. The PC possessed higher water absorption capacity, foam and emulsion capacities and stabilities with lower bulk density and oil absorption capacity than the flour. From these results, it is concluded that mushroom flour and protein concentrates may be exploited in formulation of food products requiring gel formation.

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