

## Functional properties of flours and protein concentrates of 3 strains of the edible mushroom *Pleurotus ostreatus*

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**Abstract** *Pleurotus ostreatus* is an edible mushroom with significant nutritional properties and highly valuable protein concentrates can be obtained from its fruit bodies. Functional properties of flours and protein concentrates derived from 3 *Pleurotus ostreatus* strains (PCM, POS and hybrid PCM × POS) were evaluated in this investigation. Fruit bodies were produced on wheat straw substrate, flours were obtained from dried and grinded fruit bodies and protein concentrates were extracted from flours by alkaline solubilization. For all 3 strains, pale yellow flours were obtained and protein concentrates showed a grayish brown color. Flour bulk densities ranged from 0.52 to 0.64 g/mL, a higher value than those for protein concentrates, i.e. 0.30–0.35 g/mL. The highest water absorption capacities (WAC) were observed for flours (300–418.8%) while

protein concentrates presented higher oil absorption capacity (OAC) (173.3–214.1%). Flours and protein concentrates presented a minimal gelation concentration of 2%. Protein concentrates showed a higher foam capacity formation (FC) at pH 8. Likewise, flours and protein concentrates presented higher foam stability (FS) at alkaline pH (8 and 10). Emulsion activity index (EAI) for flours ranged from 3.96 to 26.68 m<sup>2</sup> g<sup>-1</sup> whereas for protein concentrates ranged from 1.55 to 10.28 m<sup>2</sup> g<sup>-1</sup>. These results indicate that flours and protein concentrates from *P. ostreatus* have remarkable functional properties, valuable in food industry where foaming and emulsifying properties are required.

**Keywords** *Pleurotus ostreatus* · Protein concentrates · Functional properties · Edible mushrooms

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### Introduction

*Agaricus bisporus* ranks in the first place among edible cultivated fungi followed by *Lentinula edodes* and *Pleurotus ostreatus* (Corrêa et al. 2016). *Pleurotus* cultivation, however, shows many advantages, it has the shortest cultivation cycle, grows at moderate temperature, 25–30 °C, grows on a broad range of lignocellulosic materials (Sánchez 2004), and *Pleurotus* cultivation is rather simple and low-cost since sophisticated equipment, complicated technology or resources are not required (Tesfaw et al. 2015). Edible mushrooms are important in human food due to their nutritional value, they present a high protein content with a good balance in essential amino acids, high fibre and low lipid content (Khatun et al. 2012). Proximal chemical analysis of edible cultivated mushrooms shows dry weight

protein content in the range of 19 up 35% (Chang and Buswell 1996).

Fruit bodies of the edible fungus *Pleurotus* sp. 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 (Maftoun et al. 2015). Chemical composition of *P. ostreatus* fruit bodies, as recently reported by Akyüz and Kirbag (2010) on a dry matter basis, is 6% ashes, 0.5% lipids and 41.6% protein, a higher value than the corresponding for *A. bisporus* (36.3%). Additionally, proteins from these fungi contain all essential aminoacids, especially abundant in lysine and leucine, aminoacids not found in some basic grains (Kim et al. 2009). Furthermore, 98% digestibility has been reported for *Pleurotus* proteins (Valencia et al. 2006), a higher value than for *Schizophyllum commune* (53.2%) and for *Lentinula edodes* (76.3%) (Longvah and Deosthale 1998).

There is an increasing interest for low cost and high protein sources for human diet and usually vegetable proteins have been favored. However, the high quality of fungal proteins has prompted the interest in using them for fortification and enrichment of foods (Akitayo et al. 1999). For an efficient use of fungal flours and acceptance by consumers, it is important to identify their functional properties, i.e. their capacity for water and oil absorption as well as their foaming, emulsifying and gelification capacities (Adebowale and Lawal 2004). Functional properties of proteins are physicochemical properties that determine their yields and behavior in food systems during preparation, processing, storage and consumption (Panyam and Kilara 1996). Functional properties have been established for flours and protein concentrates of *Pleurotus tuberregium* sclerotia (Alobo 2003). Flours from fruit bodies of various edible fungi have been used for production of wheat bread (Okafor et al. 2012). It is therefore necessary to study functional properties of cultivated mushrooms in order to gather elements for using flours and protein concentrates in human foods. The aim of this study is to establish functional properties of flours and protein concentrates derived from fruit bodies of three different *Pleurotus ostreatus* strains.

## Materials and methods

### Biological material

Three *Pleurotus ostreatus* strains from the culture collection of the Cellular Cultivations Laboratory of Instituto Politécnico Nacional (UPIBI-IPN) were used: POS (a commercial strain), PCM (a strain from a culture

collection) and PCM × POS (an hybrid derived from the 2 previous strains).

### Preparation of protein concentrates of *Pleurotus ostreatus*

Fruit bodies from the three *Pleurotus ostreatus* strains were produced on pasteurized wheat straw substrate. Fruit bodies were dried at 40 °C and grinded with an homogenizer at 9000 rpm until a flour mash 50 (Montinox, México) was obtained. Flours were defatted using hexane 1:5 (w/v) with continuous agitation for 8 h at 4 °C. Hexane was eliminated by decantation and defatted flours were placed in an extraction cabinet for 24 h, or until solvent was completely evaporated. Flours were then sieved through mesh 80. Protein concentrates (PC) were obtained by isoelectric precipitation according to Cruz-Solorio et al. (2014).

### Determination of physical and functional properties

#### Color and bulk density

Color Reader CR-10 Konica Minolta was used to evaluate color of flours and protein concentrates. CIE-Lab values ( $L^*$ ,  $a^*$  and  $b^*$ ) were converted to a system RGB (Red, Green and Blue) with converter ColorMine.org. Hexadecimal values were obtained from RGB values with Adobe Color software and then with Munsell code, the color of flours and protein concentrates were accordingly judged. Whiteness index (WI) and Browning index (BI) (which represents the purity of brown color) were assessed with CIE-Lab values using following equations (Maskan 2001):

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$

$$BI = 100 \left( \frac{x - 0.31}{0.17} \right)$$

where

$$x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}$$

Volumetric density was assessed by weight difference and indicated as  $\text{g mL}^{-1}$ .

#### Water and oil absorption capacity

Water absorption capacity and oil absorption capacity of flours and protein concentrates were measured according to the methods proposed by Alobo (2003), with certain modifications, i.e. 5 g of flour or protein concentrates were placed in either 5 mL of water or oil and agitated for 5 min, the suspension was left standing for 30 min and then centrifuged at 3000 rpm for 30 min, the excess water

or oil was decanted. Water absorption capacity (WAC) and oil absorption capacity (OAC) were assessed according to following equations:

$$\text{Water absorption capacity (\%)} (WAC) = \frac{\text{mL of absorbed water}}{\text{weight of initial sample (g)}} * 100$$

$$\text{Oil absorption capacity (\%)} (OAC) = \frac{\text{mL of absorbed oil}}{\text{weight of initial sample (g)}} * 100$$

#### Foaming properties

Foaming capacity (FC) and foam stability (FS) was measured according to Alobo (2003). A sample of 1 g of flour or protein concentrate was mixed with 25 mL of deionized water, after adjusting pH at 6.8, the solution was homogenized at 3000 rpm for 5 min and then placed in a 100 mL test tube. Total volume was registered for 0 min to measure foam capacity and in 10 min intervals, until completing 120 min to measure foam stability. Foaming capacity was assessed according to following equation:

$$\text{Foaming capacity (\%)} (FC) = \frac{VH_2 - VH_1}{VH_1} * 100$$

where  $VH_1$  volume before homogenization,  $VH_2$  volume after homogenization.

Foam stability was assessed according to following equation:

$$\text{Foam stability (\%)} (FS) = \frac{FV_t}{FV_i} * 100$$

where  $FV_t$  foam volume after time (t),  $FV_i$  initial foam volume.

To evaluate the effect of pH on foaming properties, the same methodology was followed but adjusting pH at 2, 4, 6, 8 and 10, respectively.

#### Gelification concentration

The minimum concentration for gelification (MCG) was assessed by the methodology proposed by Alobo (2003) with slight modifications. In 10 mL test tubes, 5 mL solutions of flours and protein concentrates were prepared at 2, 4, 6, 8 and 10% concentrations. After heating test tubes in boiling water for 60 min, they were cooled down in ice and stored at 4 °C for 30 min. Test tubes were placed upside down, the sample not sliding down corresponded to the minimum concentration for gelification.

#### Emulsifying properties

Emulsifying activity index (EAI) and emulsification stability index (ESI) were assessed according to the methodology proposed by Pearce and Kinsella (1978) with minor modifications. A sample of 0.1 g of flour or protein concentrate was mixed with 10 mL of deionized water, 3.3 mL of corn oil was added and pH was adjusted at 2, 4, 6, 8, and 10, respectively. The mixture was homogenized at 20,000 rpm for 1 min and then, 50  $\mu$ L of the resulting emulsion was added to a 5 mL solution of sodium dodecyl sulphate (SDS) (0.1%). Absorbance was measured at 500 nm. Emulsifying activity index (EAI) and emulsification stability index (ESI) were assessed according to following equations:

$$\text{Emulsifying activity index (EAI)} \left( \frac{\text{m}^2}{\text{g}} \right) = \frac{2 \times 2.303 \times A_0}{0.25 \times \text{protein weight (g)}}$$

$$\text{Emulsification stability index (ESI)} (\text{min}) = \frac{A_{10} \times \Delta t}{\Delta A}$$

where  $A_0$  absorbance at 0 min after homogenization,  $A_{10}$  absorbance at 10 min after homogenization,  $\Delta t$  10 min and  $\Delta A = A_0 - A_{10}$ .

#### Statistical treatment

Data were examined for normal distribution (Shapiro–Wilk and Kolmogorov–Smirnov test) and for homogeneity of variance (Levene test). Thereafter different analysis of variance was performed for 1 or 2 factors and repeated measures. Duncan test was carried out when significant differences were found. All tests were performed with SPSS Ver. 22 software.

## Results and discussion

### Physical and functional properties

The protein content in protein concentrates produced from the parental and hybrid strains were 48.56, 49.85 and 49.94% for PCM  $\times$  POS-PC, POS-PC and PCM-PC, respectively. These values were significantly higher than the corresponding values in fungal flours, 26.81, 25.78 and 24.25%, respectively. For the first time, functional and physical properties of flours and protein concentrates were studied using three different *P. ostreatus* strains, two parental and an hybrid strain. As shown on Table 1, flour bulk

**Table 1** Functional properties (WAC, OAC and MCG) and physical properties (bulk density and color) of flour and protein concentrates of three *P. ostreatus* strains

Flour and Protein concentrate	Functional properties			Physical properties					
	WAC (%)	OAC (%)	MCG (%)	Bulk density (g/mL)	L*	a*	b*	BI	WI
WHEAT-F	109.7 ± 0.7 <sup>a</sup>	94.9 ± 0.9 <sup>a</sup>	4	0.78 ± 0.1 <sup>e</sup>	90.8 ± 0.4	0.63 ± 0.0	9.2 ± 0.1	10.9 ± 0.2 <sup>a</sup>	65.1 ± 0.3 <sup>c</sup>
PCM-F	397.8 ± 14.3 <sup>e</sup>	122.2 ± 4.8 <sup>b</sup>	2	0.64 ± 0.1 <sup>d</sup>	72.3 ± 1.3	4.07 ± 1.0	23.30 ± 0.6	42.3 ± 1.5 <sup>b</sup>	63.6 ± 1.4 <sup>b</sup>
POS-F	418.8 ± 11.1 <sup>e</sup>	125.9 ± 0.3 <sup>b</sup>	2	0.58 ± 0.2 <sup>c</sup>	73.8 ± 0.3	6.47 ± 0.5	26.17 ± 0.7	49.4 ± 0.7 <sup>b</sup>	62.3 ± 0.7 <sup>b</sup>
PCM × POS-F	300.0 ± 18.1 <sup>d</sup>	104.8 ± 2.2 <sup>a</sup>	2	0.52 ± 0.1 <sup>b</sup>	72.6 ± 0.6	5.90 ± 1.2	26.27 ± 1.0	49.9 ± 0.9 <sup>b</sup>	61.5 ± 0.9 <sup>b</sup>
PCM-PC	179.17 ± 3.7 <sup>b</sup>	173.3 ± 4.5 <sup>c</sup>	2	0.35 ± 0.1 <sup>a</sup>	32.7 ± 2.0	4.96 ± 0.7	15.63 ± 1.8	74.0 ± 1.6 <sup>d</sup>	30.6 ± 1.6 <sup>a</sup>
POS-PC	214.5 ± 3.6 <sup>c</sup>	214.1 ± 5.9 <sup>c</sup>	2	0.30 ± 0.2 <sup>a</sup>	30.8 ± 0.8	5.16 ± 0.4	13.13 ± 2.5	67.6 ± 0.7 <sup>c</sup>	29.3 ± 0.7 <sup>a</sup>
PCM × POS-PC	182.2 ± 2.3 <sup>b</sup>	195.8 ± 5.4 <sup>d</sup>	2	0.34 ± 0.1 <sup>a</sup>	29.9 ± 0.1	3.73 ± 0.3	13.83 ± 0.1	69.4 ± 0.2 <sup>c</sup>	28.5 ± 0.1 <sup>a</sup>

WAC water absorption capacity, OAC oil absorption capacity and MCG minimum concentration for gelification, L\*, a\*, b\* parameters for CIELAB color system, BI browning index, WI whiteness index. Values are expressed as mean ± standard error of means, n = 3. Different letters in a column indicate statistically significant differences (Duncan’s test  $p < 0.05$ )

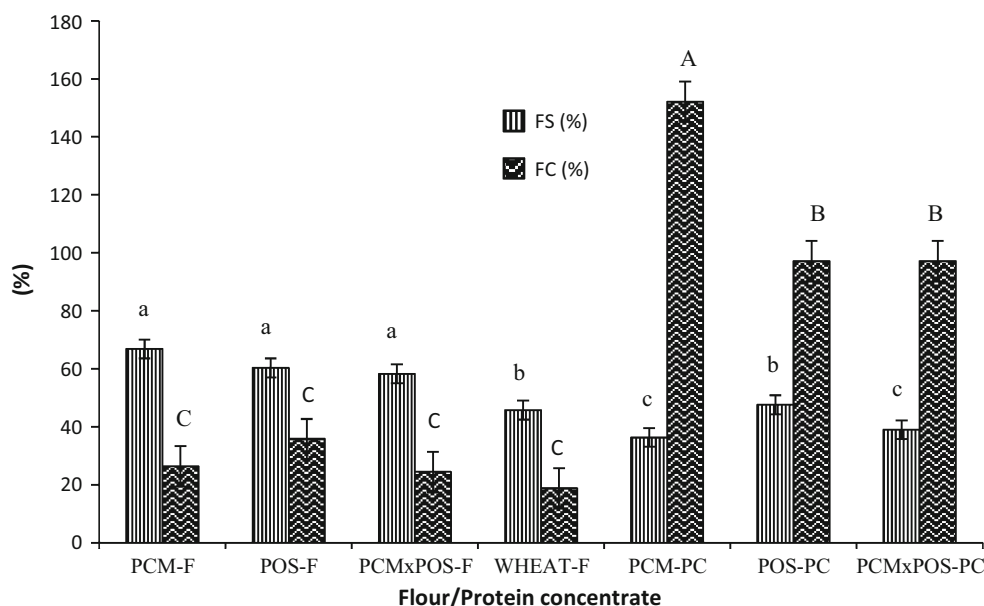
density (0.52–0.64 g/mL) were superior to the corresponding protein concentrates (0.30–0.35 g/mL), ( $F_{(6,20)} = 207.1, p = 0.0001$ ), however, they were lower than wheat flour bulk density (0.78 g/mL). Densities in this range (0.68 g/mL) have been previously reported by Islam et al. (2012) for wheat flour and various studies have reported that flours normally show higher bulk density than the protein concentrates derived from them (Yuliana et al. 2014), suggesting that this property depends upon particle size and the nature of the other components in flour as well as of their interactions. The bulk density value depends on the flour type and the use it is intended for. A large spectrum of values have been reported, as low as 0.179 g/cm<sup>3</sup> for defatted pecan flour up to 0.93 g/cm<sup>3</sup> for full fat black gram flour. Bulk density is influenced by lipid and moisture content (Joshi et al. 2015). A higher bulk density is desirable since this helps to decrease paste thickness, an important factor for child foods and convalescence patients (Padmashree et al. 1987).

*Water and oil absorption capacity*

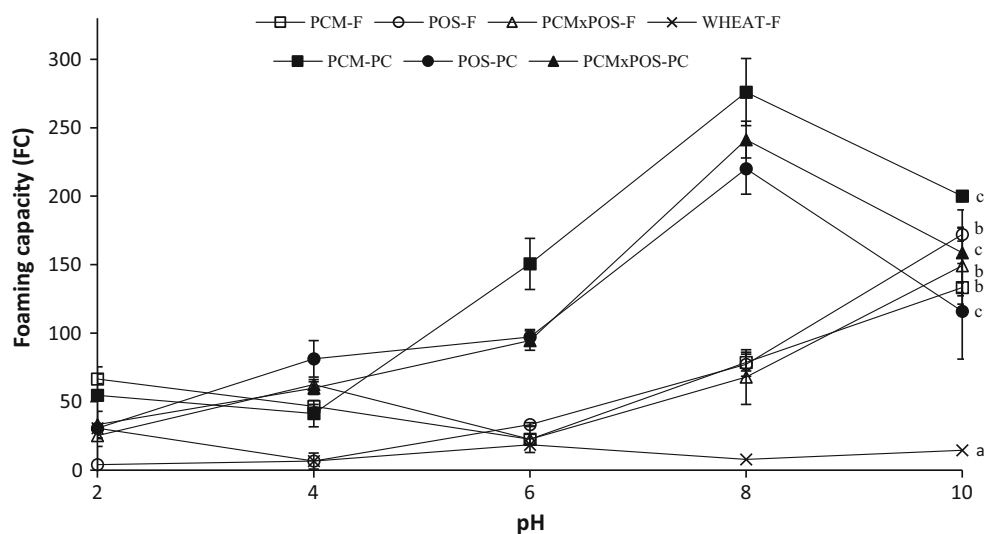
Significant differences were found in water absorption capacity (WAC) ( $F_{(6,20)} = 140.2, p = 0.0001$ ) and five groups were established by Duncan test. Wheat flour showed the lowest value (109.7%), protein concentrates show intermediate values, in groups “b” and “c” with values from 179.1 to 214.5% while flours from the 3 strains are in groups “d” and “e”, thus showing significant higher WAC values than their corresponding protein concentrates, i.e. 397.8 for protein concentrate of PCM in contrast to 179.1 for PCM flour, 418.8 for protein concentrate of POS while 214.5 for POS flour and 300 for protein concentrate of PCM × POS compared to 182.2 for PCM × POS flour.

Although similar, our results showed a clearer tendency than the data previously reported for *P. tuber-regium* sclerotia, i.e. 337% for flour and 331% for protein concentrates (Alobo 2003). WAC is the ability of proteins to hold water against gravity (Shevkani et al. 2015) and since water molecules are captured by hydroxyl groups in carbohydrates through hydrogen bonds, Chel-Guerrero et al. (2002) pointed out that carbohydrates contribute to the high WAC in legume flours, so the lower WAC of protein concentrates, as observed in Table 1, is a result of the decreased carbohydrates content (around 70%) as previously reported by Cruz-Solorio et al. (2014). Water absorption (WAC) of chickpea protein concentrates was reported to be affected by the procedure for isolation of protein concentrates as well as by protein conformation and the availability of polar amino acids for protein-water interactions (Paredes-López et al. 1991). Therefore, the high WAC values of *P. ostreatus* flours allow them to be used as ingredients in bakery and meat products (Mao and Hua 2012), noticeably more suitable than wheat flour. Similarly, wheat flour also showed the lowest oil absorption capacity (OAC), 94.9%, and in this case, protein concentrates from the three *P. ostreatus* strains showed significant higher values than their corresponding flours, i.e. 173.3 for protein concentrate of PCM against 122.2 for PCM flour, 214.1 for protein concentrate of POS while 125.9 for POS flour and finally, 195.8 for protein concentrate of PCM × POS and 104.8 for PCM × POS flour. Oil absorption capacity (OAC) is an important functional property in food technology since higher OAC values promote good flavor retention, better palatability and extended shelf life of foods (Chel-Guerrero et al. 2002) like breads, soups and meat products. The oil absorption capacity of flours is important for the development of new

**Fig. 1** Foaming capacity (FC) and foam stability (FS) at pH 6.8 of flour and protein concentrates of three *P. ostreatus* strains. Values are expressed as mean  $\pm$  standard error of means,  $n = 3$ . Different letters indicate statistically significant differences (Duncan's test  $p < 0.05$ ) for comparisons of treatment means for FC (capital letters) and for FS (lowercase letters)



**Fig. 2** Foaming capacity (FC) at different pH values of flour and protein concentrates of three *P. ostreatus* strains. Values are expressed as mean  $\pm$  standard error of means,  $n = 3$ . Different letters indicate statistically significant differences (Duncan's test  $p < 0.05$ )



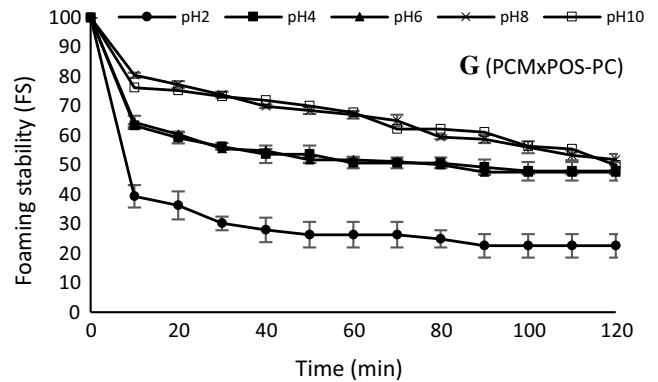
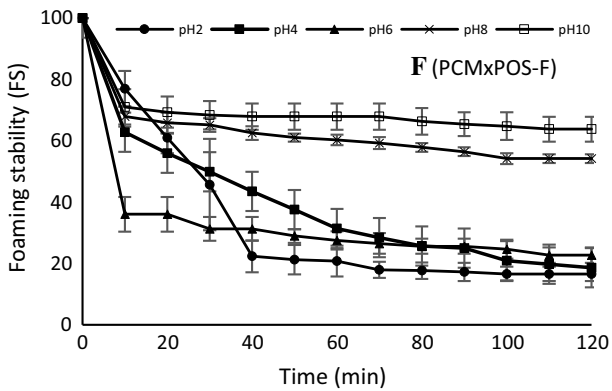
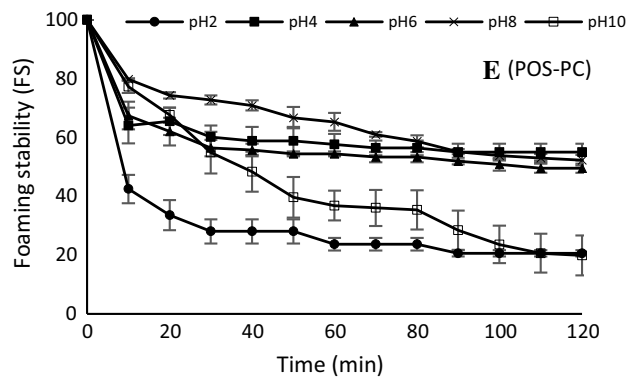
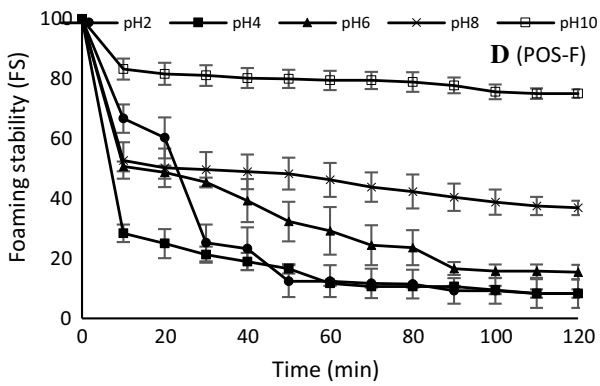
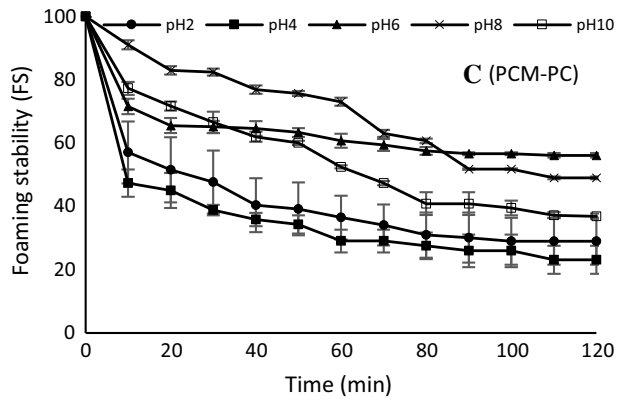
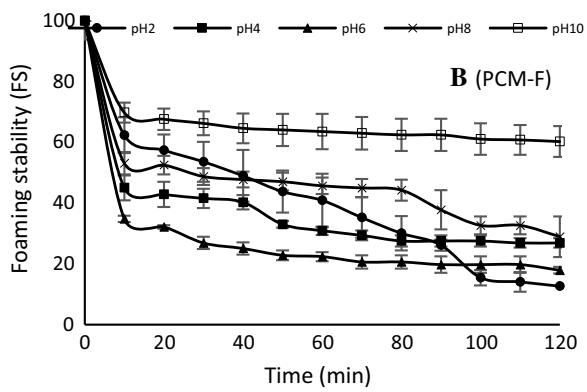
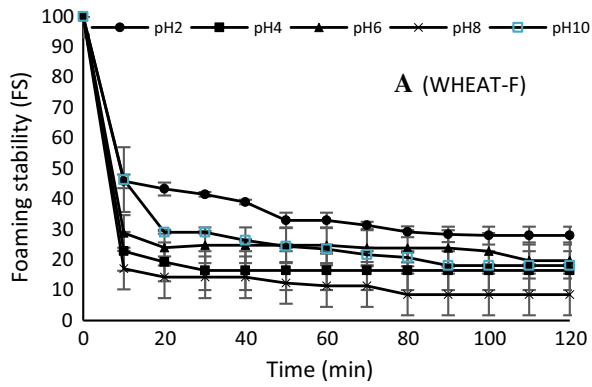
food products, particularly in regards to their storage stability since it promotes flavor binding and prevents development of oxidative rancidity (Siddiq et al. 2010).

#### Gelification concentration

*Pleurotus ostreatus* protein concentrates and flours showed a good gelification property with a lower minimum concentration for gelification (MCG), 2%, than wheat flour, 4%, but also much lower than the results previously reported by Alobi (2003) for *P. tuber-regium* sclerotia, 4% for flour and 6% for protein concentrates. Variations in gelification properties result of the different type of components in flours, i.e. proteins, lipids and carbohydrates, and according to Nithiyantham et al. (2013) gelification

mechanism and gel aspect are controlled by a balance of hydrophobic attractive and electrostatic repulsive interactions. Furthermore, gel properties are influenced by various factors like pH, type and concentrations of electrolytes and type of proteins (Alleoni 2006).

In relation to color of flours and concentrates, one factor variance analyses for browning index (BI) showed significant differences ( $F_{(5,17)} = 4.52$ ,  $p = 0.015$ ) and Duncan test indicated that protein concentrates were darker than the corresponding flours, i.e. 74 versus 42.3 for PCM, 67.6 versus 49.4 for POS and 69.4 versus 49.9 for the hybrid strain PCM  $\times$  POS. The opposite results were found with whiteness index (WI), wheat flour showed the highest value, 65.1, and flours had significant higher WI values than the corresponding protein concentrates,



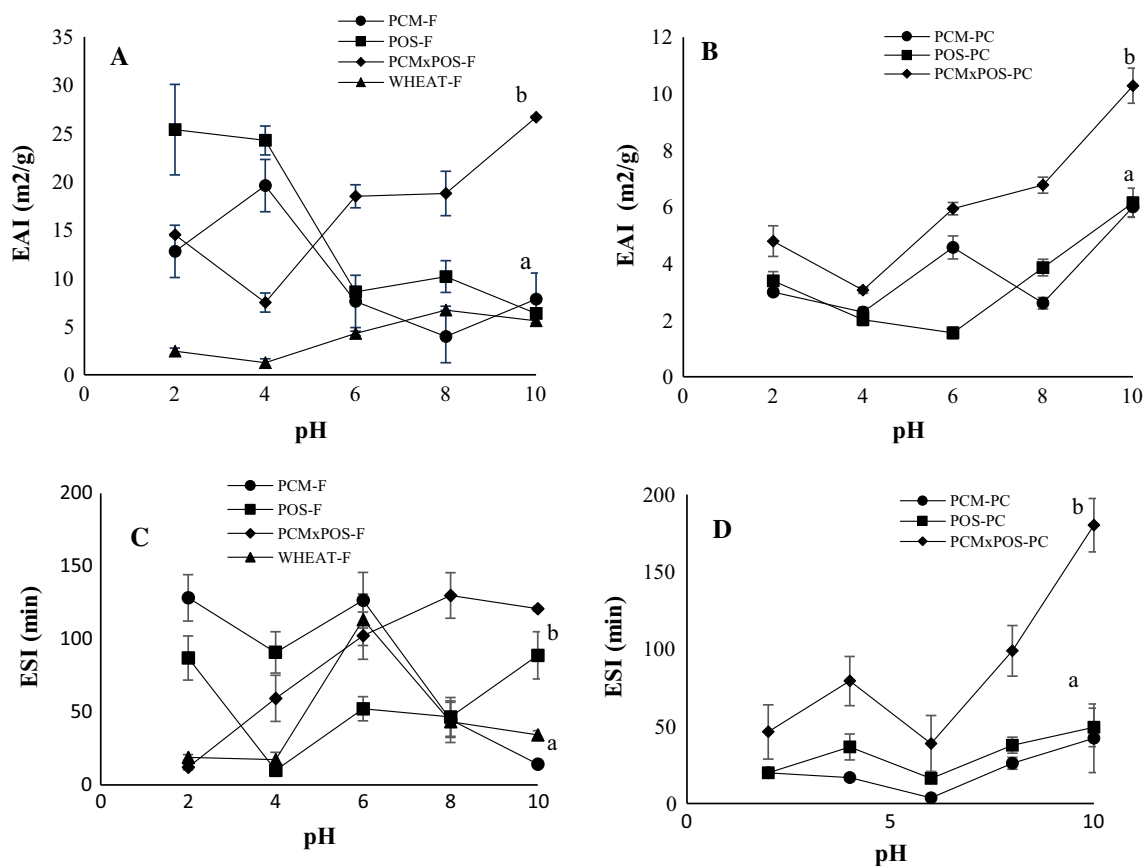
**Fig. 3** Foaming stability (FS) at different pH values of flour and protein concentrates of three *P. ostreatus* strains. Values are expressed as mean  $\pm$  standard error of means,  $n = 3$

( $F_{(5,17)} = 292.48$ ,  $p = 0.0001$ ). Color determination by Munsell code reflects and takes into account BI and WI values, so fungal flours show a pale yellow color, wheat flour a white color and protein concentrates a brownish-gray color. In some protein concentrates derived cereals, like those obtained from rice bran by Kaewka et al. (2009), brown pigments generated by Maillard reaction are responsible for their brown color, though Mwasaru et al. (1999) associated the brown color of protein concentrates from two legumes, to the recovery methods, i.e. isoelectric precipitation and micellar solubilizing. In this study, color change in protein concentrates are influenced by the method for recovery of protein concentrates and by the Millard reaction due to the presence of carbohydrates, as previously reported (Cruz-Solorio et al. 2014). Has been reported that the variations in the color characteristics of the protein isolates could possibly be due to the presence of different types and concentrations of coloring constituents,

for example polyphenols, present in the flours that might have interacted with the proteins and extracted along with them (Shevkani and Singh 2015).

#### Foaming properties

Significant differences were found in foaming capacity of flours and protein concentrates at pH 6.8 ( $F_{(6,14)} = 46.5$ ,  $p = 0.0001$ ), it was significantly higher in protein concentrates (109.1–144.5%) than in flours (60.3–69.6%) (Fig. 1). Likewise, Wu et al. (2009) found that peanut protein concentrates showed higher FC (50%) than the corresponding flours (28%) but contrarily, Alobo (2003) reported higher FC values for flours than for protein concentrates of *P. tuber-regium* sclerotia. Protein concentrates have the double content of proteins than flours (Cruz-Solorio et al. 2014) and this account for differences in FC since proteins unfold and interact among them to form a film or multilayer of proteins in the air/liquid interface protecting air bubbles from collapsing allowing formation of larger volumes of foam (Akintayo et al. 1999). Film formation by proteins may be limited by the



**Fig. 4** Emulsifying activity index (EAI) (4A and 4B) and emulsification stability index (ESI) (4C and 4D) at different pH values of flour and protein concentrates of three *P. ostreatus* strains. Values are

expressed as mean  $\pm$  standard error of means,  $n = 3$ . Different letters indicate statistically significant differences (Duncan's test  $p < 0.05$ )

hydrophobicity forces and electrostatic interactions that maintain the native state of proteins as well as by intermolecular disulfide covalent bonds (Alleoni 2006). Although protein concentrates showed larger FC values, their foam stability (FS), 36.3–47.5%, was significantly lower ( $F_{(6,14)} = 12.4, p = 0.0001$ ) than the FS values of the corresponding flours, 45.7 to 66.8% (Fig. 1). Protein concentrates of *P. tuber-regium* sclerotia showed higher FS values than the corresponding flour as reported by Alobo (2003), however, in this case *P. tuber-regium* protein concentrate had a lower lipid content (1.5%) than flour (5.95%) while in this study, *P. ostreatus* protein concentrates had a higher lipid content, 5.6–6.1%, than flours, 1.9–2.0% (Cruz-Solorio et al. 2014). Protein concentrates with higher lipid content have lower FS and, additionally, the high protein concentration favors protein–protein interactions producing foam collapse (Toews and Wang 2013). Foaming properties have been also suggested to be influenced by the presence of other components, like carbohydrates, an important component in flours (Sreerama et al. 2012).

Foaming properties of flours and protein concentrates were affected by pH, flours generally showing lower FC values than protein concentrates (Fig. 2), but between pH 6 and 8 significant differences were indicated by ANOVA ( $F_{(6,14)} = 46.56, p > 0.001$ ). As shown in Fig. 2, protein concentrates present the highest FC values at pH 8 (220–276%) decreasing to pH 10 (116–200%). Similar results were reported for protein concentrates of *Ginkgo biloba* seeds by Deng et al. (2011), i.e. a decrease of FC as pH increased from 8 to 10, and the same behavior was reported by Klompong et al. (2007) for hydrolyzed proteins of fish (*Selaroides leptolepis*) indicating lower FC values at pH 10. A high FC at a certain pH value is caused by an increased protein flexibility resulting in a faster diffusion to the air–water interface to encapsulate air bubbles, then incrementing foaming capacity (FC) (Kinsella et al. 1985). Configuration of protein molecules is also modified by pH, thus altering foaming capacity and foam stability (Makri and Doxastakis 2006). Contrariwise, foaming capacity of *P. ostreatus* flours increased from pH 6 to pH 10, from values as low as 18.66–172.00, 158.66 and 133.66% for POS-F, PCM × POS-F and PCM-F, respectively. These results agree with a study of flours from four varieties of *Phaseolus vulgaris* that showed higher FC values, 103.8–142.0%, at alkaline pH (Wani et al. 2013). Behavior differences of flours and protein concentrates is probably associated with the diversity of proteins present in flours but not in protein concentrates since these were obtained by precipitation at pH 4, resulting in recovery of certain types of proteins. Low molecular weight proteins, like albumins and globulins, might be lost during production of protein concentrates from flours of *Pleurotus* fruit bodies by

isoelectric precipitation (pH 4). Supporting evidence was published by Yalçın and Çelik (2007), they reported that SDS-PAGE patterns of barley flours differed from their corresponding protein isolates, which showed a decrease in albumins and globulins. Similarly, a loss of albumins was found in protein isolates from chickpea and *Lathyrus* seeds (Pastor-Cavada et al. 2010). The formation of protein aggregates or polymers has been reported by Shevkani and Singh (2015) as a result of covalent and non-covalent interactions, produced between hydrophobic aminoacids during isolation of protein concentrates from legume products.

Foam stability (FS) for flours and protein concentrates of the three *P. ostreatus* strains markedly decreases, ca. 40%, during the first 10 min in all cases, as shown on Fig. 3. After 60 min, protein concentrates, PCM-PC, POS-PC and PCM × POS-PC, exhibited higher foam stability at pH 8 (73.05, 65.24 and 67.06%, respectively) than the corresponding flours, which presented higher stabilities at pH 10, i.e. 63.49% for PCM-F, 79.86% for POS-F and 67.91% for PCM × POS-F. Both, protein concentrates and flours exhibited better stability at alkaline pH (pH 8 and pH 10) while it was very reduced at pH 2. Yuliana et al. (2014) reported a similar behavior for protein concentrates from cashew shell, i.e. higher foam stabilities at alkaline pH values (8–10), 76.43 and 90.01%, after 60 min. According to these authors, higher foam stability at pH values above pI is related to an increased solubility of proteins causing a viscosity increase and favoring formation of a protein cohesive multilayer at the interface. Furthermore, the negative charge of proteins at alkaline pH also reduces the tendency of foam particles to collapse and hence increases foam stability (Yuliana et al. 2014). Noteworthy, wheat flour displayed an opposite behavior than fungal flours, foam stability was higher at acid pH. Improved foaming properties of flours at acid pH in relation to protein concentrates is attributed to their better flexibility in aqueous solutions and stronger interactions at the air–water interface resulting in stable foams (Aluko et al. 2001). This factor might explain the different behavior of fungal and wheat flours in addition to the differences in regards to their amino acid profile.

#### Emulsifying properties

Emulsifying properties of *P. ostreatus* flours and protein concentrates at varying pH are shown on Fig. 4. Flours exhibited higher emulsifying activity index (EAI), 3.96–26.68 m<sup>2</sup>/g (Fig. 4a), than the corresponding protein concentrates, 1.55–10.28 m<sup>2</sup>/g (Fig. 4b). Emulsifying capacity of proteins has been reported to diminish with increasing concentration (Kinsella et al. 1985) as observed with protein concentrates of beans (Sathe et al. 1982) and



sunflower (Lin et al. 1974). Protein concentration in *P. ostreatus* protein concentrates doubles that in flours (Cruz-Solorio et al. 2014) explaining their lower EAI values. For protein concentrates, EAI decreased as the protein pI was approached (Fig. 4b) in agreement with a lowering amount of soluble protein (Cruz-Solorio et al. 2014). The highest EAI values for protein concentrates were observed at alkaline pH (8–10) while that of PCM × POS-PC was significantly higher than the corresponding EAI for PCM-PC and POS-PC ( $F_{(2,6)} = 86.7$ ,  $p = 0.0001$ ). Similarly, Jamdar et al. (2010) reported for peanut protein hydrolysate, low EAI at the isoelectric point (pH 5.6) and high values at an alkaline pH (pH 9). Likewise, Shevkani et al. (2015) reported that protein concentrates from two different legume products showed a low EAI at pH 4 and 6, which increased at pH 7. Emulsifying activity depends of exposure of hydrophobic amino acids at the oil–water interface, which affects protein solubility and determine emulsifying properties (Du et al. 2014). Emulsion stability index (ESI) for flours were in the range of 10.1–130.0 min (Fig. 4c) and for protein concentrates 3.64 to 180.27 min (Fig. 4d). While stability of protein concentrates tended to improve at alkaline pH (8 and 10), flours from parental strains, PCM-F and POS-F, showed increased stability at acid pH. Similar results were reported by Jamdar et al. (2010) for peanut protein isolates showing increasing emulsion stability index (ESI) at alkaline pH (7 and 9) and acid pH (3).

## Conclusion

Three different *P. ostreatus* strains, two parental and an hybrid strain, were used to study functional properties of fungal flours and protein concentrates. These properties were established to vary depending on strain. Both, fungal flours and protein concentrates were found highly suitable for formulation of food products requiring foaming and emulsifying properties or gel formation. Foam formation and stability, as well as emulsion activity and stability were highly dependent on pH, fungal flours and protein concentrates showed higher FC, FS, EAI and ESI at alkaline pH. Protein concentrates showed higher foaming capacity than flours but their foam stability was lower. Gelification concentration was also lower for protein concentrates than for flours. Protein concentrates showed higher browning index than flours. Experimental results indicate that *P. ostreatus* flours and protein concentrates can be used by the food industry in product formulations of food products when foaming properties, gel formation or emulsifying properties are required.

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