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# Antioxidant and antibacterial activity of coffee silverskin fermented with *Pleurotus ostreatus*

# Actividad antioxidante y antibacteriana de la cascarilla plateada de café fermentada con *Pleurotus ostreatus*

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**ABSTRACT**

This study evaluated the effect of the extraction method and the level of coffee silverskin residue addition on the metabolite composition, antioxidant activity, and antibacterial activity of aqueous extracts. Two extraction methods were employed: maceration and submerged fungal fermentation, with residue levels of 0, 1, and 2 %. The results showed that both factors significantly influenced the composition and bioactivity of the extracts. Maceration with 1 % and 2 % residue yielded the highest phenolic compound content, while fermentation with 2 % residue yielded the highest chlorogenic acid content. Extracts obtained by maceration exhibited the greatest inhibition of free radicals and ferric reducing power. In contrast, those obtained by fermentation presented the most significant inhibition of cation radicals and reducing power. Fermentation with 2 % resulted in greater inhibition of *Staphylococcus aureus*, while maceration and fermentation with 2 % were effective against *Listeria monocytogenes* and *Salmonella typhimurium*. Extracts obtained by submerged fungal fermentation are potential additives for the meat industry.

**KEY WORDS:** Agro-industrial residue, Maceration, Fermentation, Bioactivity, *Pleurotus ostreatus*.

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**R E S U M E N**

Este estudio evaluó el efecto del método de extracción y el nivel de adición de residuo de la cascarilla plateada de café en la composición de metabolitos, actividad antioxidante y actividad antibacteriana de extractos acuosos. Se emplearon dos métodos de extracción: maceración y fermentación fúngica sumergida, con niveles de residuo de 0, 1 y 2 %. Los resultados mostraron que ambos factores influyeron significativamente en la composición y bioactividad de los extractos. La maceración con 1 % y 2 % de residuo presentó el contenido más alto de compuestos fenólicos, mientras que la fermentación con 2 % de residuo tuvo el ácido clorogénico más alto. Los extractos obtenidos por maceración exhibieron la inhibición más significativa de radicales libres y poder reductor férrico. Por el contrario, los obtenidos por fermentación presentaron la inhibición más significativa de radicales catiónicos y poder reductor. La fermentación con 2 % resultó en una mayor inhibición de *Staphylococcus aureus*, mientras que la maceración y fermentación con 2 % fueron efectivas contra *Listeria monocytogenes* y *Salmonella typhimurium*. Los extractos obtenidos por fermentación fúngica sumergida son aditivos potenciales para la industria cárnica.

**P A L A B R A S  C L A V E :** Residuos agroindustriales, Maceración, Fermentación, Bioactividad, *Pleurotus ostreatus*.

## Introduction

The coffee industry generates a significant number of by-products at different stages of its processing, more than six million tons per year, including coffee pulp, husk, and silver husk. These by-products are considered underutilized, as they are a source of carbohydrates, proteins, fat, fiber (soluble and insoluble), cellulose (mainly glucose units), hemicellulose (arabinose, mannose, galactose, and xylose), lignin (soluble and insoluble), caffeine, tannins, and chlorogenic acid. Furthermore, the presence of bioactive compounds such as polyphenols has been associated with the biological properties of these by-products (Ballesteros *et al*., 2014; Serna-Jiménez *et al*., 2022).

Unlike coffee husks, which are obtained from coffee cherries during dry processing, coffee silver husks (seminal tegument or testa) are the main by-product generated during the roasting process (Serna-Jiménez *et al*., 2022). It is estimated that, between 2015 and 2023, global coffee production grew from 8.5 to 11 million tons, resulting in an accumulation of silver husks between 200,000 and 400,000 tons per year (Boninsegna *et al*., 2025). Due to its composition and functional properties, this by-product represents an alternative raw material with potential for value-added applications in the food industry (Boninsegna *et al*., 2025; Serna-Jiménez *et al*., 2022).

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Regarding its functional properties, a previous study showed that silver husk flour exhibits techno-functional properties such as water retention capacity, oil retention capacity, emulsification capacity, and emulsion stability. In contrast, the aqueous-methanolic extract obtained from silver husk by maceration extraction showed antiradical activity as well as reducing power (Ballesteros *et al*., 2014).

In another study, the effectiveness of the aqueous extract of silverskin, obtained by maceration extraction, as an antioxidant in uncooked pork homogenates was evaluated. To this end, the polyphenol content and antioxidant activity of the extract were determined, and the extract was incorporated into the meat homogenate at concentrations of 0, 250, and 500 ppm, using induced oxidation. The results showed that the extract reduced lipid oxidation, metmyoglobin content, and color changes, which were related to the presence of polyphenols, suggesting its potential use as an antioxidant additive in meat products (Vargas-Sánchez *et al*., 2024).

However, the extraction of antioxidant and antibacterial compounds through biotechnological processes presents an opportunity to develop new food additives (Martí-Quijal *et al*., 2021; Ogidi *et al*., 2020). Fermentation, both in solid-state and submerged culture, has been explored as a tool to recover bioactive compounds (Martí-Quijal *et al*., 2021). The use of fungi to ferment agro-industrial by-products can facilitate the release of these compounds through enzymatic activity, becoming an alternative to conventional solvent extraction, since this often does not completely release the compounds bound to the plant matrix (Ogidi *et al*., 2020; Terán-Rivera *et al*., 2025). Submerged fermentation, in particular, offers several advantages over solid-state fermentation, such as uniform inoculum distribution and shorter processing times (Dey *et al*., 2016). However, its effectiveness depends on several factors, including the fungal species used, the substrate composition, and the culture conditions (Martí-Quijal *et al*., 2021; Terán-Rivera *et al*., 2025).

Despite these findings, no research has been reported on the recovery of antioxidant compounds from silverskin by submerged fermentation using Pleurotus spp. Therefore, in this study, the objective was to evaluate the metabolite composition and antioxidant activity of aqueous extracts of silverskin fermented in submerged culture with *Pleurotus ostreatus* mycelium.

## Materials and Methods

### Plant material

The coffee residue (silver husk), donated by a local supplier (Caffenio®, Hermosillo, Mexico), was dried at 60 °C (DX402, Yamato, Japan) until it reached 10 % humidity, and then sterilized at 121 °C for 20 min (SM300, Yamato, Japan).

### Fermentation in a submerged medium

*P. ostreatus* mycelium was grown on Petri dishes using potato dextrose agar as a medium at 25 °C for five days (IC602, Yamato, Japan), until the mycelium completely covered the surface.

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The fermentation medium (pH = 5.4) was sterilized at 121 °C for 30 min and consisted of glucose (20 g/L), yeast extract (5 g/L), potassium phosphate (1 g/L), magnesium sulfate (0.5 g/L), and ascorbic acid (0.05 g/L). Fermentation was carried out using 100 mL of medium, to which different proportions of silverskin (0, 1, and 2 %, w/v) were added. Two types of tests were performed: 1) without the addition of mycelium, called ME (maceration extraction), and 2) with the addition of 1.5 mg of mycelium, called FE (fermentation extraction). The samples were incubated at 150 rpm (28 °C; 2.54 cm orbital shaking) for 10 days (MaxQ-5000, Fisher, Canada), in the dark (Terán-Rivera *et al*., 2025).

### Extract obtention

The fermented culture medium was homogenized at 10,000 rpm (4 °C) for 1 min (T25, IKA®, Germany) and vacuum-filtered through Whatman 1 filter paper (MVP6, Soosung Vacuum, South Korea). The resulting solution was then freeze-dried (DC401, Yamato, Japan), and the aqueous extracts obtained were stored in plastic containers until analysis (Terán-Rivera *et al*., 2025).

### Metabolite's content

Total carbohydrate content (TCC) was determined using the phenol-sulfuric acid method (Albalasmeh *et al*., 2024), with slight modifications. Aqueous extracts (50 µL, 5 mg/mL) were homogenized with 25 µL of phenol solution (5 %, v/v) and 125 µL of concentrated sulfuric acid. The reaction mixture was incubated at room temperature (25 °C) in the dark for 10 min. The absorbance was measured at 490 nm in a spectrophotometer (Multiskan-FC, Thermo Scientific, Japan), and the results were expressed as glucose equivalents (g GE/L).

The total phenol content (TPC) was determined using the Folin-Ciocalteu method (Matić & Jakobek, 2021), with slight modifications. The aqueous extracts (20 µL, 5 mg/mL) were homogenized with 160 µL of distilled water, 40 µL of Folin-Ciocalteu reagent (2 M), and 60 µL of sodium carbonate solution (7 %, w/v). The reaction mixture was incubated at 25 °C in the dark for 1 h. The absorbance was measured at 750 nm, and the results were expressed as gallic acid equivalents (g GAE/L).

The total flavonoid content (TFC) was determined using the aluminum chloride complexation method (Matić & Jakobek, 2021), with slight modifications. The aqueous extract (20 µL, 5 mg/mL) was homogenized with 130 µL of methanol and 20 µL of aluminum chloride (5 %, w/v). The reaction mixture was incubated at 25 °C for 30 min in the dark. The absorbance was measured at 415 nm, and the results were expressed as quercetin equivalents (g QE/L).

The total chlorogenic acid content (CAC) was also determined (Nguyen *et al*., 2022), with slight modifications. The aqueous extracts (100 µL, 5 mg/mL) were homogenized with 500 µL of distilled water, 200 µL of urea (0.17 M), and 200 µL of glacial acetic acid (0.1 M). The obtained solution was mixed with 500 µL of sodium nitrite (0.14 M) and 500 µL of sodium hydroxide (1 M), a mixture that was centrifuged at 2250× g (5 °C) for 10 min (ST18R, Thermo Fisher Scientific,

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USA). The absorbance was measured at 510 nm, and the results were expressed as chlorogenic acid equivalents (g CAE/L).

### Antiradical activity

Free radical scavenging activity was measured using the DPPH method (Carrera-Felipe *et al*., 2020), with slight modifications. Aqueous extracts (100 µL, 5 mg/mL) were homogenized with 100 µL of DPPH solution (0.03 M). The reaction mixture was incubated at 25 °C for 30 min in the dark. Absorbance was measured at 517 nm, and the results were expressed as a percentage of radical inhibition.

The radical-scavenging activity was determined using the ABTS method (Biskup *et al*., 2013),with slight modifications. Aqueous extracts (20 µL, 5 mg/mL) were homogenized with 200 µL of ABTS solution (absorbance = 0.8 at 730 nm, diluted in ethanol). The reaction mixture was incubated at 25 °C for 10 min in the dark. The absorbance was measured at 730 nm, and the results were expressed as a percentage of radical inhibition.

### Reducing power

The reducing power was determined using the ferric reducing antioxidant power (FRAP) method (Berker *et al*., 2010), with slight modifications. Aqueous extracts (20 µL, 5 mg/mL) were homogenized with 200 µL of FRAP solution [10:1:1, sodium acetate buffer (0.3 M) in glacial acetic acid (pH 3.6) and 2,4,6-Tris(2-pyridyl)-s-triazine (0.01 M) in hydrochloric acid (0.04 M) and ferrous chloride (0.02 M)]. The reaction mixture was incubated at 25 °C for 8 min in the dark. The absorbance was measured at 595 nm, and the results were expressed as $Fe^{2+}$ equivalents (g $Fe^{2+}$/L).

The reducing power ability (RPA) was also determined using the ferricyanide/Prussian blue method (Berker *et al*., 2010), with slight modifications. The aqueous extracts (100 µL, 5 mg/mL) were homogenized with 300 µL of phosphate buffer (0.2 M, pH 6.6) and 300 µL of potassium ferrocyanide (1 %, w/v). The reaction mixture was incubated at 50 °C for 20 min in the dark. Subsequently, the solution was mixed with 300 µL of TCA (10 %, w/v) and centrifuged at 4200× g (4 °C) for 10 min. The supernatant (100 µL) was mixed with 100 µL of distilled water and 250 µL of iron chloride (0.1 %, w/v). The absorbance was measured at 700 nm, and the results were expressed as absorbance at the same wavelength.

### Antibacterial activity

Antibacterial activity was determined using the microdilution method (Terán-Rivera *et al*., 2025), with slight modifications. The pathogens used were Gram-positive, including *Staphylococcus aureus* ATCC 29213B (SAU) and *Listeria monocytogenes* ATCC 33090 (LMO), and Gram-negative, like *Escherichia coli* ATCC 25922 (ECO) and *Salmonella typhimurium* ATCC 14028 (STY) bacterial strains, which were initially reactivated in BHI broth at 37 °C for 24 h (IC403C, Yamato, Japan). The aqueous extracts (20 µL, 5 mg/mL) were homogenized with 100 µL

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of the bacterial suspension ($1.5 \times 10^8$ CFU/mL) and incubated at 37 °C for 24 h. BHI broth solution was used as a negative control. The absorbance was measured at 630 nm, and the results were expressed as percentage inhibition of the bacteria.

## Statistical analysis

Values are presented as mean ± standard deviation (n = 6). Data were subjected to a two-way ANOVA, considering the level of silverskin addition and the use or absence of mycelium as factors. Statistical differences between treatments were determined using a Tukey-Kramer means comparison test at $p < 0.05$. In addition, principal components analysis (PCA) (SPSS version 21, IBM, USA) was used to evaluate the relationship between variables.

## Results and Discussion

Table 1 presents the results of the metabolite contents (TCC, TPC, TFC, and CAC) in the evaluated extracts. The data indicate that these values were significantly affected by the extraction method and the residue level used ($p < 0.001$). In particular, the FE-1 % and FE-2 % extracts had the lowest TCC, with a 26.5 % reduction compared to ME-0 %. On the other hand, ME-1 % and ME-2 % showed the highest TPC, with a 41.1 % increase compared to ME-0 %. Furthermore, the ME-2 %, FE-1 %, and FE-2 % extracts had the highest TFC (93.5 % increase), and FE-2 % showed the highest CAC, with a 99.8 % increase compared to ME-0 %.

**Table 1. Metabolite content of coffee silverskin extracts obtained by ME and FE.**

| Parameter | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | ME-0 % | ME-1 % | ME-2 % | FE-0 % | FE-1 % | FE-2 % |
| TCC | 20.84$^d$ | 19.41$^c$ | 18.58$^c$ | 16.77$^b$ | 15.04$^a$ | 15.61$^a$ |
| (g GE/L) | (0.47) | (0.38) | (0.57) | (0.52) | (0.50) | (0.49) |
| TPC | 0.38$^c$ | 0.64$^d$ | 0.65$^d$ | 0.20$^a$ | 0.21$^a$ | 0.25$^b$ |
| (g GAE/L) | (0.02) | (0.04) | (0.05) | (0.01) | (0.01) | (0.01) |
| TFC | 0.002$^a$ | 0.025$^b$ | 0.030$^c$ | 0.025$^b$ | 0.031$^c$ | 0.032$^c$ |
| (g QE/L) | (0.001) | (0.001) | (0.001) | (0.001) | (0.001) | (0.001) |
| CAC | 0.06$^a$ | 0.11$^b$ | 0.12$^b$ | 0.40$^c$ | 0.44$^d$ | 0.52$^e$ |
| (g CAE/L) | (0.01) | (0.01) | (0.01) | (0.01) | (0.02) | (0.01) |

Results are expressed as mean (standard deviation) (n = 6). TTC, total carbohydrate content; TPC, total phenolic content; TFC, total flavonoids content; CAC, chlorogenic acid content; GE, glucose equivalents; GAE, gallic acid equivalents; QE, quercetin equivalents; CAE, chlorogenic acid equivalents; ME, maceration extraction; FE, fermentation extraction. The literals indicate significant differences due to the interaction of the extraction method and the level of residue addition (Tukey, $p < 0.05$).

The results obtained are consistent with those reported by Vargas-Sánchez *et al*. (2023) and Terán-Rivera *et al*. (2025), who observed that extracts obtained by submerged fungal fermentation presented higher TPC, TFC, and CAC values, while those obtained by maceration showed higher TCC values. This behavior can be attributed to the ability of the fermentation process to release bioactive compounds bound to the plant matrix through extracellular enzymatic activity (Dey *et al*., 2016). Chlorogenic acid is a phenolic compound widely present in plant extracts, particularly in coffee, and has high functional relevance because it is a potent free radical scavenger and exhibits antibacterial activity (Ballesteros *et al*., 2014; Terán-Rivera *et al*., 2025).

Table 2 presents the results of the antioxidant activity of the evaluated extracts. It is observed that the DPPH and ABTS values are significantly affected by the extraction method and the residue level used ($p < 0.001$). In contrast, the FRAP and RPA values are not affected by the extraction method ($p = 0.262$). Still, they are affected by the level of residue addition ($p < 0.001$), with no significant interaction between the two factors observed ($p = 0.809$). The ME-2 % extract presents the greatest inhibition of the DPPH radical, with a 78.3 % increase compared to ME-0 %. On the other hand, FE-2 % shows the greatest inhibition of the ABTS radical (20.1 % increase) and the highest reducing power values (FRAP and RPA) with an increase of 18.78 and 48.8 % compared to ME-0 %, respectively.

**Table 2. Antioxidant activity of coffee silverskin extracts obtained by ME and FE.**

| Parameter | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | ME-0 % | ME-1 % | ME-2 % | FE-0% | FE-1 % | FE-2 % |
| DPPH (% inhibition) | 9.32[b] (0.25) | 31.18[d] (1.36) | 43.13[e] (2.30) | 2.53[a] (0.30) | 8.72[b] (1.31) | 20.13[c] (1.70) |
| ABTS (% inhibition) | 50.68[a] (0.31) | 52.98[b] (1.05) | 57.52[c] (0.99) | 50.12[ab] (1.04) | 57.10[c] (0.85) | 63.43[d] (0.83) |
| FRAP (g $Fe^{2+}$/L) | 0.160[a] (0.010) | 0.175[b] (0.011) | 0.198[c] (0.016) | 0.153[a] (0.002) | 0.170[b] (0.016) | 0.197[c] (0.011) |
| RPA (700 nm) | 0.173[a] (0.009) | 0.195[b] (0.010) | 0.247[c] (0.003) | 0.185[a] (0.003) | 0.239[c] (0.006) | 0.338[d] (0.010) |

Results are expressed as mean (standard deviation) (n = 6). DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; FRAP, ferric reducing antioxidant power; RPA, reducing power ability; ME, maceration extraction; FE, fermentation extraction. The literals indicate significant differences due to the interaction of the extraction method and the level of residue addition (Tukey, $p < 0.05$).

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The results obtained are consistent with previous studies, which have reported a direct relationship between the phenolic content and the antioxidant activity of extracts obtained by submerged fungal fermentation, highlighting the production of natural antioxidants with potential for industrial applications (Özdal *et al*., 2019; Jiamworanunkul, 2020). In this context, a previous study determined the metabolite content and antioxidant activity of the ethanolic extract obtained by submerged fungal fermentation using mycelium of edible mushrooms of the genus Pleurotus (*P*. *eryngii*, *P*. *ostreatus*, *P*. *florida*, and *P*. *sajor-caju*). The results of this study showed that the extracts obtained presented activity against the DPPH radical (*P*. *ostreatus* > *P*. *eryngii* = *P*. *florida* = *P*. *sajor-caju*) and reducing power (*P*. *ostreatus* = *P*. *eryngii* > *P*. *florida* = *P*. *sajor-caju*), respectively (Dundar *et al*., 2013).

Table 3 presents the antibacterial activity results of the extracts evaluated against Gram-positive and Gram-negative bacteria. It is observed that SAU inhibition is significantly affected by the extraction method and the residue level used ($p < 0.001$). In contrast, LMO inhibition is independently affected by both factors ($p < 0.001$), with no interaction ($p = 0.672$). On the other hand, the inhibition of ECO is not affected by the extraction method ($p = 0.560$). Still, it is affected by the level of residue addition ($p < 0.001$), with no interaction effect ($p = 0.771$). Similarly, STY inhibition was not affected by the extraction method ($p = 0.897$), but it was affected by the level of residue addition ($p < 0.001$), with no interaction effect observed ($p = 0.596$). Regarding antibacterial efficacy, the FE-2 % extract showed the highest inhibition values against SAU, with an 88 % reduction compared to ME-0 %, while ME-2 %, FE-1 %, and FE-2 % showed the greatest inhibition of LMO (58.5 % reduction). Likewise, ME-2 % and FE-2 % were shown to be more effective against ECO (85.1 % reduction), and ME-2 %, FE-1 %, and FE-2 % showed the highest inhibition values against STY, with a 72 % reduction compared to ME-0 %.

The results obtained partially agree with those reported by Nicolcioiu *et al*. (2017) and Hiroko Hassegawa *et al*. (2005), who found that fungal extracts showed greater activity against Gram-positive bacteria. However, in contrast to Terán-Rivera *et al*. (2025), in this study, the fungal fermentation extracts showed activity against Gram-negative bacteria, particularly ECO and STY.

In another study, the antibacterial activity of the aqueous extract obtained by submerged fungal fermentation using mycelium of *P*. *eryngii*, *P*. *sajor-caju*, *P*. *citrinopileatus*, *P*. *ostreatus,* and *P*. *florida* was determined. The results of this study showed that all the extracts exhibited an inhibitory activity against Gram-positive (*Bacillus cereus*) and Gram-negative bacteria (*Azomonas agilis*, *Helicobacter pylori*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Xanthomonas campestris*) (Özdal *et al*., 2019).

It has been shown that Gram-negative bacteria are more resistant than Gram-positive bacteria due to the presence of an outer membrane rich in lipopolysaccharides that acts as a barrier against antibacterial compounds. However, some bioactive compounds (e.g., flavonoids, terpenes, and others) present in natural extracts may be more effective against Gram-negative bacteria by inducing oxidative stress and altering membrane permeability (Efenberger-Szmechtyk *et al*., 2021; Terán-Rivera *et al*., 2025). Combating this type of bacteria is especially important for the meat industry, since many pathogenic or spoilage species associated with meat and its products belong to this group (Efenberger-Szmechtyk *et al*., 2021).

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The results of the Pearson correlation matrix (Table 4) show significant associations between the different variables (metabolite content, antioxidant activity, and antibacterial activity). It is observed that the positive correlation between TPC and the inhibition of the DPPH radical was very strong (r = 0.868; ****p* < 0.001). However, the inverse correlation between TPC and TCC and CAC was very strong (r = -0.926 and -0.786, respectively; ****p* < 0.001).

**Table 3. Antibacterial activity of coffee silverskin extracts obtained by ME and FE.**

| Parameter | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | ME-0 % | ME-1 % | ME-2 % | FE-0 % | FE-1 % | FE-2 % |
| SAU | 9.32[b] | 31.18[d] | 43.13[e] | 2.53[a] | 8.72[b] | 20.13[c] |
| (% inhibition) | (0.25) | (1.36) | (2.30) | (0.30) | (1.31) | (1.70) |
| LMO | 50.68[a] | 52.98[b] | 57.52[c] | 50.12[ab] | 57.10[c] | 63.43[d] |
| (% inhibition) | (0.31) | (1.05) | (0.99) | (1.04) | (0.85) | (0.83) |
| ECO | 0.160[a] | 0.175[b] | 0.198[c] | 0.153[a] | 0.170[b] | 0.197[c] |
| (% inhibition) | (0.010) | (0.011) | (0.016) | (0.002) | (0.016) | (0.011) |
| STY | 0.173[a] | 0.195[b] | 0.247[c] | 0.185[a] | 0.239[c] | 0.338[d] |
| (% inhibition) | (0.009) | (0.010) | (0.003) | (0.003) | (0.006) | (0.010) |

Results are expressed as mean (standard deviation) (n = 6). SAU, *S. aureus*; LMO, *L. monocytogenes*; ECO, *E. coli*; STY, *S. typhimurium*; ME, maceration extraction; FE, fermentation extraction. The literals indicate significant differences due to the interaction of the extraction method and the level of residue addition (Tukey, $p < 0.05$).

On the other hand, the positive correlation between TFC and CAC was strong (r = 0.586v; ****p* < 0.001), while the positive correlations for the inhibition of SAU and LMO were very strong (r = 0.963 and 0.764, respectively, ****p* < 0.001). Furthermore, the positive correlation between TFC with respect to ABTS and RPA (r = 0.619 and 0.620, respectively, ****p* < 0.001), as well as for the inhibition of ECO and STY, was strong (r = 0.697 and 0.700, respectively, ***p < 0.001).

These results suggest that the phenolic compounds present in the extracts contribute significantly to the antioxidant activity and antibacterial effect against relevant pathogens in the food industry.

Various studies have consistently reported an association between metabolites and the antioxidant and antibacterial activity of extracts from natural sources. For example, a previous study reported a strong positive correlation between TPC and TFC and the inhibition of DPPH (r = 0.81 and 0.84) and ABTS (r = 0.86 and 0.83) radicals, in addition to the FRAP (r = 0.88 and 0.77) values of extracts obtained from different parts of meadowsweet (*Filipendula ulmaria*).

Additionally, a strong positive correlation was found between the content of phenolic acids and the values of DPPH (r = 0.93), ABTS (r = 0.94), and FRAP (r = 0.84) (Savina *et al*., 2023).

In the case of coffee industry products, specifically espresso coffee, the highest positive correlation was reported between TPC and DPPH and FRAP antiradical activities (r = 0.664 and 0.723, respectively), compared to ABTS radical inhibition (r = 0.130). On the other hand, TFC and CAC were most strongly correlated with FRAP (r = 0.751 and 0.843) (Jung *et al*., 2021).

**Table 4. Pearson correlation matrix of the evaluated parameters.**

| | TCC | TPC | TFC | CAC | DPPH | ABTS | FRAP | RPA | SAU | LMO | ECO | STY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TCC | 1.000 | 0.627 *** | -0.720 *** | -0.926 *** | 0.292 * | -0.573 *** | -0.153 ns | -0.607 *** | -0.717 *** | -0.488 ** | -0.351 * | -0.355 * |
| TPC | | 1.000 | -0.021 ns | -0.786 *** | 0.868 *** | -0.067 ns | 0.339 * | -0.176 ns | -0.058 ns | 0.209 ns | 0.321 * | 0.362 * |
| TFC | | Alor | 1.000 | 0.586 *** | 0.341 * | 0.619 *** | 0.445 ** | 0.620 *** | 0.963 *** | 0.764 *** | 0.697 *** | 0.700 *** |
| CAC | | | | 1.000 | -0.461 ** | 0.510 ** | 0.055 ns | 0.603 *** | 0.637 *** | 0.342 * | 0.210 ns | 0.164 ns |
| DPPH | | | | | 1.000 | 0.359 * | 0.677 *** | 0.272 ns | 0.319 * | 0.570 *** | 0.711 *** | 0.642 *** |
| ABTS | | | | | | 1.000 | 0.678 *** | 0.959 *** | 0.651 *** | 0.811 *** | 0.873 *** | 0.730 *** |
| FRAP | | | | | | | 1.000 | 0.663 *** | 0.484 ** | 0.711 *** | 0.764 *** | 0.656 *** |
| RPA | | | | | | | | 1.000 | 0.685 *** | 0.769 *** | 0.807 *** | 0.626 *** |
| SAU | | | | | | | | | 1.000 | 0.771 *** | 0.699 *** | 0.681 *** |
| LMO | | | | | | | | | | 1.000 | 0.858 *** | 0.911 *** |
| ECO | | | | | | | | | | | 1.000 | 0.844 *** |
| STY | | | | | | | | | | | | 1.000 |
| + | Very strong | | | Strong | | | Moderate | | | Light | | |
| - | Very strong | | | Strong | | | Moderate | | | Light | | |

TCC, carbohydrates; TPC, phenols; TFC, flavonoids; CAC, chlorogenic acid; DPPH, 2,2-difenil-1-picrilhidrazilo; ABTS, ácido 2,2-azino-bis(3-etilbenzotiazolina-6-sulfónico); FRAP, ferric reducing antioxidant power; RPA, reducing power ability; SAU, *S. aureus*; LMO, *L. monocytogenes*; ECO, *E. coli*; STY, *S. typhimurium*. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ns, not significant.

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Likewise, a high positive correlation was observed between TPC and DPPH, ABTS, and FRAP values (r = 0.979, 0.954, and 0.881, respectively), as well as between CAC and RPA (r = 0.986) in extracts obtained from coffee bagasse. In addition, a strong positive correlation was reported between phenolic content and SAU and LMO inhibition (r = 0.934 and 0.864, respectively), as well as between TFC and CAC and ECO and STY inhibition (r > 0.90) (García-Larez *et al*., 2021).

Figure 1 presents the PCA that explains the variability of the evaluated treatments.

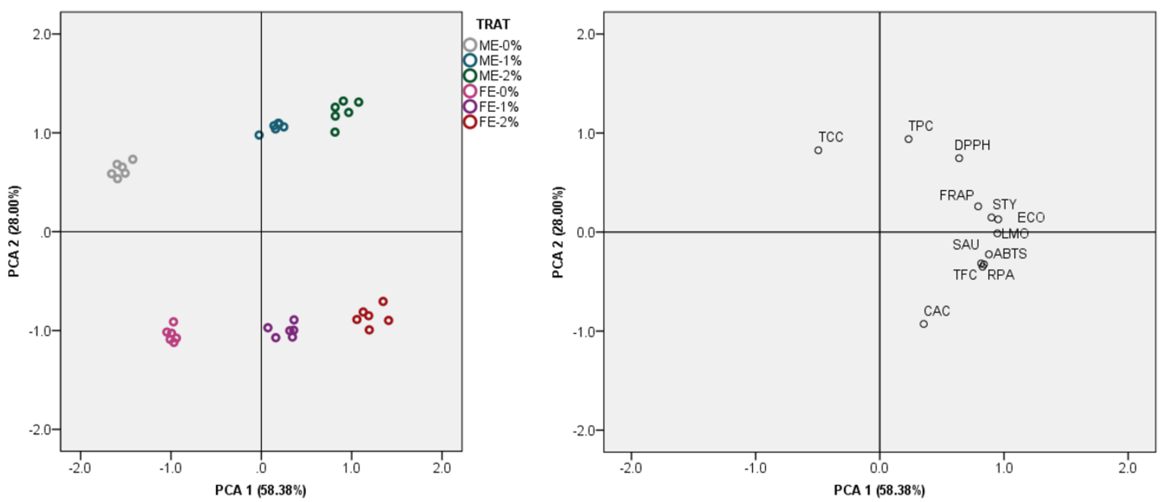


**Figure 1. PCA of evaluated parameters.**

The first component explains 58.38 % of the variation, while the second component accounts for 28.00 %, adding up to 86.38 % of the variability described in the system. The left panel shows a separation between treatments obtained through maceration and submerged fungal fermentation. In the upper right panel, the distribution of the variables suggests that TPC, antioxidant activity measured by DPPH and FRAP, and STY inhibition are positively correlated with the extracts obtained through maceration (ME-2 % > ME-1 %). In contrast, in the lower right panel, TFC and CAC, antioxidant activity measured by ABTS and RPA, and SAU inhibition are positively correlated with the extracts obtained through submerged fungal fermentation (FE-2 % > FE-1 %).

## Conclusions

This study demonstrated that both the extraction method and residue level influenced the metabolite content and biological activity of the extracts. Maceration favored higher phenol content and antioxidant activity (DPPH and FRAP), while submerged fungal fermentation favored flavonoid and chlorogenic acid content and antioxidant capacity (ABTS and RPA). In terms of antibacterial activity, extracts obtained by submerged fungal fermentation were primarily active against Gram-positive bacteria rather than Gram-negative bacteria. Correlation analysis revealed a significant relationship between metabolites and bioactivity, while principal component analysis highlighted the importance of the extraction method in distinguishing between the extracts. In conclusion, extracts obtained by submerged fungal fermentation can be considered a natural additive with potential use in the meat industry.

### Authors contribution

Conceptualization of work, WAAE, ASE, FJIA, RDVS; development of the methodology, WAAE, RDVS; software management, BMTM, RDVS; analysis of results, WAAE, BMTM, GRTU, ASE, RDVS; writing and preparing the manuscript, WAAE, BMTM, GRTU, ASE, RDVS; writing, reviewing, and editing, WAAE, BMTM, GRTU, ASE, RDVS.

All authors of this manuscript have read and agreed to the published version of the manuscript.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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