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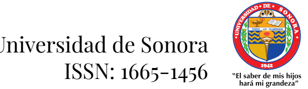

*Original Article*

# Recovery of an additive for pork meat from *Pleurotus ostreatus* grown in agro-industrial wastes

Recuperación de un aditivo para carne de cerdo a partir de *Pleurotus ostreatus* cultivado en residuos agroindustriales

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## ABSTRACT

In recent years, there has been growing interest in using agro-industrial by-products to produce edible mushrooms and recovering functional food ingredients from them. This study aimed to evaluate the polyphenol content and antioxidant activity of *Pleurotus ostreatus* grown in different agro-industrial wastes (wheat straw partially replaced with spent coffee grounds and potato peel) and its potential as an additive to increase the oxidative stability of a meat product subjected to thermal-treatment and *in vitro* gastrointestinal digestion. The mushroom aqueous extract was subjected to polyphenol content and antioxidant activity assays. In addition, pork meat homogenates were antioxidant treated (mushroom extract and synthetic antioxidant), stored (65 °C for 120 min), and subjected to *in vitro* gastrointestinal digestion for oxidative stability evaluation. Results demonstrated that the type of substrate used to produce *P. ostreatus* affects ($p < 0.05$) the polyphenol content and antioxidant activity. Incorporating the extract of *P. ostreatus* cultivated with agro-industrial waste reduced ($p < 0.05$) changes in pH values, lipid oxidation, and color of meat samples. The meat samples' tannins, phenols, flavonoids, chlorogenic acid contents, antiradical activity, and reducing power increased ($p < 0.05$) during gastrointestinal digestion. *P. ostreatus* can be considered a novel additive for the meat industry.

**Keywords:** Edible mushroom; antioxidant; meat quality; heating time; *in vitro* gastrointestinal digestion.

## RESUMEN

En los últimos años ha habido un creciente interés en el uso de subproductos agroindustriales para producir hongos comestibles y la recuperación de ingredientes alimentarios funcionales a partir de ellos. El objetivo de este estudio fue evaluar el contenido de polifenoles y la actividad antioxidante de *Pleurotus ostreatus* cultivado en diferentes residuos agroindustriales (paja de trigo parcialmente reemplazada por granos de café gastados y cáscara de papa) y su potencial como aditivo para aumentar la estabilidad oxidativa de un producto cárnico sometido a tratamiento térmico y digestión gastrointestinal *in vitro*. El extracto acuoso del hongo fue sometido a evaluación del contenido de polifenoles y actividad antioxidante. Además, los homogenizados de carne de cerdo se trataron con antioxidantes (extracto de hongo y antioxidante sintético), se almacenaron (65 °C durante 120 min) y se sometieron a digestión gastrointestinal *in vitro* para evaluar la estabilidad oxidativa. Los resultados demostraron que el tipo de sustrato utilizado para producir *P. ostreatus* afectó ($p < 0.05$) el contenido de polifenoles y la actividad antioxidante. La incorporación del extracto de *P. ostreatus* cultivado con residuos agroindustriales redujo ($p < 0.05$) los cambios en los valores de pH, oxidación de lípidos y color de las muestras de carne. El contenido de taninos, fenoles, flavonoides y ácido clorogénico, actividad antirradicalaria y poder reductor aumentaron ($p < 0.05$) en las muestras de carne durante la digestión gastrointestinal. *P. ostreatus* puede considerarse un aditivo novedoso para la industria cárnica.

**Palabras clave:** hongo comestible; antioxidante; calidad de la carne; tiempo de almacenamiento; digestión gastrointestinal *in vitro*

## INTRODUCTION

One of the most widely consumed foods worldwide is meat; it is highly nutritious but also highly perishable. According to FAO, it is estimated that more than 20 % of the meat produced worldwide is not consumed due to losses or waste. As a result, several preservatives and chemicals are used to preserve or enhance the quality, safety, wholesomeness, and consumer appeal of meat (FAO, 2015; Nair *et al*., 2020). The U.S. Food & Drug Administration indicates that food additives are added to foods in order to preserve or improve freshness,

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safety, and nutritional and sensory attributes (FDA, 2023). In the meat industry, oxidative stability (which depends on changes caused by both internal and external factors) can affect the freshness and safety of meat and meat products. It is, therefore, at specific concentrations that chemical additives like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) improve the oxidative stability of meat and meat products (FDA, 2023). Although these additives are considered safe for human health at authorized levels, published information regarding their toxicological effects is of great concern to consumers (Nieva-Echevarría *et al*., 2014).

In this context, studies have been reported focusing on the search for efficient and sustainable alternatives to improve the oxidative stability of meat products during their cooking treatment and consumption. Based on this, using powders or extracts obtained from plants and, recently, edible mushrooms from the *Pleurotus* genus as a source of additives, has become a promising area of research. For example, it was demonstrated that the incorporation of *Pleurotus sajor-caju* flour in cooked chicken burgers increases oxidative stability (Wan Rosli *et al*., 2011); another study demonstrated that the incorporation of *Pleurotus* spp. powder into cooked Northern Thai style sausages increases the presence of polyphenols and improves antioxidant status (Mazumder *et al*., 2024). In a previous study by our research group, it was demonstrated that incorporating commercial *Pleurotus ostreatus* powder, increased the oxidative stability of a meat product during the cooking process and its gastrointestinal digestion. However, it is important to better understand the mechanism behind this beneficial effect, under controlled conditions, to maximize its bioactive compound content since mushroom powder used for this study was acquired commercially (Torres-Martínez *et al*., 2022).

*Pleurotus ostreatus* stands out as one of the most studied species within the genus. This edible mushroom is popular worldwide due to its beneficial nutrients and delicious flavor. It has been evidenced by multiple studies that the composition of nutrients within the substrate, such as the carbon/nitrogen ratio, pH, and the presence of phenolic compounds, among other factors, can influence the production of secondary metabolites such as polyphenols and consequently affect their bioactivity (Devi *et al*., 2024; Silva *et al*., 2024). Because of its saprophytic nature, this mushroom can grow on various substrates, such as agro-industrial waste. Due to this attribute, it emerges as a promising choice for the sustainable development of food additives. However, multiple studies show that the nutrient composition of the substrate, the carbon/nitrogen ratio, pH, and the presence of phenolic compounds, among other factors, can influence the production of secondary metabolites such as polyphenols and consequently affect their bioactivity (Silva *et al*., 2024). For example, it has been shown that spent coffee grounds and potato peel are essential sources of nutrients and bioactive compounds, so that they can be used as substrates for the growth of the *Pleurotus* species (Alsanad *et al*., 2020; Sabri *et al*., 2019).

Regarding the above, the present study aimed to evaluate the polyphenol content and antioxidant activity of the aqueous extract of *Pleurotus ostreatus* grown in different agro-industrial wastes (spent coffee grounds and potato peel), and its potential as an additive to increase the oxidative stability of a meat product during its thermal treatment and gastrointestinal digestion.

## MATERIAL AND METHODS

### Mushroom production

*P. ostreatus* strain IE-8 (CIAD-Plant based Food Technology Department) was grown on potato dextrose agar medium (DifcoTM) at 25 °C for 5 d (model IC403C, Yamato, Japan). Then, the mycelium (approx. 1.5 mg) was incorporated into the inoculum seed. For the preparation of the inoculum seed, wheat grains (*Triticum aestivum* L.) were hydrated for 16 h, drained to remove water, and sterilized at 121 °C for 1 h (model SM300, Yamato, Japan). The inoculated bags were stored at 28 °C in the dark until the mycelium completely covered the surface of the seed (white coloration). Then, wheat straw was used as basal substrate and mixed at different ratios of supplementing residues: T1, wheat straw (100 %); T2, wheat straw (80 %) + spent coffee grounds (10 %) + potato peel (10 %); T3, wheat straw (70 %) + spent coffee grounds (15 %) + potato peel (15 %); T4, wheat straw (60 %) + spent coffee grounds (20 %) + potato peel (20 %). The wet and sterilized substrates were inoculated with the inoculum seed covered with mycelium (10 %, *w/w*), and stored at 28 °C in the dark until mycelium covered the surface. For mushroom fructification, samples were stored at 25 °C for 24 days (12 h photoperiod/80-90 % RH/$CO_2$ <1,200 ppm/in the dark). The obtained samples were dried (60 °C for 12 h), pulverized (20 mesh), and vacuum packaged until use (Sánchez *et al*., 2002).

### Extracts preparation

The bioactive compounds were recovered from each dried mushroom powder using water as solvent extraction (1:10 ratio, *w/v*), through an ultrasound-assisted system at 40 kHz/25 °C/1 h (model 3800, Branson, Germany). The obtained solution was filtered (model FE-1500, Felisa, México), concentrated at 150 rpm/65 °C/15 min (model RE301BW, Yamato, Japan), and dried at 20 Pa/-40 °C/48 h (model DC401, Yamato, Japan) (Torres-Martínez *et al*., 2022).

### Polyphenol content assays

The total tannin content (TTC) was measured by the vanillin test (Price and Butler, 1977) with minor modifications. The aqueous extract (20 µL, 5 mg/mL) was homogenized with 100 µL of vanillin (1 %, *w/v*) and 100 µL of hydrochloric acid (8 %, *v/v*) and subsequently incubated (25 °C/20 min/in the dark). The absorbance at 500 nm was measured (model Multiskan GO, Thermo Scientific, EUA). Catechin was used as a standard to express the results (mg CAT/mL).

The total phenolic content (TPHC) was measured by the Folin-Ciocalteu test (Matić and Jakobek, 2021), with minor modifications. The aqueous extract (20 µL, 5 mg/mL) was

homogenized with 160 µL of d-water, 40 µL of Folin reagent (2 M), and 60 µL of sodium carbonate (7 %, *w/v*). The obtained solution was incubated (25 °C/1 h/in the dark), and the absorbance was measured at 750 nm. Gallic acid was used as a standard to express the results (mg GAE/mL).

The total flavonoid content (TFC) was measured by the aluminum-complex test (Matić and Jakobek, 2021) with minor modifications. The aqueous extract (20 µL, 5 mg/mL) was homogenized with 130 µL of methanol (Fagalab®) and 20 µL of $AlCl_3$ (5 %, w/v), and subsequently incubated (25 °C/30 min/in the dark). A spectrophotometer instrument was used to measure the absorbance at 415 nm. Quercetin was used as a standard to express the results (mg QE/mL).

The total chlorogenic acid content (TCGA) test was evaluated (Griffiths *et al*., 1992), with minor modifications. The aqueous extract (20 µL, 5 mg/mL) was homogenized with 100 µL of urea (0.17 M), 100 µL of glacial acetic acid (0.1 M), and 250 µL of d-water. Subsequently, the resultant solution was mixed with 250 µL of sodium nitrite (0.14 M) and 250 µL of sodium hydroxide (1 M), centrifuged at 2250 ×g/4 °C/10 min (model Sorvall ST18R, Thermo Fisher Scientific, USA), and the absorbance was determined at 510 nm. Chlorogenic acid was used as a standard to express the results (mg CGA/mL).

**Antioxidant assays**

The free-radical scavenging activity (FRSA) was measured by the DPPH test (Ozgen *et al*., 2006), with minor modifications. The aqueous extract (20 µL, 5 mg/mL) was homogenized with 100 µL of DPPH ethanol solution (300 µM) and subsequently incubated (25 °C/30 min/in the dark). Absorbance was measured at 517 nm. BHT (100 µg/mL) was used as a positive control. The inhibition percentage was calculated to express the results: [(DPPH absorbance at 0 min) – (DPPH absorbance + antiradical at 30 min) / (DPPH absorbance at 0 min)] x 100.

The radical cation scavenging activity (RCSA) was determined by the ABTS test (Ogen *et al*., 2006) with minor modifications. The aqueous extract (20 µL, 5 mg/mL) was homogenized with 180 µL of ABTS solution and subsequently incubated (25 °C/8 min/in the dark), and the absorbance was measured at 730 nm. BHT (100 µg/mL) was used as a positive control. The inhibition percentage was calculated to express the results: [(ABTS absorbance at 0 min) – (ABTS absorbance + antiradical at 30 min) / (ABS absorbance at 0 min)] x 100.

The ferric-reducing antioxidant power (FRAP) test was evaluated (Berker *et al*., 2010), with minor modifications. The aqueous extract (20 µL, 5 mg/mL) was homogenized with 180 µL of FRAP solution [10:1:1, 300 mM buffer sodium acetate in glacial acetic acid at pH 3.6 and 4,4,6-tripyridyl-S-triazine (10 mM) in hydrochloric acid (40 nM) and iron chloride ($FeCl_3$, 20 mM)], and subsequently incubated (25 °C/8 min/in the dark). The absorbance of the sample was measured at 595 nm. BHT (100 µg/mL) was used as a positive control. $Fe^{2+}$ was used as a standard to express the results (mg $Fe^{2+}$/mL).

The reducing power ability (RPA) was determined by the ferricyanide/Prussian blue test (Berker *et al*., 2010), with minor modifications. The aqueous extract (20 µL, 5 mg/mL) was homogenized with 300 µL of phosphate buffer (0.2 M, pH 6.6) and 500 µL of potassium ferrocyanide (1 %, *w/v*), and incubated (50 °C/20 min/in the dark). The obtained solution was mixed with 500 µL of trichloroacetic acid (10 %, *w/v*) and centrifuged at 2300 ×g at 4 °C for 10 min. After that, the supernatant (100 µL) was mixed with 100 µL of $FeCl_3$ (0.1 %, *w/v*). The absorbance was measured with a spectrophotometer at 700 nm. BHT (100 µg/mL) was used as a positive control. The optical density at 700 nm was used to express the results (abs at 700 nm).

**Oxidative stability of meat samples**

Fresh minced pork meat (*Semimembranosus* muscle) was purchased from a local processor (Norson®, Hermosillo, Mexico). Minced pork meat (1 g) was homogenized with 10 mL of d-water at 6000 rpm (5 °C) for 1 min, and 1 mL of the respective antioxidants: Control, without antioxidant; T1-T4, aqueous extracts (500 µg/g); BHT, butylated hydroxytoluene (500 µg/g). The obtained meat homogenates were stored at 65 °C for 0, 60, and 120 min. After that, pork meat homogenates were subjected to meat quality assays.

The pH and color of pork meat homogenates were determined as previously described (AOAC 2020; Hernández *et al*., 2016), with minor modifications. In addition, the TBARS test was used to measure lipid oxidation (Pfalzgraf *et al*., 1995). Meat samples (0.5 mL) were homogenized with 1 mL of trichloroacetic acid (10 %, w/v) at 4500 rpm/5 °C/1 min and centrifuged (2500 ×g/5 °C/20 min). After that, the filtered supernatant (1 mL) was mixed with 1 mL of 2-thiobarbituric acid solution (20 mM) and incubated (98 °C/20 min). The specific wavelength used was 531 nm, measured with a spectrophotometer. 1, 1, 3, 3-tetramethoxypropane was used as a standard to express the results in mg of malonaldehyde/kg of sample (mg MDA/kg).

***In vitro* gastrointestinal digestion of meat samples**

*In vitro* gastrointestinal digestion (IGD) of pork homogenates was also evaluated (Torres-Martínez *et al*., 2022), with minor modifications. The physiological solution was prepared with d-water (200 mL) and placed in a screw-top flask inside a shaking incubator (model MaxQ-5000, Fisher Scientific, Canada) at 150 rpm until it reached an internal temperature of 37 °C in the dark. After that, pH was adjusted between 2.0 – 2.5 with hydrochloric acid (5 M), while the stomach phase was simulated by adding pepsin (0.33 g) at a 1:10,000 (enzyme-substrate) ratio. Subsequently, minced pork patties (120 g) were homogenized with 200 mL of physiological solution and subjected to a continuous digestion process (150 rpm/37 °C/2 h). The mixture (50 mL) was adjusted to a pH of 5.0 – 5.5 with sodium hydroxide (3 M) to inactivate the enzyme. At the same time, the small intestine phase was simulated by adding 50 mL of d-water containing pancreatin (0.19 g, 25,000 UI), lipase (0.001 g, type II, 100 – 500 units/mg of protein), and Oxgall (1 g). After IGD (150 rpm/37 °C/4 h), enzyme activity was inhibited by heating (95 °C/10 min/

in the dark). The digested sample was centrifuged (4200 ×g/4 °C/20 min) and filtered (Milli-pore filter 0.22 μm). The obtained supernatant was used to carry out phytochemical and antioxidant activity tests.

### Statistical analysis

Data from metabolites and antioxidant activity were subjected to a one-way ANOVA. In contrast, data from oxidative stability were subjected to a two-way ANOVA using the treatments and the heating time as the fixed effects and their two-way interaction. Also, data from IGD were subjected to a two-way ANOVA using the treatments and the IGD phases as the fixed effects and their interaction. A mean comparison test (Tukey-Kramer) was performed at $p < 0.05$. All results (mean ± standard deviation) were obtained from at least three independent experimental traits (n = 6). The relationship between treatments and evaluated parameters was also determined through a principal component analysis (SPSS21).

## RESULTS AND DISCUSSION

### Polyphenol content and antioxidant activity

The results of the analysis of polyphenol content and antioxidant activity in aqueous extracts of *P. ostreatus* cultivated on agro-industrial residues are presented in Table 1. Regarding polyphenol assays, the results demonstrate that T2 samples showed the highest ($p < 0.05$) TTC and TCGA values, while T1, T2, and T3 presented the highest ($p < 0.05$) TPHC values. While no significant ($p > 0.05$) variations were observed in TFC across the treatments, the antioxidant activity assays revealed distinct patterns. T2 exhibited the strongest ($p < 0.05$) FRSA and RPA activity, whereas groups T2, T3, and T4 all displayed the highest ($p < 0.05$) RCSA values. Also, T1 showed the highest ($p < 0.05$) FRAP values.

These findings are consistent with previous research indicating that combining corncobs and herb residues in the substrate formulation of the *P. ostreatus* cultivation, resulted in a significant increase in the polyphenol concentration and antiradical properties of the obtained aqueous extract (Jin *et al*., 2018). Another study explored the influence of replacing tree sawdust with tea waste in the growth medium on the antioxidant properties of *P. ostreatus* methanol extracts. The study results revealed that when 20% of the original substrate was replaced with tea waste, the polyphenol content increased, there were changes in the antiradical properties and reducing power measurements (Bozdeveci *et al*., 2022). Miahi *et al*. (2022) also demonstrated that the antiradical properties of *P. ostreatus* depend on the growth substrate. Another study demonstrated that combining sugarcane bagasse and banana leaves, in the formulation of the substrate for *Pleurotus djamor* growth, increases the RCSA values of the obtained aqueous-ethanol extract (Medeiros *et al*., 2024). The methods commonly used to evaluate antioxidant activity are based on three mechanisms: 1) metal ion chelation,

**Table 1.** Polyphenol content and antioxidant activity of the aqueous extract obtained from *Pleurotus ostreatus* grown in agro-industrial residues.
**Tabla 1**. Contenido de polifenoles y actividad antioxidante del extracto acuoso obtenido de *Pleurotus ostreatus* cultivado en residuos agroindustriales.

| Item | Treatments | | | | Std | P-value |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | | |
| TTC (mg CAT/mL) | 0.34 ± 0.01$^{b}$ | 0.47 ± 0.02$^{c}$ | 0.27 ± 0.02$^{a}$ | 0.29 ± 0.01$^{a}$ | | <0.001 |
| TPHC (mg GAE/mL) | 0.20 ± 0.02$^{b}$ | 0.17 ± 0.02$^{ab}$ | 0.17 ± 0.01$^{ab}$ | 0.16 ± 0.01$^{a}$ | | <0.001 |
| TFC (mg QE/mL) | 0.01 ± 0.001 | 0.01 ± 0.001 | 0.01 ± 0.001 | 0.01 ± 0.001 | | 0.679 |
| TCGA (mg CGA/mL) | 0.24 ± 0.01$^{b}$ | 0.27 ± 0.01$^{c}$ | 0.23 ± 0.02$^{ab}$ | 0.22 ± 0.01$^{a}$ | | <0.001 |
| FRSA (%) | 22.24 ± 0.74$^{a}$ | 25.06 ± 1.06$^{b}$ | 21.46 ± 1.79$^{a}$ | 20.07 ± 1.48$^{a}$ | 90.09 ± 1.28$^{c}$ | <0.001 |
| RCSA (%) | 13.92 ± 1.15$^{a}$ | 21.78 ± 2.66$^{b}$ | 22.90 ± 1.57$^{b}$ | 22.32 ± 2.96$^{b}$ | 70.20 ± 0.74$^{c}$ | <0.001 |
| FRAP (mg $Fe^{2+}$/mL) | 0.09 ± 0.01$^{b}$ | 0.04 ± 0.01$^{a}$ | 0.04 ± 0.01$^{a}$ | 0.02 ± 0.01$^{a}$ | 1.50 ± 0.01$^{c}$ | <0.001 |
| RPA (Abs) | 0.34 ± 0.01$^{a}$ | 0.36 ± 0.02$^{b}$ | 0.35 ± 0.01$^{ab}$ | 0.34 ± 0.01$^{a}$ | 1.09 ± 0.04$^{c}$ | <0.001 |

Data expressed as mean ± SD. TTC, total tannin content; TPHC, total phenolic content; TFC, total flavonoid content; TCGA, total chlorogenic acid content; FRSA, free-radical scavenging activity; RCSA, radical cation scavenging activity; FRAP, ferric-reducing antioxidant power; RPA, reducing power ability. T1, wheat straw (100 %); T2, wheat straw (80 %) + spent coffee grounds (10 %) + potato peel (10 %); T3, wheat straw (70 %) + spent coffee grounds (15 %) + potato peel (15 %); T4, wheat straw (60 %) + spent coffee grounds (20 %) + potato peel (20 %); Std, standard (BHT). Lowercase letters indicate differences between treatments ($p < 0.05$).
Datos expresados como media ± DE. TTC, contenido total de taninos; TPHC, contenido total de fenoles; TFC, contenido total de flavonoides; TCGA, contenido total de ácido clorogénico; FRSA, actividad eliminadora de radicales libres; RCSA, actividad eliminadora de radicales cationes; FRAP, poder antioxidante reductor férrico; RPA, poder reductor. T1, paja de trigo (100 %); T2, paja de trigo (80 %) + granos de café gastados (10 %) + cáscara de papa (10 %); T3, paja de trigo (70 %) + granos de café gastados (15 %) + cáscara de papa (15 %); T4, paja de trigo (60 %) + granos de café gastados (20 %) + cáscara de papa (20 %); Std, estándar (BHT). Las letras minúsculas indican diferencias entre tratamientos ($p < 0.05$).

2) electron transfer (ET), and 3) hydrogen atom transfer (HAT) (Ivanova *et al*., 2020).

Edible and medicinal mushrooms can release phenolic compounds from the substrate through several mechanisms, such as the production of ligninolytic enzymes. These compounds can be absorbed by mycelium and conjugated with other molecules. In addition, although at a biochemical level, some mechanisms of action are not clearly known, the production of secondary metabolites in fungi responds to an environmental stimulus; these stimulate the biosynthesis of metabolites by regulating the expression of genes, transcription factors, and signaling factors (Lin *et al*., 2020; Silva *et al*., 2024).

## Oxidative stability of meat samples

Table 2 showcases the effect of both, the treatment applied and the heating time, on the oxidative stability of homogenized pork meat. The analysis identified a noteworthy interaction between these two factors ($p < 0.001$), affecting both pH and TBARS measurements. These parameters exhibited an increase throughout the heating time. After heating, in treatments T2 and T3, samples exhibited the highest ($p < 0.05$) pH and the lowest TBARS values. Concerning color parameters, the analysis revealed a synergistic influence between the applied treatment and duration of thermal exposure ($p < 0.001$) on L*, a*, and b* values, of which L* values increased, and the others decreased by the effect of the heating time. At the end of the heating period, similarly, BHT, T1-T4 samples showed lower ($p < 0.05$) L* values than Control samples, while T1-T3 samples presented the highest ($p < 0.05$) a* values. In addition, T1-T4 showed higher ($p < 0.05$) b* values than Control samples.

The human diet requires meat and animal products as a vital source of nutrients and endogenous antioxidants, yet

**Table 2.** Treatment and storage time effect on the oxidative stability of pork meat homogenates.
**Tabla 2**. Efecto del tratamiento y del tiempo de almacenamiento sobre la estabilidad oxidativa de homogenizados de carne de cerdo.

| Item | Treatments | Heating time (at 65 °C) | | |
|---|---|---|---|---|
| | | 0 min | 60 min | 120 min |
| pH | Control | 5.62 ± 0.02 $^{aA}$ | 5.97 ± 0.01 $^{aB}$ | 6.09 ± 0.01 $^{aC}$ |
| | BHT | 5.64 ± 0.01 $^{aA}$ | 5.99 ± 0.01 $^{aB}$ | 6.13 ± 0.05 $^{aC}$ |
| | T1 | 5.81 ± 0.02 $^{bA}$ | 6.27 ± 0.01 $^{bB}$ | 6.27 ± 0.00 $^{bB}$ |
| | T2 | 5.85 ± 0.01 $^{cA}$ | 6.29 ± 0.01 $^{bB}$ | 6.34 ± 0.01 $^{cB}$ |
| | T3 | 5.89 ± 0.01 $^{dA}$ | 6.32 ± 0.01 $^{cB}$ | 6.34 ± 0.01 $^{cB}$ |
| | T4 | 5.64 ± 0.01 $^{aA}$ | 5.99 ± 0.01 $^{aB}$ | 6.10 ± 0.01 $^{aC}$ |
| TBARS | Control | 0.23 ± 0.01 $^{cA}$ | 0.47 ± 0.01 $^{cB}$ | 0.63 ± 0.03 $^{eC}$ |
| | BHT | 0.22 ± 0.01 $^{cA}$ | 0.41 ± 0.01 $^{bB}$ | 0.53 ± 0.02 $^{dC}$ |
| | T1 | 0.02 ± 0.01 $^{aA}$ | 0.09 ± 0.02 $^{aB}$ | 0.25 ± 0.02 $^{cC}$ |
| | T2 | 0.04 ± 0.01 $^{bA}$ | 0.12 ± 0.04 $^{aB}$ | 0.09 ± 0.03 $^{aB}$ |
| | T3 | 0.06 ± 0.03 $^{bA}$ | 0.10 ± 0.02 $^{aB}$ | 0.09 ± 0.03 $^{aB}$ |
| | T4 | 0.04 ± 0.02 $^{bA}$ | 0.07 ± 0.02 $^{aB}$ | 0.14 ± 0.02 $^{bC}$ |
| L* | Control | 36.05 ± 1.60 $^{aA}$ | 54.93 ± 2.02 $^{aB}$ | 61.74 ± 1.11 $^{bC}$ |
| | BHT | 38.99 ± 0.28 $^{bA}$ | 55.72 ± 2.38 $^{aB}$ | 58.24 ± 0.85 $^{aB}$ |
| | T1 | 36.10 ± 1.61 $^{aA}$ | 58.31 ± 1.07 $^{aB}$ | 59.03 ± 1.24 $^{aB}$ |
| | T2 | 36.13 ± 1.62 $^{aA}$ | 58.06 ± 0.73 $^{aB}$ | 60.42 ± 0.96 $^{aB}$ |
| | T3 | 39.05 ± 0.28 $^{bA}$ | 57.21 ± 4.79 $^{aB}$ | 57.86 ± 0.94 $^{aB}$ |
| | T4 | 39.08 ± 0.28 $^{bA}$ | 56.56 ± 2.19 $^{aB}$ | 57.65 ± 1.09 $^{aB}$ |
| a* | Control | 7.16 ± 0.49 $^{aB}$ | -2.15 ± 0.27 $^{aA}$ | -2.31 ± 0.11 $^{aA}$ |
| | BHT | 6.56 ± 0.46 $^{aB}$ | -2.29 ± 0.17 $^{aA}$ | -2.40 ± 0.04 $^{aA}$ |
| | T1 | 7.18 ± 0.50 $^{aB}$ | -2.11 ± 0.23 $^{aA}$ | -2.17 ± 0.26 $^{abA}$ |
| | T2 | 7.18 ± 0.49 $^{aB}$ | -1.95 ± 0.21 $^{aA}$ | -1.98 ± 0.18 $^{bA}$ |
| | T3 | 6.57 ± 0.46 $^{aB}$ | -2.30 ± 0.19 $^{aA}$ | -2.25 ± 0.11 $^{abA}$ |
| | T4 | 6.58 ± 0.45 $^{aB}$ | -2.22 ± 0.21 $^{aA}$ | -2.54 ± 0.40 $^{aA}$ |
| b* | Control | 9.56 ± 0.86 $^{aC}$ | 6.57 ± 0.93 $^{bB}$ | 3.96 ± 0.69 $^{aA}$ |
| | BHT | 9.09 ± 0.64 $^{aB}$ | 4.23 ± 0.18 $^{aA}$ | 4.56 ± 0.52 $^{aA}$ |
| | T1 | 9.58 ± 0.86 $^{aB}$ | 6.79 ± 0.59 $^{bA}$ | 6.35 ± 0.68 $^{bA}$ |
| | T2 | 9.59 ± 0.86 $^{aB}$ | 7.20 ± 0.24 $^{bA}$ | 6.99 ± 0.49 $^{bA}$ |
| | T3 | 9.11 ± 0.63 $^{aB}$ | 6.17 ± 0.98 $^{bA}$ | 6.39 ± 0.51 $^{bA}$ |
| | T4 | 9.10 ± 0.63 $^{aB}$ | 6.24 ± 0.51 $^{bA}$ | 5.84 ± 0.65 $^{bA}$ |

Data expressed as mean ± SD. TBARS, thiobarbituric acid reactive substances. T1, wheat straw (100 %); T2, wheat straw (80 %) + spent coffee grounds (10 %) + potato peel (10 %); T3, wheat straw (70 %) + spent coffee grounds (15 %) + potato peel (15 %); T4, wheat straw (60 %) + spent coffee grounds (20 %) + potato peel (20 %); Std, standard (BHT). Lowercase letters indicate differences between treatments in each sampling time; capital letters indicate differences in each treatment through the storage time ($p < 0.05$). Datos expresados como media ± DE. TBARS, sustancias reactivas al ácido tiobarbitúrico. T1, paja de trigo (100 %); T2, paja de trigo (80 %) + granos de café gastados (10 %) + cáscara de papa (10 %); T3, paja de trigo (70 %) + granos de café gastados (15 %) + cáscara de papa (15 %); T4, paja de trigo (60 %) + granos de café gastados (20 %) + cáscara de papa (20 %); Std, estándar (BHT). Las letras minúsculas indican diferencias entre tratamientos en cada tiempo de muestreo; las letras mayúsculas indican diferencias en cada tratamiento a través del tiempo de almacenamiento ($p < 0.05$).

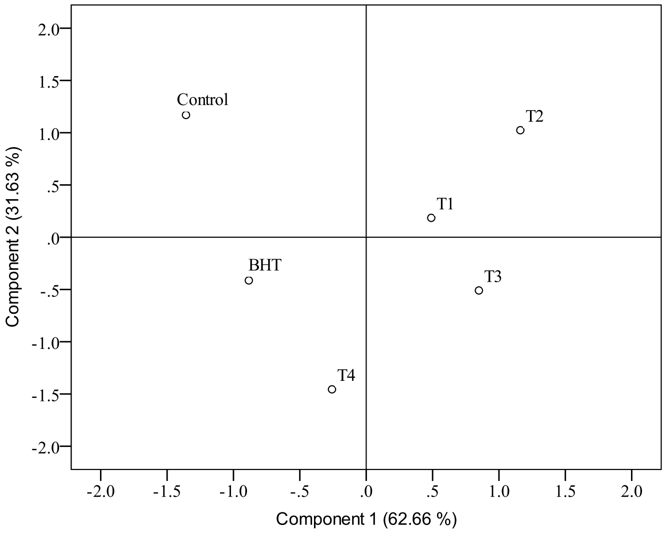
the components of meat are susceptible to deterioration. The oxidative deterioration of meat and meat products during manufacturing, processing, storage, and heat treatment can be evaluated using various effective techniques to assess changes in pH, lipid-protein oxidation, and color. The inclusion of naturally sourced antioxidants (exogenous antioxidants) in meat and meat productsn represents a highly promising approach to minimizing alterations in oxidative stability (Rangel-Vargas *et al*., 2021; Zhu *et al*., 2022). Research has demonstrated that incorporating *P. ostreatus* powder (2-5 %) in the formulation of uncooked beef patties, reduced changes in pH, TBARS, and color values throughout refrigerated storage (4 °C/13 days) (Cerón-Guevara *et al*., 2019). A similar effect was observed on these parameters when adding *P. djamor* powder (5-7.5 %) within the composition of beef patties refrigerated at 4 °C/ 12 days (Bermúdez *et al*., 2023). In cooked meat products, it was demonstrated that adding *P. ostreatus* powder (1-5 %) in the formulation of pork sausages, reduced TBARS and color values during storage at 2 °C/ 90 days (Özünlü and Ergezer, 2020). Furthermore, studies have indicated that the incorporation of extracts derived from *P. ostreatus* and *P. pulmonarious* (250 ppm) in the formulation of pork patties, reduced TBARS values during heating at 65 °C/60 min (Torres-Martínez *et al*., 2023).

pH values in antioxidant-treated pork minced meat may be slightly higher than in untreated ones but still show lower lipid oxidation. This is because certain phenolic compounds and plant extracts may be more effective under less acidic conditions, which reinforces their antioxidant activity (Cheng *et al*., 2007; Di Majo *et al*., 2011). In addition, there are different pathways in which polyphenols inhibit hemoprotein-mediated lipid oxidation in muscle foods: 1) the reducing capacity of polyphenols towards oxidized forms of hemoglobin and myoglobin (the reactions between polyphenols and ferryl heme proteins, met-heme proteins, oxy-heme proteins); 2) the covalent and non-covalent interactions; 3) partitioning of polyphenols into cellular membranes of muscle (physical barrier and membrane fluidity change); 4) polyphenols as reactive oxygen species- and free-radical-scavengers; 5) Role of polyphenols as reactive carbonyl species scavengers; 6) indirect pathways (regeneration of alpha-tocopherol, chelating free ions and decomposition of lipid hydroperoxides) (Wu *et al*., 2024).

The distinctions among experimental conditions and evaluated meat quality indicators are illustrated in Figure 1. The initial two principal components accounted for 62.66 % and 31.63 % of the total variance, respectively; the two components explained 94.29 % of the total variation. The results indicate that T2 samples, grouped in the graph towards the upper right quadrant, presented the highest values of pH and color (a* and b*) and the lowest values of L* and TBARS. Consistent with these findings, previous research has documented that these quality parameters are highly associated (Zhu *et al*., 2022). A high association has been observed between these quality parameters of meat products treated with natural antioxidants (Ramírez-Rojo *et al*., 2022).

**_In vitro_ gastrointestinal digestion of meat samples**

The impact of experimental treatments and digestive stages on the phenolic compound concentrations in porcine tissue homogenates is illustrated in Figure 2. Analysis revealed a synergistic effect between the applied treatments and the stages of digestion ($p < 0.001$), on TTC, TPHC, TFC, and TCGA values, which increased by the digestion phase effect. The results of the digestion experiment indicated that group T2 possessed the highest ($p < 0.05$) TTC and TCGA values, while T1-T4 presented higher ($p < 0.05$) TPHC and TFC values than the control and BHT. The impact of the treatment and digestion phase on the antioxidant activity of homogenized pork meat is illustrated in Figure 3. According to the results ($p < 0.001$) on FRSA, RCSA, FRAP, and RPA values, there was a significant interaction between the treatment applied and



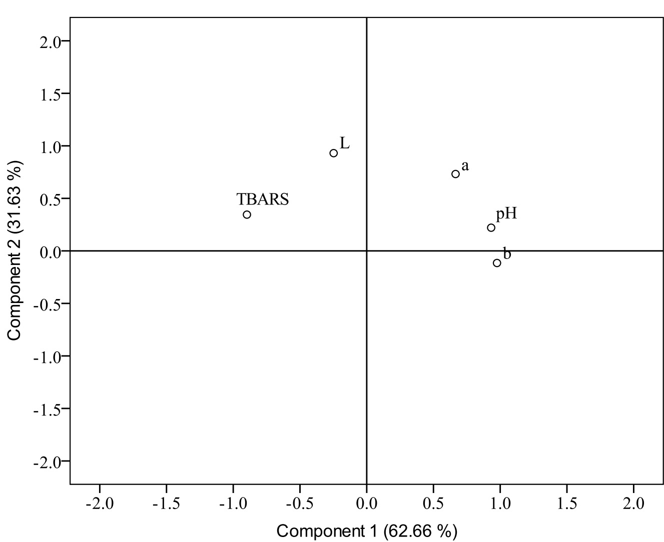




**Figure 1.** Principal component analysis of treatments and evaluated parameters.
**Figura 1**. Análisis de componentes principales de los tratamientos y de los parámetros evaluados.

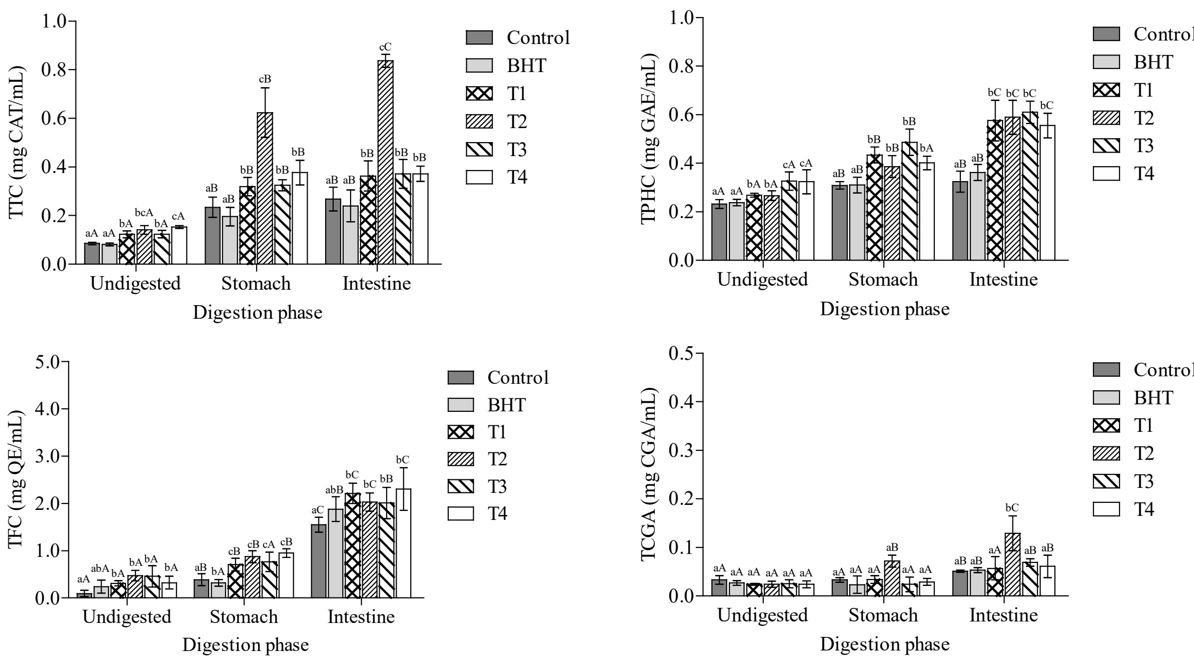



**Figure 2.** Treatment and digestion phase effect on polyphenol content of pork meat homogenates. Lowercase letters indicate differences between treatments in each digestion phase; capital letters indicate differences in each treatment through the digestion process ($p < 0.05$).
**Figura 2**. Efecto del tratamiento y de la fase de digestión sobre el contenido de polifenoles de homogenizados de carne de cerdo. Las letras minúsculas indican diferencias entre tratamientos en cada fase de digestión; las letras mayúsculas indican diferencias en cada tratamiento a través del proceso de digestión ($p < 0.05$).

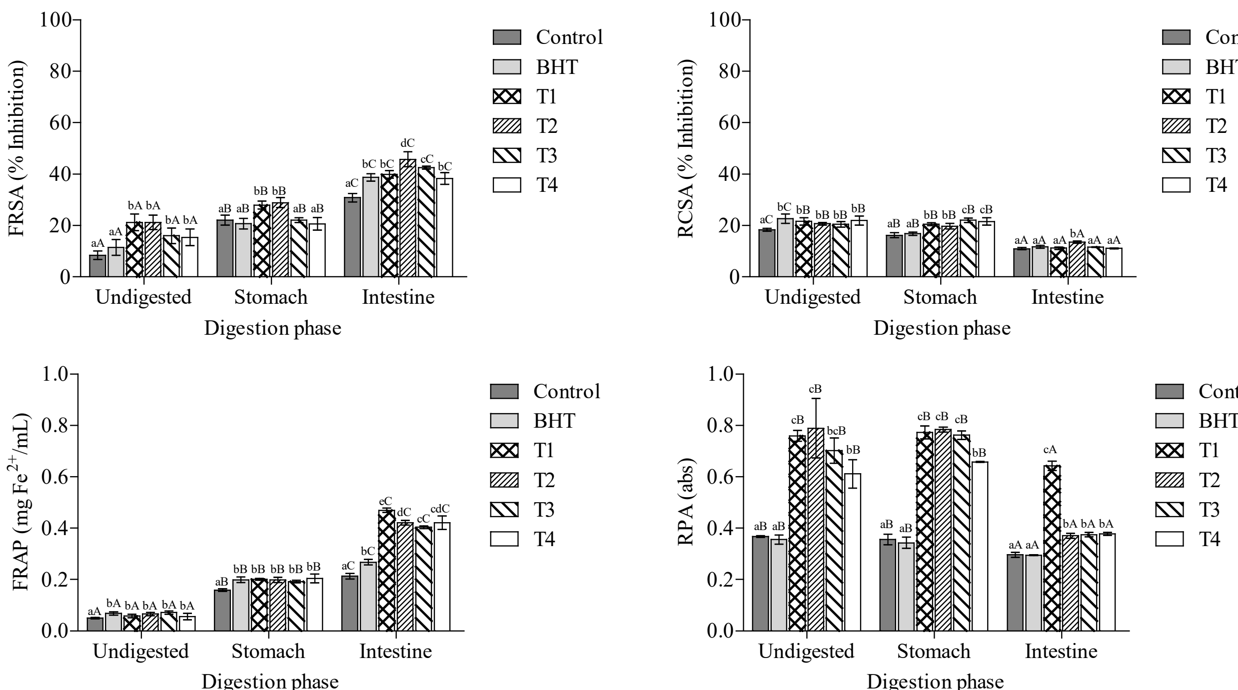



**Figure 3.** Treatment and digestion phase effect on antioxidant activity of pork meat homogenates. Lowercase letters indicate differences between treatments in each digestion phase; capital letters indicate differences in each treatment through the digestion process ($p < 0.05$).
**Figura 3**. Efecto del tratamiento y de la fase de digestión sobre la actividad antioxidante de homogenizados de carne de cerdo. Las letras minúsculas indican diferencias entre tratamientos en cada fase de digestión; las letras mayúsculas indican diferencias en cada tratamiento a través del proceso de digestión ($p < 0.05$).

the digestion phase. FRSA and FRAP values increased ($p < 0.05$) by the digestion phase effect, while RCSA and RPA values decreased ($p < 0.05$). The analysis revealed that T2 samples possessed the greatest ($p < 0.05$) FRSA, RCSA, and RPA activity at the conclusion of the digestion phase. Conversely, T1 samples demonstrated the highest ($p < 0.05$) FRAP values. Furthermore, Figure 4 shows the contrast between treatments and the assessed parameters (polyphenol content and antioxidant activity). The first and second principal components accounted for 68.23% and 26.12% of the variance, respectively, explaining a total of 94.35% of the variation in the data. The results indicate that T2 samples, grouped in the graph towards the upper right quadrant, presented the highest polyphenol and antioxidant activity values.

Prior investigations have shown that how meat and meat products are broken down in the digestive system, can affect their susceptibility to oxidative damage. Therefore, adding or formulating meat products with additives of natural origin is recommended to increase the presence of antioxidant compounds and oxidative stability (Nieva-Echevaría *et al*., 2018). Previous research suggests that the aqueous extract of *Pleurotus ostreatus* exhibits increased antiradical activity and enhanced reducing power after undergoing gastrointestinal digestion (Brugnari *et al*., 2018). A similar effect was observed on polyphenol content, antiradical, and reducing power activity of *Pleurotus sajor-caju* after *in vitro* gastrointestinal digestion (Ng and Rosman, 2019). When incorporated into a food matrix, reports indicate that adding *P*. *ostreatus* powder (2 and 5%) in the formulation of pork patties increased polyphenol content and antioxidant activity after *in vitro* gastrointestinal digestion (Torres-Martínez *et al*., 2022). The bioavailability of polyphenols is key to determining the content of polyphenols released from the meat matrix. After digestion, an increase in the content of these compounds has been observed in patties added with extracts from natural origin, which is associated with greater solubilization in digestive fluids. In addition, the acid hydrolysis of glucosides during digestion, which forms aglycone structures, enhances antioxidant activity (Antonini *et al*., 2020).

## CONCLUSIONS

Our findings demonstrate a significant influence of the type of agro-industrial waste added to the cultivation substrate, on the polyphenol content and antioxidant activity from *P. ostreatus* aqueous extract (T2). The aqueous (T2) extract helps maintain pH, lipid oxidation, and meat color. Furthermore, during gastrointestinal digestion, polyphenol content and antioxidant activity increase in meat samples, suggesting that it could be a promising additive for the meat industry.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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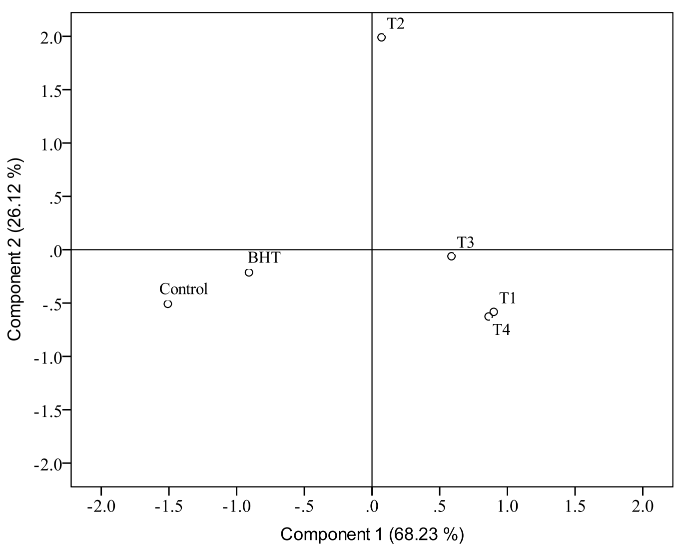


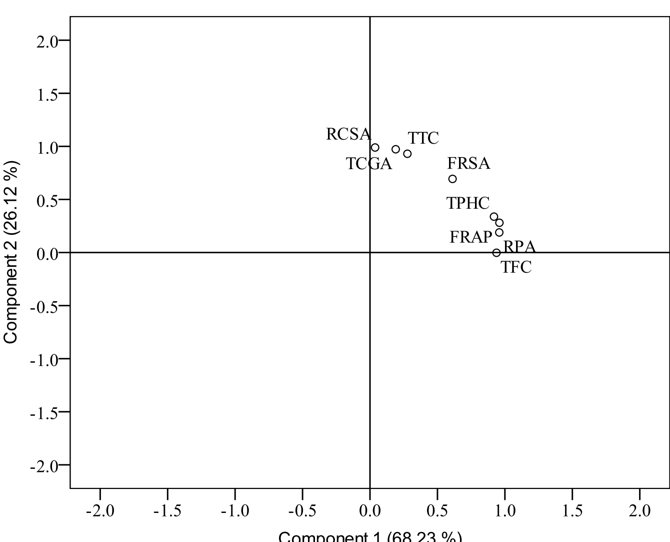


**Figure 4.** Principal component analysis of treatments and evaluated parameters.
**Figura 4**. Análisis de componentes principales de los tratamientos y de los parámetros evaluados.

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