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Proximate Composition of *Pleurotus* Fruit Body Flour and Protein Concentrate

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Abstract: Protein concentrates are important in the food industries because added to food they increase the nutritional value and provide specific functional attributes. Edible mushrooms represent a viable alternative to obtain protein concentrates with an acceptable quality. In the present study protein concentrates were obtained from defatted flour of fruit bodies form two parental *Pleurotus* strain (POS and PCM) and their hybrid (PCM1xPOS1). The chemical analysis of flours and protein concentrates was carried out and also the content of soluble protein as function of pH was determined. The results indicate that the total biological efficiency for two flushes of PCM1xPOS1, PCM y POS strains was 77.8, 81.36 y 85.11%, respectively and the Mean mushroom size (MS) of POS strain (7.03 g) was higher than the others strains. The protein content in the three strains increased two fold in the protein concentrate ranged from 48.56 to 49.94% of protein. The ash and moisture content was similar in both flours and protein concentrate, while the fiber and carbohydrate content decreased; in contrast the fat content was lower in flours than in protein concentrates. Regarding the pH the higher concentration of soluble protein in flours (0.20 to 0.24g/L) and protein concentrates (2.25 to 2.68g/L) was presented with pH 12. The results show the potential of *Pleurotus* mushrooms in protein concentrates preparation.

Keywords: *Pleurotus*, protein concentrates, hybrid strain, protein soluble, fruit body

INTRODUCTION

Proteins are an important component of living cells due to their structural and functional properties, i.e. catalytic, transport, protection, regulation. Proteins are macro molecules built up of amino acids and its nutritional value is determined by the sequence and type of amino acids present in a protein. Alternative sources of food proteins, different to animal origin, are of utmost importance, and edible fungi represent a viable option for high quality proteins for human nutrition. Chemical analysis of some species of edible fungi show a dry matter protein content ranging [1-3] from 19 up to 35%. Additionally, protein nitrogen represents more than 50% of total nitrogen[4] and its final content is influenced by substrate composition, pileus size, cropping time and fungal species[5]. Thus, production of easily growing edible mushrooms, like *Pleurotus* spp., may well represent an alternative source of food proteins.

Defatted flours from oil seeds show a dry matter protein content in the range of 45 up to 60 % and protein concentrates are more refined products showing a higher dry matter protein content, i.e. more than 70%, this resulting from elimination of almost 50% carbohydrates and other minor components[6-8]. Protein concentrates have become important in food industry and due to their high protein content; they can be added to foods to increase nutritional value and to provide specific functional properties[9]. Success of plant protein concentrates has mainly depended upon their functional properties, which is influenced by intrinsic factors (i.e. protein composition and conformation), by environmental factors (growing conditions) and by isolation and purification methodologies.

The edible mushroom *Pleurotus tuberregium* has been proposed as a source of protein concentrates[10], therefore, production and characterization of protein concentrates obtained from parental and hybrid strains of commercially grown *Pleurotus* spp. species was relevant. Thus, this study was aimed to determine protein content of flours and protein concentrates obtained from parental and hybrid *Pleurotus* sp. strains.

METHODS

Biological material: Hybrid strain PCM_1xPOS_1 was obtained by pairing compatible neohaplonts recovered by dedikaryotization of two parental strains, i.e. PCM (supplied from Faculty of Chemistry, UNAM) and POS (commercial strain). Stocks of all three strains are deposited at the fungal collection of the Bioconversion Laboratory of the Biotechnology Interdisciplinary Professional Unit (UPIBI) IPN.

***Pleurotus* cultivation:** Substrates were prepared by immersing chopped wheat straw in boiling water for 2 h. After cooling down, 150 g grain spawn (completely colonized with mycelium) was mixed with 2 kg pasteurized straw and packed in 32 cm x 49 cm polypropylene plastic bags. Bags filled with substrate were incubated in darkness for 15 to 25 days and once mycelium has completely invaded the substrate, bags were transferred to a fruiting chamber with 15-30°C, 85-95% relative humidity, 12h /day lighting, and continuous ventilation to keep CO_2 concentration lower than 0.5%. Mature fruit bodies were cropped and weighted individually. Two flushes were cropped for each bag in a 7 day interval. Fresh fruit body weights were used for productivity measurements, i.e. Biological efficiency (BE %) = g of fresh fruit bodies / 100 g dry substrate; Production rate (PR %) = Biological efficiency / cropping time (days); Mean mushroom size (MS) = total weight of harvested fresh mushrooms /total number of harvested mushrooms. Mushrooms were dried

at 40°C and then milled to a fine powder (mesh 50 Montinox, México) and stored in sealed air tight containers.

Proximate chemical analysis: AOAC [11] methodology was used for water, fat, crude fiber and ash content in flours and protein concentrates. Micro Kjeldahl methodology was used for total protein content with a conversion factor[12] of 4.38. Carbohydrates were assessed by difference (Carbohydrates (%) = 100% - (ashes (%) + lipids (%) + crude fiber (%) + protein (%)).Three samples were analyzed for all determinations.

Production of protein concentrates: Alkaline extraction and isoelectric precipitation was used[9]. Mushroom flour samples were defatted with hexane 1:5 (w/v) for 8 h at 4°C. Solvent was evaporated and defatted flour was sieved through 80 mesh (Montinox, México). Defatted flour (30 g) was dissolved in 200 mL distilled water and then NaOH 2M was added until pH 12 was attained, thereafter, samples were centrifuged (J2-MC Beckman Coulter) at 12400 g and 4°C for 30 min. Supernatant was adjusted to the corresponding isoelectric pH of proteins, previously assessed (Table 1). The resulting pellet was frozen at -35°C and immediately lyophilized (Labconco). This procedure was separately followed for each 3 flours.

Table 1: Isoelectric point of defatted flours from *Pleurotus* spp. strains

	Ip (pH)
F-PCM	4.15
F-POS	4.0
F-PCM_1xPOS_1	3.96
Ip : Isoelectric point	

Soluble protein: Soluble protein in mushroom flours and protein concentrates was determined following the methodology proposed by Ogunwolu *et al.*[13] with certain modifications; 1% solutions of flour or protein concentrates were adjusted to pH 2, 4, 6, 8, 10 and 12. Solutions were agitated for 30 min and then centrifuged at 5514 g for 30 min and soluble protein was determined in supernatant following Bradford methodology[14].

Statistical analysis: Data were analyzed with one way ANOVA and thereafter classified according to Duncan test. SPSS ver. 15 was used to compare proximal composition of mushroom flours and protein concentrates.

RESULTS

***Pleurotus* cultivation:** Table 2 shows productivity parameters of the 3 *Pleurotus* spp. strains for the first crop and the accumulated values for the second crop. No significant differences were found among 3 strains regarding neither biological efficiencies (BE) nor production rate (PR). However, after the second crop POS strain showed a significantly higher mushroom size (MS), 7.03 g /mushroom, than the other 2 strains. Total biological efficiency was 77.8, 81.36 and 85.11% for strains PCM_1xPOS_1, PCM and POS, respectively.

Salmones *et al.*[15] reported that after 3 to 5 flushes, hybrid strains of *P. djamor* (Fr.) Boedijn showed higher biological efficiencies (73.6 to 114.4%) than the corresponding parental strains (53.6 to 86.4%). In this study,

hybrid strain PCM_1xPOS_1 produced similar yields than parental strains. More recently, Patil *et al.*[16]. cultivated *Pleurotus ostreatus* and reported that after 3 flushes, biological efficiency on soya bean substrate was 85.16% and 72.06% on wheat straw substrate. Remarkably, in this study, parental strains yielded 81.36 and 85.11% biological efficiencies but just after 2 flushes. Moreover, the biological efficiencies of parental and hybrid strains obtained in this study are similar to those reported by Salmones *et al.*[15] for parental and hybrid *P. djamor* strains after 2 flushes.

Table 2: Productivity parameters of *Pleurotus* spp. strains

Strain	BE_1 (%)	BE_T (%)	PR_1 (%)	PR_T (%)	MS
PCM	48.53 ± 3.93	81.36 ± 5.94	1.36 ± 0.90	2.39 ± 0.15	5.42 ± 0.38[a]
POS	57.68 ± 3.36	85.11 ± 4.93	1.71 ± 0.12	2.81 ± 0.19	7.03 ± 0.47[b]
PCM_1xPOS_1	54.49 ± 3.12	77.80 ± 4.65	1.48 ± 0.09	2.41 ± 0.15	5.72 ± 0.31[a]

Note: BE is Biological efficiency, PR rate production and MS mushroom size. Values are mean ± ESM. Values with different letters in the same column indicate significant differences (Duncan, $p<0.05$), n=10.

Regarding mushroom size (MS), significant differences ($F_{(2,62)} = 4.61$, $p=0.014$) were found and 2 groups were formed after *Post Hoc* Duncan test, PCM and PCM_1xPOS_1 are in group "a" with 5.42 and 5.72 g / mushroom whereas strain POS is in group "b" with 7.03 g / mushroom. Onupha and Obi-Adumanya [17] reported MS values of 6.0 g and 8.4 g / mushroom for *Pleurotus tuberregium* produced on sawdust, humus and wood chips while Royse *et al.* [18] reported values ranging from 9.2 to 13.0 g / mushroom for a *Pleurotus cornucopiae* strain.

Proximal analysis of mushroom flour and protein concentrates: Table 3 shows chemical composition of mushroom flours obtained from the three *Pleurotus* spp. strains. No significant differences were found in regards to composition. Water content ranged from 7.62 to 7.93 %, these values are lower to those reported by Akyuz & Kirbag[19] for *P. sajor-caju* and *P. ostreatus*, 10.0% and 10.3%, respectively, probably as a result of differences in storage conditions. Ash content of mushroom flours ranged from 7.94 to 8.01 %, similar values to previous reports for different *Pleurotus* species. Crisan and Sands[2] reported 6.1 to 9.8 % for *P. ostreatus*, and Gupta *et al.* [20] indicated 7.92 to 8.16 % for *P. sajor-caju* while, in another study, Chirinang & Intarapichet [21] reported ash contents of 7.95 % for *P. sajor-caju* and 5.81% for *P. ostreatus.*

Mushrooms are considered a high quality food since they are a good source of digestible proteins and show a remarkably low lipid content. The main classes of lipid compounds present in mushrooms include phospholipids, sterols, sterol esters, acylglycerols as well as free fatty acids[22] . Lipid content in mushroom flours ranged from 1.92 to 2.0 %, similar value to *P. ostreatus* flours reported by Hung & Nhi[23], 2.5 %, and Crisan y Sands[2], 1.6 to 2.2 %. Crude fiber content in mushroom flours ranged from 8.60 to 9.29% for the 3 strains, Crisan and Sands[2] reported 7.5 to 8.7% for *P. ostreatus* while 12.3% crude fiber has been reported for *P. sajor-caju*[24].

Carbohydrates are the major constituents of edible mushrooms, 16 to 85 g / 100g fresh weight[6]. Carbohydrate content in mushroom flours of parental and hybrid strains ranged from 54.62 to 56.65 %. In

previous studies, carbohydrate content in *P. ostreatus* (Jacq. Fr.) flours was reported[25] to be 64.1 % and 57.6 to 81.8 by Crisan y Sands [2] while Dundar *et al.* [26] reported 39.39% for *P. eryngii*.

Table 3: Proximate composition (g/100g) of flour (F) and protein concentrate (PC) of *Pleurotus* spp. strains

	F-PCM	F-POS	F-CM$_1$xPOS$_1$	PC-PCM	PC-POS	PC-PCM$_1$xPOS$_1$
Water	7.93 ±0.36	7.64±0.27	7.62±0.34	7.60±0.31	7.39±0.19	7.22±0.21
Ash	7.94±0.06	7.96±0.04	8.01±0.08	7.50±0.29	7.59± 0.31	7.59±0.29
Protein	24.25±0.85	25.78±1.00	26.81±0.86	49.85±1.90	49.94± 2.42	48.56± 0.17
Fat	1.97±0.15	2.00±0.16	1.92±0.09	5.96±1.17	6.11± 1.05	5.66±1.22
Crude fiber	9.29±0.38	8.60±0.14	8.64±0.42	0.00	0.00	0.00
Carbohydrates	56.55±1.10	55.65±1.03	54.62±0.42	36.69±2.21	36.36± 3.48	38.19±1.09
Vales are mean ± MSE, with n=3.						

In general, wild mushrooms have shown to be richer sources of protein with a lower amount of fat than commercial mushrooms[27]. Crude protein content of edible mushrooms varies greatly and ranges from 15 to 35% of dry weight, depending on the species, varieties, and stage of development of the fruiting body[1]. In this study, protein content in mushroom flours ranged from 24.25 to 26.81 %, similar values to previous reports by Dunkwal & Jood[28] for *P. sajor-caju*, 25.3 %, by Valencia del Toro *et al.* [29] for three *Pleurotus* spp strains, 26.7 to 28.2 %, and by Crisan and Sands[13] for *P. ostreatus*, 10.5 to 30.4 %.

Proximal composition of protein concentrates from the 3 *Pleurotus* spp. strains is also shown on Table 3. Crude fiber was not obtained in protein concentrates. Ash content in concentrates was in the range of 7.50 to 7.59 %, and individual values were was significantly lower than those of the corresponding mushroom flour ($F_{(1,\ 18)}$ =5.50, p= 0.037). In regards to water content no statistical differences were found between concentrates and flours. Fat content in protein concentrates ranged from 5.66 to 6.11 %, they were significantly higher than those of the corresponding flours ($F_{(1,\ 18)}$ =34.90, p= 0.0001). A large increase in lipid content, almost 3 fold, has been reported in protein concentrates produced from legume seeds flours[30,31] Protein isolates (or concentrates) from legume seeds normally have high lipid content due to a binding mechanism between protein and lipids that may result from emulsification of the lipids by the protein[24,31] .

Protein concentrates produced from the parental and hybrid strains showed significantly higher protein content (48.56 to 49.94 %) than the corresponding values in mushroom flours ($F_{(1,18)}$=428.05, p= 0.0001). These values are higher than those reported by Alobo[10] for *P. tuber-regium* protein concentrates, i.e. 40.4 %. As suggested by Wani *et al.* [32], such differences might be a result of the effect of substrate composition, pileus size, harvest time and strains on mushrooms protein content. On the other hand, protein concentrates obtained from various plant sources show higher protein contents. For cereals, Chandi & Sogi [33] reported protein contents of 54.08, 58.92 and 52.46 % for 3 different sources of rice bran. Furthermore, protein concentrates produced by isoelectric precipitation, reached 72.3 % protein content for *Phaseolus vulgaris* and 69.2% for *Phaseolus coccineus*[23] whereas , 93.2% for *Lupinus campestris* and 92.3% for soya bean[34] .

As expected, carbohydrate content in protein concentrates (36.36 to 38.19 %) was significantly lower, almost 65%, than in mushroom flours ($F_{(1,18)}$=149.56, p= 0.0001). Likewise, Oliviera-Castilllo *et al.*[31] reported a 5 fold reduction of carbohydrate content in protein concentrates as regards to the corresponding flours.

Soluble protein in mushroom flours and protein concentrates: Figure 1 shows protein solubility of mushroom flours and protein concentrates at different pH values. Maximum solubility was attained at pH 12 for both, mushroom flours (0.20 to 0.24 g/L) and protein concentrates (2.25 to 2.68g/L), and as expected, higher concentrations of soluble protein were observed with protein concentrates than with mushroom flours. Protein concentrates still showed good solubility at pH8 but it decreased as pH reached mushroom proteins isoelectric point (pH 4). As observed in Table 3, parental strains showed higher protein solubility than the corresponding hybrid strain. Mushroom flours produced from *P. sajor caju* showed 0.64 g/L soluble protein and 0.687 g/L in the case of *P. cornucopiae* flours[35], however no studies are available reporting soluble protein from *Pleurotus* spp. protein concentrates.

Similar results were mentioned for protein concentrates from sesame seeds reaching 72% soluble protein[36] at pH 10 and 96% solubility for *Vigna unguculata*[37]. According to Gheibi *et al.* [38], environmental factors such as pH, viscosity, ionic strength, temperature, prosthetic groups and solvent composition can affect protein dynamics and structure, especially protein flexibility, and thus protein solubility.

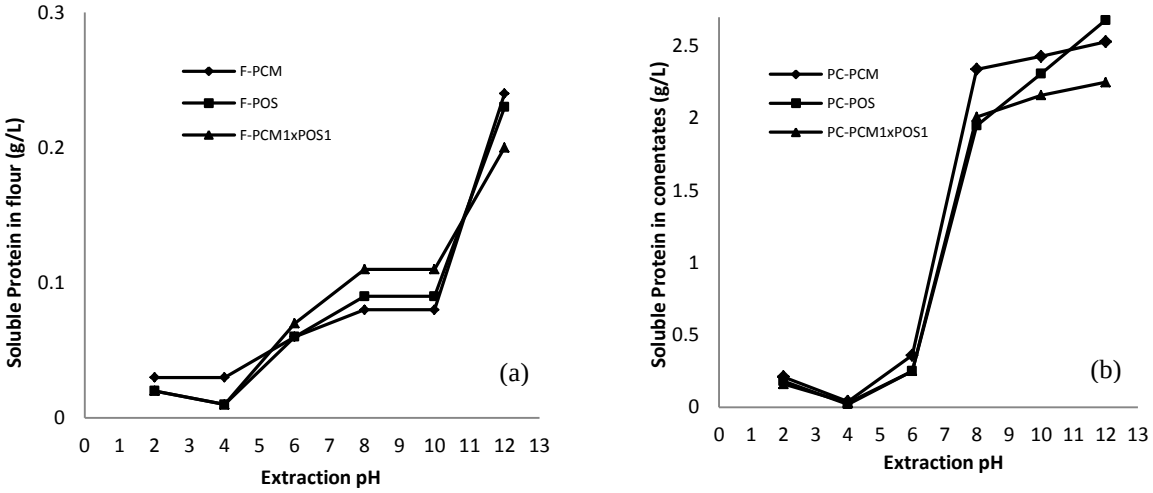

Figure 1: Effects of pH on protein solubility of mushroom flours (a) and protein concentrates (b) from different *Pleurotus* spp. strains (PCM, POS and PCM_1xPOS_1)

Varying solubility as pH changes is explained in terms of electric charges on proteins in concentrates. At extreme pH values, protein chains present a net negative or positive charge resulting in repulsion of protein molecules, thus promoting protein solubility[39]. In addition, hydrophopic interactions in proteins occur between nonpolar regions of their amino acid residues through Van der Waals forces and protein molecules are then driven by the gain in free energy that results from their movement from polar (aqueous) to nonpolar environment[3].

CONCLUSIONS

Maximum protein solubility of mushroom flours and protein concentrates was attained at alkaline pH (12). Although no significant differences in protein solubility were observed among the flours and concentrates

obtained from the two parental strains and their respective hybrid, protein content of protein concentrates showed a two fold increase in relation to the corresponding flour. These results contribute to the knowledge of protein concentrates derived from *Pleurotus* spp. fruiting bodies.

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