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## Physicochemical, techno-functional and antioxidant properties of *Pleurotus* spp. powders

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### ABSTRACT

Edible mushrooms are considered an important source of nutritional and functional components; therefore, they have been proposed as promissory ingredients for foods. The aim of this study was to evaluate the physicochemical, techno-functional, and antioxidant properties of two *Pleurotus* spp. powders (*P. ostreatus* and *P. pulmonarius*). Regarding physicochemical properties, *Pleurotus* spp. powders showed ( $p < 0.05$ ) pH values near to neutrality in comparison to soy protein (control), and all samples showed slightly brown color. Concerning their techno-functional properties they retained water and oil, and there was swelling, foaming and gelling capacities ( $p < 0.05$ ). Results demonstrated the presence of primary (carbohydrates and protein) and secondary metabolites (phenols, flavonoids, and chlorogenic acid), as well as antioxidant capacity (free-radical and cations scavenging activity and reducing power) in dependence of solvent extraction ( $p < 0.05$ ). Also, all powdered mushroom extracts reduced ( $p < 0.05$ ) lipid oxidation of meat homogenates subjected to thermal treatment. In conclusion, the results confirm that *P. ostreatus* and *P. pulmonarius* powders are useful as functional ingredients for meat products.

**Keywords:** edible mushrooms, *Pleurotus ostreatus*, *Pleurotus pulmonarius*, functional properties, biological activity.

### Propiedades fisicoquímicas, tecnofuncionales y antioxidantes de harinas de *Pleurotus* spp.

### RESUMEN

Los hongos comestibles son considerados una fuente importante de componentes nutricionales y funcionales; por ello, se han propuesto como ingredientes potenciales para los alimentos. El objetivo de este estudio fue evaluar las propiedades fisicoquímicas, tecnofuncionales y antioxidantes de dos harinas de *Pleurotus* spp. (*P. ostreatus* y *P. pulmonarius*). En cuanto a las propiedades fisicoquímicas, las harinas de *Pleurotus* spp. mostraron ( $p < 0.05$ ) valores de pH cercanos a la neutralidad en comparación con la proteína de soja (control), y en todas las muestras el color fue ligeramente marrón. En cuanto a sus propiedades tecnofuncionales, retuvieron agua y aceite, hubo hinchamiento, formación de espuma y gelificación ( $p < 0.05$ ). Los resultados demostraron la presencia de metabolitos primarios (carbohidratos y proteínas) y secundarios (fenoles, flavonoides y ácido clorogénico), así como capacidad antioxidante (eliminación de radicales libres y cationes, y poder reductor) dependiendo del disolvente utilizado en la extracción ( $p < 0.05$ ). Además, todos los extractos de las harinas de hongos disminuyeron ( $p < 0.05$ ) la oxidación lipídica de los homogeneizados de carne sometidos a un tratamiento térmico. En conclusión, se confirma que las harinas de *P. ostreatus* y *P. pulmonarius* son útiles como ingredientes funcionales para los productos cárnicos.

**Palabras clave:** hongos comestibles, *Pleurotus ostreatus*, *Pleurotus pulmonarius*, propiedades funcionales, actividad biológica.

## INTRODUCTION

**E**dible mushrooms are widely cultivated, marketed, and consumed worldwide. In Mexico, *Agaricus* (white button), *Lentinula* (shiitake), and *Pleurotus* species (oyster) are extensively cultivated, and some of their main brands include fresh mushrooms like *Agaricus* (champignon, portobello, portobellini, and cremini), *Lentinula* and *Pleurotus*. While some canned mushrooms like *Agaricus* (whole, sliced, pieces, sauces, marinated, among others) and *Pleurotus* (whole) have been commercialized (Mayett *et al.*, 2006; Verdugo-Silva, Martínez-Ruiz & Rojo-Martínez, 2014).

Oyster mushrooms (*Pleurotus* spp.) have been recognized as an important source of proteins and amino acids, lipids and fatty acids, minerals, carbohydrates and fibers, vitamins, and secondary metabolites. Some bioactive compounds in the genus *Pleurotus* have been associated with their pharmacological properties, including anticancer, anti-inflammatory, anti-hypertensive, anti-diabetic, anti-allergic, antiviral, antifungal, antibacterial, and antioxidant activities. Due to their nutritional and health benefits *Pleurotus* spp. has been proposed as a functional ingredient (Adebayo & Oloke, 2017; Montes, Rangel-Vargas, Lorenzo, Romero & Santos, 2020).

In this context, *P. enryngii* powder has been used as antioxidants to decrease lipid peroxidation and improve meat quality in poultry (Lee, Ciou, Chiang, Chao & Yu, 2012). At the same time, powders and extracts obtained from *P. enryngii*, *P. sajor-caju*, and *P. ostreatus* have been recommended to replace protein, fat, salt, and phosphates in the formulation of meat products, to produce healthier products and improve shelf-life without affecting their physicochemical and sensory properties (Montes *et al.*, 2020). Similarly, the incorporation of oyster mushroom powders in products like bread, cakes, and biscuits, among others has been investigated (Salehi, 2019).

However, data on the physicochemical, techno-functional, and bioactive properties of powders obtained from *Pleurotus* spp. are still limited. Therefore, this study aimed to evaluate the physicochemical (pH and color), techno-functional (water-holding, oil-holding, swelling, gelling, emulsifying, and foaming capacities), and antioxidant properties (metabolites profile, antiradical, and reducing power activities), as well as lipid oxidation inhibition activity of powders obtained from *P. ostreatus* and *P. pulmonarius*.

## MATERIALS AND METHODS

### Chemicals and reagents

All the chemical reagents used were analytical grade. Ethanol, methanol, phenol, ferric chloride ( $\text{FeCl}_3$ ), Folin-Ciocalteu's phenol reagent, sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), potassium persulfate ( $\text{K}_2\text{S}_2\text{O}_8$ ), potassium ferricyanide ( $\text{K}_3[\text{Fe}(\text{CN})_6]$ ), phosphate buffer, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation ( $\text{ABTS}^+$ ) and 1,1-diphenyl-

2-picrylhydrazyl (DPPH<sup>\*</sup>), iron(II) sulfate 7-hydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ), glacial acetic acid; trichloroacetic acid (TCA), sodium nitrite ( $\text{NaNO}_2$ ), 4,4,6-tripyridyl-S-triazine (TPTZ), and iron (III) chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) were purchased from Sigma Chemical (St. Louis, MO, USA). Butylhydroxytoluene (BHT), glucose, gallic acid, quercetin, and chlorogenic acid, were also procured from Sigma Chemical. While sodium hydroxide (NaOH), sulfuric acid ( $\text{H}_2\text{SO}_4$ ), aluminum chloride ( $\text{AlCl}_3$ ), and hydrochloric acid (HCl) were obtained from J.T. Baker®.

### Mushrooms Production and Processing

Two *Pleurotus* strains were evaluated, *P. ostreatus* (IE-8) and *P. pulmonarius* (IE-115) and propagated in Petri dishes with malt agar extract (MAE). Wheat grains (*Triticum aestivum* L.) previously hydrated and sterilized (121 °C for 1 h) were used to prepare the seed inoculum. The seeds were inoculated with portions of mycelium from each strain grown in MAE (approx. 1.5 mg of mycelium) and incubated at 27 °C until full colonization. The growth and development of *Pleurotus* spp. was carried out on wheat straw (crushed into sections of 5 – 7 cm), hydrated, pasteurized, and inoculated with 5% inoculum seeds into plastic bags (40 x 60 cm). After completely colonizing the substrate under darkness at 28 °C, the culture room was changed to a photoperiod (12 h light/12 h darkness), room temperature (25 °C), relative humidity of 80 – 90%, and  $\text{CO}_2$  concentration < 1,200 ppm until fruiting bodies were obtained.

In order to obtain the mushroom powders, the fruiting bodies from *P. ostreatus* and *P. pulmonarius* were dried at 60 °C for 12 h and then pulverized at 20 mesh of particle size (hammer mill, Pulvex 200, D. F., Mexico). Both materials were packed under vacuum (vacuum sealer, Food Saver®, FLA., USA) and stored at 25 °C until analysis. The samples were subjected to physicochemical and techno-functional properties, as well as to metabolites content evaluation and compared with a commercial standard (texturized soy protein), regarding antioxidant evaluation was used a positive control as standard (BHT).

### Physicochemical characterization

**Measurement of pH.** The powdered mushrooms were homogenized at 2,500 rpm/4 °C/1 min (Ultraturrax T25, IKA, Heitersheim, Germany) with distilled water at a ratio of 1:10 (w/v). After homogenization, the pH was measured using a potentiometer (Model pH211, Hanna Instruments Inc., Woonsocket, Rhode Island, USA) with automatic temperature control [method 943.02]. (AOAC, 2020).

**Color measurement (CIELab).** The powdered mushrooms were subjected to a color measurement using a spectrophotometer (model CM 508d, Konica Minolta Inc., Tokyo, Japan). The registered values were lightness ( $L^*$ , ranging from 0-black to 100-white), redness ( $a^*$ , which takes positive values for reddish colors and negative values for greenish colors), yellowness

(b\*, which takes positive for yellowish colors, and negative for bluish colors), Chroma (C\*), and hue angle (h\*). The samples were placed in 3.5 cm diameter Petri dishes, and ten measurements were performed on the surface. In addition, Hex color (hexadecimal format code for identifying colors), color name (common name) and RGB (red, green and blue) were acquired (López-Marcos, Bailina, Viuda-Martos, Pérez-Álvarez & Fernández-López, 2015).

#### Techno-functional characterization

The techno-functional properties of the powders obtained from *Pleurotus* spp. were measured, as previously described, with slight modifications. A commercial soy protein was used as a control (López-Marcos *et al.*, 2015; Zou *et al.*, 2022).

**Water-holding capacity (WHC).** The powdered mushrooms (0.1 g) were homogenized with 1 mL of distilled water at 10,000 rpm/25 °C/1 min (Analog vortex mixer, Fisher Scientific TM, Nueva Jersey, USA) and kept at 25 °C for 30 min. Subsequently, the test tubes were centrifuged at 15,000x g/4 °C/20 min (Sorvall ST18R, Thermo Fisher Scientific, Massachusetts, USA). The supernatant was decanted, and the test tubes with the sediment were weighed. The WHC was calculated as follows:  $WHC (\%) = [(Final\ weigh\ of\ the\ tube\ with\ the\ sample - Initial\ weigh\ of\ the\ tube\ with\ the\ sample) / (Weigh\ of\ the\ sample)]$ .

**Oil-holding capacity (OHC).** The powdered mushrooms (0.1 g) were homogenized with 1 mL of commercial corn oil at 10,000 rpm/25 °C/1 min and kept at 25 °C for 30 min. Subsequently, the test tubes were centrifuged at 15,000x g/4 °C/20 min. The supernatant was decanted, and the test tubes with the sediment were weighed. The OHC was calculated as follows:  $OHC (\%) = [(Final\ weight\ of\ the\ tube\ with\ the\ sample - Initial\ weight\ of\ the\ tube\ with\ the\ sample) / (Weight\ of\ the\ sample)]$ .

**Swelling capacity (SC).** The powdered mushrooms (0.1 g) were homogenized with 1 mL of distilled water at 10,000 rpm/25 °C/1 min, using a graduated tube, and kept at 25 °C for 1 h. The initial and final volume occupied by the samples was measured. An increase in sample volume after incubation indicates swelling capacity.

**Foaming capacity (FC).** The powdered mushrooms (0.1 g) were homogenized with 1 mL of distilled water at 5,000 rpm/4 °C/5 min. The foam produced within 30 seconds indicates foaming capacity.

**Emulsifying capacity (EC).** The powdered mushrooms (0.1 g) were homogenized with 1 mL of distilled water at 8,000 rpm/4 °C/1 min. The resultant solution was homogenized with 10 mL of commercial corn oil under the same conditions. A two-phase suspension formation indicates emulsification ability.

**Gelling capacity (GC).** The powdered mushrooms (0.1 g) were homogenized with 1 mL of distilled water and boiled in a water bath at 100 °C for 1 h (Aquabath, Thermo Fisher Scientific TM, Nueva Jersey, USA). Subsequently, test tubes were cooled at 0 °C for 1 h. The tubes were then reversed to observe gel formation.

#### Metabolites content

**Extract preparation.** The bioactive compounds of powders from *Pleurotus* spp. were obtained with different solvents (water, ethanol, and a mixture of water-ethanol) in a ratio of 1:10 (w/v), using ultrasound-assisted equipment at 40 kHz/25 °C/1 h (Branson 3,800 Ultrasonic bath, Branson, Germany). The resultant mixture was filtered (Whatman #1 filter paper) using a vacuum pump (MVP 6, Jeju, Korea) and concentrated in a rotary evaporator at 65 °C (Yamato RE301BW, Tokyo, Japan). The powdered mushroom extracts were lyophilized (Yamato DC401 Freeze Dryer, Tokyo, Japan) and stored at -20 °C in the dark until analysis (Torres-Martínez *et al.*, 2021).

**Metabolites content.** Total carbohydrate content (TCC) was determined by the phenol-sulfuric acid method (Albalasmeh, Berhe & Ghezzehei, 2013), with slight modifications. An aliquot of each dried mushroom extract (250 µL, 5 mg/mL) was homogenized in a vortex mixer with 125 µL of aqueous phenol solution (5%, v/v) and 625 µL of concentrated H<sub>2</sub>SO<sub>4</sub>. Subsequently, the reaction mixture (200 µL) was transferred into each well of a flat microplate (96-well) and stored at 25 °C/20 min/in darkness. After, absorbance was measured at 490 nm in a spectrophotometer (Multiskan FC UV-Vis, Thermo Scientific, Tokyo, Japan). Results were expressed as mg of glucose equivalent/g of dried extract (mg GE/g).

Total protein content (TPC) was measured using the Biuret method (Bouregghda, Satha & Bendebane, 2021), with slight modifications. An aliquot of each dried mushroom extract (20 µL, 5 mg/mL) was homogenized with 100 µL of Biuret reagent and incubated at 25 °C/15 min/in darkness. After that, the absorbance was measured at 595 nm. Results were expressed as mg of bovine serum albumin/g of dried extract (mg BSA/g).

Total phenolic content (TPHC) was measured according to the Folin-Ciocalteu method (Matić & Jakobek, 2021), with slight modifications. An aliquot of each dried mushroom extract (10 µL, 5 mg/mL) was homogenized with 80 µL of distilled water, 40 µL of Folin-Ciocalteu's reagent (0.25 N), and 60 µL of Na<sub>2</sub>CO<sub>3</sub> solution (7%, w/v). The resultant mixture was homogenized with 80 µL of distilled water and incubated at 25 °C/1 h/in darkness. Then, the absorbance was measured at 750 nm. Results were expressed as mg of gallic acid equivalent/g of dried extract (mg GAE/g).

Total flavonoid content (TFC) was measured based on the formation of aluminum chloride complexes (Matić & Jakobek,

2021), with slight modifications. An aliquot of each dried mushroom extract (10  $\mu$ L, 5 mg/mL) was mixed with 130  $\mu$ L of methanol and 10  $\mu$ L AlCl<sub>3</sub> 5% (w/v) and incubated at 25 °C/30 min/in darkness. Then, the absorbance was measured at 412 nm. Results were expressed as mg of quercetin equivalent/g of dried extract (mg QE/g).

Chlorogenic acid content (CAC) was also determined (Griffiths, Bain & Dale, 1992), with slight modifications. An aliquot of each dried mushroom extract (100  $\mu$ L, mg/mL) was homogenized with 200  $\mu$ L of urea (0.17 M), 200  $\mu$ L of glacial acetic acid (0.1 M), and 500  $\mu$ L of distilled water. The resultant mixture was mixed with 500  $\mu$ L of NaNO<sub>2</sub> (0.14 M) and 500  $\mu$ L of NaOH and subsequently centrifuged (2,250x g/4 °C/10 min). Then, the absorbance was measured at 510 nm. Results were expressed as mg equivalents of chlorogenic acid/g of dried extract (mg CAE/g).

### Antioxidant properties

**Free-radical scavenging activity (FRSA).** FRSA was determined by the 2,2-diphenyl-1-picrylhydrazyl method (Takatsuka, Goto, Kobayashi, Otsuka & Shimada, 2022), with slight modifications. An aliquot of each dried mushroom extract (100  $\mu$ L, 100  $\mu$ g/mL) was mixed with an equal volume of DPPH<sup>•</sup> solution (300  $\mu$ mol) and incubated at 25 °C/130 min/in darkness. BHT (50  $\mu$ g/mL) was used as a positive control. The absorbance was measured at 520 nm. The inhibition percentage was calculated as follows:  $FRSA (\%) = [1 - Abs(S)/Abs(0)] \times 100$ , where Abs(S) is the absorbance of the antioxidant at t = 30 min, and Abs(0) is the absorbance of the control at t = 0 min.

**Radical-cation scavenging activity (RCSA).** RCSA was determined by the 2,2'-azinobis-3 ethylbenzothiazoline-6-sulfonic acid radical cation method (Takatsuka *et al.*, 2022), with slight modifications. The radical cation was formed by mixing equal parts of ABTS solution (7 mM) and potassium persulfate (2.45 mM), which was incubated at 25 °C for 16 h in darkness. The obtained mixture was diluted with ethanol until an absorbance of 0.8 was reached. An aliquot of each dried mushroom extract (100  $\mu$ g/mL) was mixed with the formed radical cation (1:99 ratio). BHT (50  $\mu$ g/mL) was used as a positive control, and absorbance was measured at 730 nm. The RCSA was calculated as follows:  $RCSA (\%) = [1 - Abs(S)/Abs(0)] \times 100$ , where Abs(S) is the absorbance of the antioxidant at t = 30 min, and Abs(0) is the absorbance of the control at t = 0 min.

**Reducing power activity (RPA).** RPA was determined by the Prussian blue method (Berker *et al.*, 2010). An aliquot of each dried mushroom extract (200  $\mu$ L, 100  $\mu$ g/mL) was homogenized with 500  $\mu$ L of phosphate buffer (50 mM, pH 7.0) and 500  $\mu$ L of potassium ferricyanide (1%, w/v) and incubated at 50 °C/20 min/in darkness. After, the samples were mixed with 500  $\mu$ L of TCA (10%, w/v) and centrifuged at 3,500x g/4 °C/10 min.

The supernatant (500  $\mu$ L) was mixed with 500  $\mu$ L of distilled water and 100  $\mu$ L FeCl<sub>3</sub> (0.1%, w/v). The obtained mixture (200  $\mu$ L) was transferred into each well of a flat microplate (96-well). BHT (50  $\mu$ g/mL) was used as a positive control, and the absorbance was measured at 700 nm. Results were expressed as an absorbance increase at the same wavelength.

**Ferric reducing antioxidant power (FRAP).** FRAP was also determined (Berker, Güçlü, Demirata & Apak, 2010), with slight modifications. An aliquot of each extract (5  $\mu$ L, 100  $\mu$ g/mL) was homogenized with 150  $\mu$ L of FRAP solution [10:1:1, 300 mM buffer sodium acetate in glacial acetic acid at pH 3.6 and 10 mM TPTZ in 40 nM HCl and 20 mM FeCl<sub>3</sub>] and incubated at 25 °C/8 min/in darkness. BHT (50  $\mu$ g/mL) was used as a positive control, and the absorbance was measured at 595 nm. Results were expressed as mg equivalents of Fe<sup>2+</sup>/g of dried extract (mg Fe<sup>2+</sup>/g).

**Lipid oxidation inhibition.** To prepare meat homogenates, fresh pork meat (*Semimembranosus* muscle, 48 h *postmortem*) was purchased from a local processor (Norson<sup>®</sup>, Hermosillo, Mexico), visible extra muscular fat was trimmed, and the meat was minced through a 4.5 mm-hole plate (meat grinder 4152, 4 Hobart Dayton, Ohio, USA). The minced pork meat was mixed with salt (1.5%, w/w) and pork back fat (10% in the final formulation, w/w). Subsequently, the meat homogenate was obtained by homogenizing (4,500 rpm/5 °C/1 min) 1 g of minced meat with 1 mL of the respective antioxidants (Control, without antioxidant; BHT, synthetic antioxidant at 0.02% fat basis; as well as each dried mushroom extract at 250 ppm, fresh weight) and 9 mL of distilled water, using an Ultraturrax. After, the resultant solution was incubated (65 °C, at different periods of 0, 30, and 60 min) in a water bath (Torres-Martínez *et al.*, 2021).

Lipid oxidation of meat homogenates was measured by the thiobarbituric acid reactive substances (TBARS) method (Kim, Ahn, Eun & Moon, 2016), with slight modifications. The meat homogenates (0.5 mL), subjected previously and incubated at 25 °C/15 min/in darkness, were mixed with 1 mL of TCA solution (10%, w/v) in a vortex mixer at 10,000 rpm/25 °C/1 min. After, 1 mL of the resultant filtered solution (Whatman 4 filter paper) was homogenized with 1 mL of TBA solution (0.02 M) and placed in a water bath (97 °C for 20 min), and subsequently cooled. The absorbance was measured at 531 nm. The results were expressed as mg of malondialdehyde/kg of meat (mg MDA/kg).

### Statistical analysis

Mean  $\pm$  standard deviation values were used as descriptive statistics. Physicochemical and techno-functional data obtained from the three independent trials were subjected to a one-way analysis of variance (ANOVA). Data from phenolic content and antioxidant assays were subjected to a two-way factorial



ANOVA, in which the powdered mushrooms and solvent extraction were the effects in the model, while the treatment and storage time were considered the effects in the model for lipid oxidation inhibition activity. Tukey tests were carried out for means comparison at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Physicochemical characteristics

Table I shows the physicochemical properties (pH and CIELab color coordinates) of the powders obtained from *Pleurotus* spp., compared to the commercial standard (Soy protein). The results showed that the pH of Soy protein was acidic, while the *Pleurotus* spp. powders exhibited significantly higher pH values ( $p < 0.05$ ). The pH is an important factor in natural ingredients because it determines into which type of food matrix it can be incorporated without affecting their techno-functional properties (Barać, Pešić, Stanojević, Kostić & Čabrilo, 2015; López-Marcos et al., 2015). According to our results, *Pleurotus* spp. powders showed pH near neutrality, hence could be used as a food ingredient for meat products (near neutral food matrix), and it is not recommended for use in acid food matrix (López-Marcos et al., 2015).

Color is one of the key attributes of foods and beverages that can influence consumer perception and behavior. Therefore, food processors focus on the design or formulation of ingredients to maintain a consistent visual appearance during the shelf-life of the processed product and attend to changes in legislation on the use of artificial color ingredients (Spence, 2018; Spence, 2019). The tested powdered mushrooms and Soy protein showed high  $L^*$  values ( $> 70$ ); however, the highest ( $p < 0.05$ )  $L^*$  value was found in *P. pulmonarius* powder. In addition, *Pleurotus* spp. powders showed low ( $p < 0.05$ )  $a^*$ ,  $b^*$ , and  $C^*$  values respect to Soy protein, while the highest ( $p < 0.05$ )  $h^*$  value was shown in *P. ostreatus* powder respect to *P. pulmonarius* powder and Soy protein. In addition, RGB and Hex color codes indicate the

color names for *P. ostreatus*, *P. pulmonarius* and Soy protein (Sorrell Brown, Tan, and Tints of Calico, respectively).

The mushrooms color can be influenced by different factors, including species, strain, culture conditions, and the used method of their preservation, e.g., drying, irradiation, packaging, and storage temperature, among others (Kortei, Tawia Odamtten, Obodai, Appiah & Toah Akonor, 2015; Ruiz-Rodriguez, Soler-Rivas, Polonia & Whichers, 2010; Villaescusa & Gil, 2003). For example, it has been reported that *P. ostreatus* (fresh harvested fruiting body) possess high  $L^*$  values ( $> 90$ ) and low  $a^*$  and  $b^*$  ( $< 1$  and  $< 5$ , respectively) (Villaescusa & Gil, 2003). Also, *P. ostreatus* and *P. pulmonarius* fruiting bodies cultivated in a medium including olive mill waste showed high  $L^*$  (average = 65) and  $b^*$  values (average = 1), as well as a reduction in  $a^*$  value (average = 1), in concentration dependence of the substrate used (Ruiz-Rodriguez et al., 2010). While, *P. sajor-caju* powder showed values close to those found in our study ( $L^* = 73$ ,  $a^* = 3.8$ ,  $b^* = 24.7$ ,  $C = 25.0$ ,  $h = 81.3$ ) (Han, Amhad & Ishak, 2016).

### Techno-functional properties

Moreover, the techno-functional properties of natural ingredients used in food formulation are variable. The most important are solubility, viscosity, water absorption, water retention, fat absorption, flavor retention, gelation, cohesion-adhesion, elasticity, foam formation, and emulsification. In this context, an ingredient with multiple functionalities is considered the most optimal to be used. However, these properties could be affected by intrinsic (chemical composition, structure-reactivity, hydrophobicity-hydrophilicity ratio, among others) and extrinsic (pH, temperature, particle size, and extraction method, among others) factors (Barać et al., 2015). Table II shows the techno-functional properties of the powders obtained from *Pleurotus* spp., compared to Soy protein. The results indicate that powder mushrooms showed the lowest

Table I. Physicochemical characteristics of powders obtained from *Pleurotus* spp.

Item	<i>P. ostreatus</i>	<i>P. pulmonarius</i>	Soy protein
pH	7.23 ± 0.03 <sup>b</sup>	7.15 ± 0.01 <sup>b</sup>	6.44 ± 0.03 <sup>a</sup>
Color			
$L^*$	73.98 ± 1.17 <sup>a</sup>	77.44 ± 1.27 <sup>b</sup>	73.40 ± 1.02 <sup>a</sup>
$a^*$	3.09 ± 0.28 <sup>a</sup>	3.83 ± 0.23 <sup>b</sup>	5.47 ± 0.05 <sup>c</sup>
$b^*$	21.15 ± 0.35 <sup>a</sup>	23.12 ± 0.32 <sup>b</sup>	27.47 ± 0.08 <sup>c</sup>
C	21.37 ± 0.38 <sup>a</sup>	23.43 ± 0.34 <sup>b</sup>	27.97 ± 0.09 <sup>c</sup>
h	81.69 ± 0.65 <sup>b</sup>	80.59 ± 0.51 <sup>a</sup>	79.59 ± 0.71 <sup>a</sup>
Hex color	#C7B38F	#D2BC95	#CBB082
Color name	Sorrell Brown	Tan	Tints of Calico
RGB			

Results are expressed as mean ± standard deviation (n=6). Different superscript (a-c) in the same row indicates significant differences ( $p < 0.05$ ).

**Table II. Techno-functional properties of powders obtained from *Pleurotus* spp.**

Item	<i>P. ostreatus</i>	<i>P. pulmonarius</i>	Soy protein
WHC (%)	34.09 ± 2.17 <sup>a</sup>	36.97 ± 2.58 <sup>a</sup>	50.23 ± 1.10 <sup>b</sup>
OHC (%)	24.23 ± 1.23 <sup>b</sup>	28.48 ± 2.36 <sup>c</sup>	16.01 ± 0.48 <sup>a</sup>
SC	+	+	+
FC	+	+	+
EC	-	-	+++
GC	+	+	+++

Results are expressed as mean ± standard deviation (n=6). WHC, water-holding capacity; OHC, oil-holding capacity; SC, swelling capacity; FC, foaming capacity; EC, emulsifying capacity; gelling capacity. Different superscript (a-c) in the same row indicates significant differences (p<0.05). (+), slight property; (++) , moderate property; (+++), high property; (-), absent property.

WHC, and the highest OHC respect to Soy protein (p<0.05). Non-differences (p>0.05) were found in SC and FC between treatments (slight properties). While Soy protein showed high EC and GC respect mushrooms powders.

In agreement, Han, Amhad & Ishak (2016) demonstrated that *P. sajor-caju* powder possesses functional properties including WHC, OHC, SC, and EC, which were related to the

polysaccharide content of the mushroom. Also, it has been shown that *P. ostreatus* powder exert WHC, OHC, SC, which were associated with the hydrophilic components of the powder including polysaccharides and proteins and supported by the porous morphology of the material (Veléz-Urbe *et al.*, 2023). In another work, it has been reported that *P. tuberregium* powder exerts gelation properties which were associated with its proteins; and suggested that this mushroom powder could be used as a protein supplement and functional ingredient in food formulations (Alobo, 2003). Additionally, it has been reported that major protein fractions (glutelin>globulin>albumin) are associated with FC of edible mushrooms and indicate that effectiveness can be related to the amino acid composition, surface hydrophobicity, and structural feature (Yu *et al.*, 2021).

**Metabolites content**

In the other hand, edible mushrooms’ nutritional components (primary metabolites) have been described as rich sources of secondary metabolites like phenolic compounds (Montes *et al.*, 2020). Table III shows the metabolites content of the powder extracts obtained from *Pleurotus* spp., compared to Soy protein. Regarding primary metabolites, Soy protein extracts (ethanol>water>1:1) showed the highest (p<0.05) TCC respect *Pleurotus* spp. powders. While Soy protein water extract showed the highest (p<0.05) TPC values when compared to others extracts. Respect secondary metabolites, the highest (p<0.05) TPHC and TFC values were observed in

**Table III. Metabolites content of powders extracts obtained from *Pleurotus* spp.**

Item	Solvent	<i>P. ostreatus</i>	<i>P. pulmonarius</i>	Soy protein
TCC	Water	21.66 ± 1.48 <sup>b</sup>	21.54 ± 1.59 <sup>b</sup>	31.07 ± 1.01 <sup>d</sup>
	Ethanol	31.94 ± 0.89 <sup>d</sup>	30.54 ± 2.55 <sup>d</sup>	35.80 ± 0.72 <sup>e</sup>
	1:1	8.51 ± 1.66 <sup>a</sup>	9.51 ± 1.30 <sup>a</sup>	25.33 ± 0.58 <sup>c</sup>
TPC	Water	24.87 ± 0.81 <sup>c</sup>	25.07 ± 1.10 <sup>c</sup>	34.73 ± 0.64 <sup>d</sup>
	Ethanol	13.80 ± 0.52 <sup>a</sup>	13.17 ± 1.04 <sup>a</sup>	14.68 ± 0.59 <sup>a</sup>
	1:1	21.33 ± 1.15 <sup>b</sup>	22.07 ± 1.79 <sup>b</sup>	21.70 ± 0.60 <sup>b</sup>
TPHC	Water	1.32 ± 0.45 <sup>a</sup>	2.98 ± 0.85 <sup>ab</sup>	2.06 ± 0.52 <sup>a</sup>
	Ethanol	7.93 ± 1.46 <sup>d</sup>	7.96 ± 0.69 <sup>d</sup>	4.96 ± 0.06 <sup>c</sup>
	1:1	5.03 ± 0.51 <sup>c</sup>	4.52 ± 0.89 <sup>c</sup>	3.05 ± 0.08 <sup>b</sup>
TFC	Water	61.07 ± 0.38 <sup>c</sup>	63.02 ± 2.47 <sup>c</sup>	N.D.
	Ethanol	183.47 ± 2.09 <sup>d</sup>	182.50 ± 1.28 <sup>d</sup>	N.D.
	1:1	36.50 ± 1.73 <sup>b</sup>	23.12 ± 1.64 <sup>a</sup>	N.D.
CAC	Water	42.47 ± 2.68 <sup>e</sup>	28.39 ± 2.38 <sup>d</sup>	1.03 ± 0.06 <sup>b</sup>
	Ethanol	0.73 ± 0.20 <sup>ab</sup>	0.58 ± 0.07 <sup>a</sup>	1.07 ± 0.12 <sup>b</sup>
	1:1	5.74 ± 0.67 <sup>c</sup>	5.80 ± 0.38 <sup>c</sup>	1.10 ± 0.17 <sup>b</sup>

Results are expressed as mean ± standard deviation (n=6). TCC, total carbohydrate content (mg GE/g); TPC, total protein content (mg BSA/g); TPHC, total phenolic content (mg GAE/g); TFC, total flavonoid content (mg QE/g); CAC, chlorogenic acid content (mg CAE/g). Different superscript (a-e) through treatment × solvent extraction indicates significant differences (p<0.05).

*Pleurotus* spp. ethanol extracts (*P. ostreatus* = *P. pulmonarius*). While the higher ( $p < 0.05$ ) CGA value was found in water extracts (*P. ostreatus* > *P. pulmonarius*) respect to Soy protein water extract.

In agreement with our study, a solvent extraction effect (ethanol > water > ethanol-water) was observed on carbohydrates values of a commercial *P. ostreatus* powder, however, although in this study it is reported that *P. ostreatus* powder is an important source of protein (Torres-Martínez *et al.*, 2022), the effect of the extraction solvent on protein content has not been reported. Also, it has been reported a solvent extraction effect (ethanol > water) on TPHC and TFC values of *P. citrinopileatus* (Gogoi, Chutia, Singh & Mahanta, 2019). Similarly, a solvent extraction effect (ethanol = ethanol-water > water) was observed in TPHC values from *P. ostreatus* (Torres-Martínez *et al.*, 2021). Additionally, it has been demonstrated that *P. citrinopileatus* water extract showed higher CAC than ethanol extract (Yin *et al.*, 2019).

#### Antioxidant properties

Although researchers have thoroughly discussed the biosynthesis of phenolic compounds, it has been known to exhibit nutritional and health-functional properties (Carocho, Barreiro, Morales & Ferreira, 2014; Montes *et al.*, 2020). In Mexico, it has been reported that a significant number (approximately 50%) of consumers from the Central Region have high preferences for the consumption of fresh mushrooms and indicate that they know their nutritional and medicinal importance (Mayett *et al.*, 2006). According to Table IV, the results indicate that ethanol extracts (*P. ostreatus* > *P. pulmonarius*) showed the highest ( $p < 0.05$ )

FRSA, RPA, and FRAP values, while the water-ethanol extracts (*P. ostreatus* > *P. pulmonarius*) showed the highest ( $p < 0.05$ ) RCSA values. In agreement with our study, it has been reported a solvent extraction effect (ethanol > water) on FRSA and FRAP values of *P. citrinopileatus* (Gogoi *et al.*, 2019). Also, a solvent extraction effect (ethanol > ethanol-water > water) was observed on RCSA, RPA and FRAP values of a commercial *P. ostreatus* powder (Torres-Martínez *et al.*, 2022).

Edible mushrooms have antioxidant properties, so they have been proposed as functional ingredients for the food industry to improve shelf-life (Montes *et al.*, 2020; Pérez-Montes, Rangel-Vargas, Lorenzo, Romero & Santos, 2021). In this context, it has been extensively documented that lipid oxidation is the main non-microbial cause of quality loss in meat and meat products (Domínguez *et al.*, 2019). In this context, Figure 1 shows the effect of *Pleurotus* spp. extracts and storage time on lipid oxidation inhibition activity of pork homogenates incubated at 65 °C, compared to a synthetic antioxidant (BHT). At the end of storage, the lowest ( $p < 0.05$ ) lipid oxidation values were found in meat samples treated with synthetic antioxidant (BHT) and powdered mushrooms extracts in comparison to control samples.

In agreement with our results, it was demonstrated that the incorporation of *P. ostreatus* extracts (water, ethanol, and water-ethanol) reduced the lipid oxidation values in meat samples (Torres-Martínez *et al.*, 2021). While Wan-Mohtar *et al.* (2020) reported higher antioxidant activity on a chicken patty by adding *P. sapidus* flour to the formulation. Similarly, incorporating dried *P. ostreatus* into beef salami prevented

**Table IV. Antioxidant activity of powders extracts obtained from *Pleurotus* spp.**

Item	Solvent	<i>P. ostreatus</i>	<i>P. pulmonarius</i>	BHT
FRSA	Water	15.30 ± 0.87 <sup>b</sup>	11.59 ± 0.68 <sup>a</sup>	73.85 ± 0.99
	Ethanol	47.40 ± 1.24 <sup>f</sup>	43.51 ± 1.20 <sup>e</sup>	
	1:1	25.09 ± 1.23 <sup>d</sup>	16.07 ± 0.58 <sup>c</sup>	
RCSA	Water	62.13 ± 1.49 <sup>e</sup>	62.96 ± 1.73 <sup>c</sup>	64.10 ± 0.98
	Ethanol	58.64 ± 1.33 <sup>b</sup>	54.05 ± 1.87 <sup>a</sup>	
	1:1	75.01 ± 1.90 <sup>e</sup>	66.79 ± 1.27 <sup>d</sup>	
RPA	Water	0.92 ± 0.02 <sup>d</sup>	0.85 ± 0.01 <sup>c</sup>	0.75 ± 0.01
	Ethanol	1.50 ± 0.02 <sup>e</sup>	1.51 ± 0.01 <sup>e</sup>	
	1:1	0.53 ± 0.01 <sup>b</sup>	0.48 ± 0.01 <sup>a</sup>	
FRAP	Water	1.82 ± 0.11 <sup>c</sup>	1.33 ± 0.10 <sup>b</sup>	2.51 ± 0.99
	Ethanol	3.52 ± 0.02 <sup>e</sup>	2.01 ± 0.05 <sup>d</sup>	
	1:1	1.33 ± 0.06 <sup>b</sup>	1.12 ± 0.08 <sup>a</sup>	

Results are expressed as mean ± standard deviation (n=6). FRSA, free-radical scavenging activity (% inhibition); RCSA, radical-cation scavenging activity (% inhibition); RPA, reducing power ability (absorbance at 700 nm); FRAP, ferric reducing antioxidant power (mg Fe<sup>2+</sup>/g). BHT, butylhydroxytoluene (positive control). Different superscript (a-f) through treatment × solvent extraction indicates significant differences ( $p < 0.05$ ).

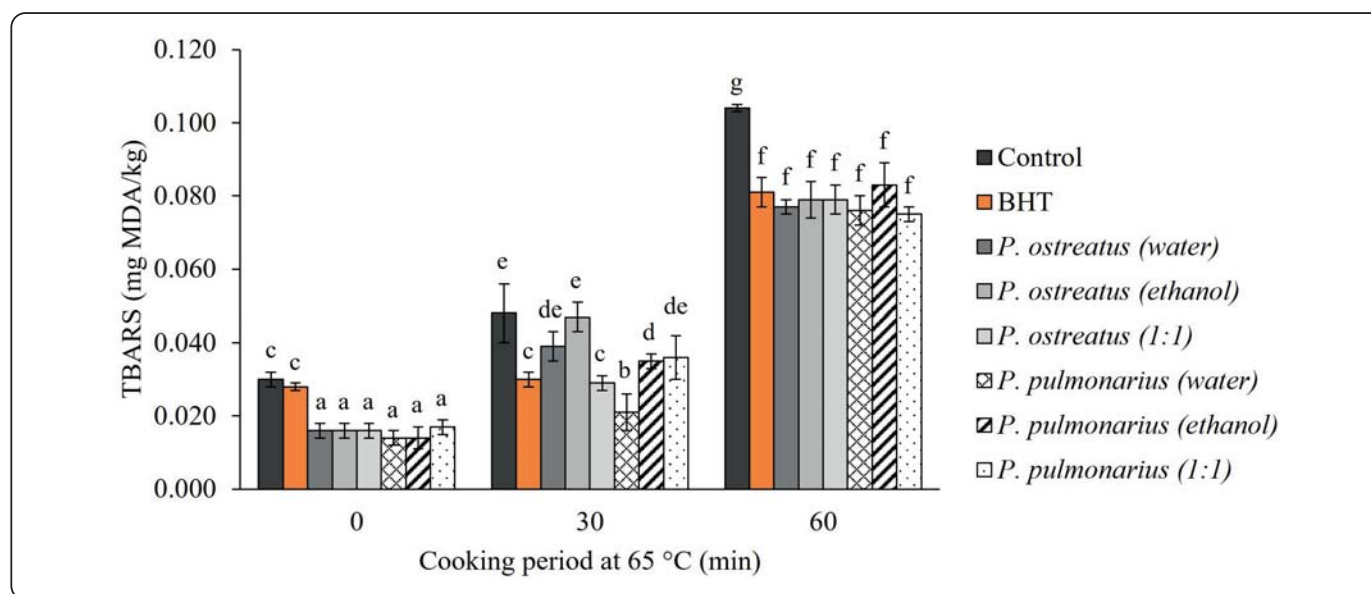


Figure 1. Effect of powder mushroom extracts and storage time on lipid oxidation inhibition of pork homogenates incubated at 65 °C. Different superscript (a-g) through treatment × solvent extraction indicates significant differences (p<0.05).

lipid and protein oxidation (Özünlü & Ergezer, 2021). Besides, adding *P. ostreatus* powder to fresh and cooked pork patties reduced lipid oxidation during storage (Torres-Martínez *et al.*, 2022).

### Pearson’s correlation analysis

Regarding Pearson’s correlation analysis between physicochemical, techno-functional and metabolites content results demonstrated a positive correlation among pH respect to h\* (0.897), OHC (0.907), TPHC (0.995), TFC (0.996) and CAC (0.969) parameters. While a negative correlation among pH respect a\* (-0.977), b\* (-0.976), C\* (-0.976), WHC (-0.997), SC (-0.908), FC (-0.908), EC (-0.996), GC (-0.999), TCC (-0.939) and TPC (-0.997) was demonstrated. In addition, results of metabolites content and antioxidant activity demonstrated a positive correlation between TPHC respect to FRSA (0.920) and TBARS (0.895) parameters, and TFC respect to FRSA (0.903), RPA (0.981), FRAP (0.823) and TBARS (0.869) parameters. However, a negative correlation was observed between TPHC and TFC respect to RCSA (-0.392 and -0.811, respectively). These results show the potential rol of pH in color values and techno-functional properties. Furthermore, the association between antioxidant activity and the presence of phenolic compounds in the samples was demonstrated.

### CONCLUSIONS

Physicochemical results indicated that *Pleurotus* spp. powders showed pH near neutrality and slightly brown color. At the same time, techno-functional determinations revealed that the powders exert water and oil-holding, swelling, foaming and gelling capacities. Regarding *Pleurotus* spp. extracts,

metabolites composition assays revealed the presence of primary (protein and carbohydrates) and secondary metabolites (phenols, flavonoids, and chlorogenic acid) in dependence of solvent extraction. In addition, the results showed that ethanol extracts showed the highest FRSA, RPA and FRAP values, while the aqueous-ethanol extract exerted the highest RPA values. Respect lipid oxidation, similarly, all powdered mushroom extracts reduced lipid oxidation of meat homogenates subjected to thermal treatment. In conclusion, *Pleurotus* spp. powders can be proposed as functional ingredients for meat products.

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