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METABOLITE CONTENT AND ANTIOXIDANT ACTIVITY OF SPENT COFFEE GRAIN FERMENTED WITH *Pleurotus pulmonarius* MYCELIUM

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ABSTRACT

Spent coffee beans are agro-industrial waste that contain nutrients and bioactive compounds that can be recovered by fungal fermentation-assisted extraction using edible fungal strains. In this study, the metabolite content and antioxidant activity of the aqueous extract of spent coffee beans fermented in a submerged culture using the mycelium of *Pleurotus pulmonarius* were evaluated. The total carbohydrate, phenol, flavonoid, and caffeoylquinic acid contents of the extract were determined, as well as the antioxidant activity by free radical and cation inhibition, reducing power, and lipid oxidation inhibition. The experimental design was completely randomized using a factorial arrangement, with three independent experimental replicates. The data were examined using analysis of variance (ANOVA) and the Tukey-Kramer mean comparison test ($p \leq 0.05$). To determine the association between variables and parameters evaluated, a principal component analysis was used. The results showed that the aqueous extract obtained by submerged culture fermentation using *P. pulmonarius* and different levels of spent coffee beans presented a high content of metabolites such as carbohydrates (70.2 %), phenols (64.5 %), flavonoids (61.9 %), and caffeoylquinic acid (90.8 %), as well as a higher antioxidant activity by inhibiting the formation of 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS$^{\bullet+}$) radicals (31.6 and 31.9 %, respectively), lipid oxidation (70 %), reducing power (14.9 %), and Ferric Reducing Antioxidant Power (FRAP) (89.4 %), compared to the control. Furthermore, the first two main components explained 91.3 % of the variation, revealing that fermented samples with and without mycelium differed in terms of metabolite content and antioxidant activity, which were dependent on the proportion of wasted coffee beans. In conclusion, the fungal fermentation of spent coffee beans is a potential strategy for the recovery of antioxidant ingredients.

Keywords: coffee residues, fungal fermentation, chemical composition, bioactivity.

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INTRODUCTION

Coffee is a perennial plant belonging to the *Coffea* genus of the Rubiaceae family. The processing of its fruit into liquid coffee is the main commercial use of this plant. Coffee is considered one of the most consumed beverages in the world and is classified as the second most commercialized product after petroleum. Dispensing coffee generates large quantities of by-products such as pulp, husk, silver skin, and spent coffee beans (Murthy and Naidu, 2012).

Spent coffee beans are the residue obtained in the process of making "instant coffee," a beverage prepared by the extraction in boiling water of the soluble material from roasted and ground coffee beans (Mussatto *et al*., 2011). This by-product is an important source of nitrogenous components (proteins, free amino acids, and caffeine), fat and fatty acids (palmitic acid and linoleic acid), carbohydrates (fructose, glucose, and galactose), and fiber (cellulose and hemicellulose), as well as bioactive compounds such as melanoidins and phenolic acids (Mussatto *et al*., 2011; Campos-Vega *et al*., 2015).

Coffee by-products are used in limited applications such as livestock feed, composts, and fertilizers. However, the recovery of these compounds with antioxidant potential through chemical and biotechnological processes can be considered a strategy for obtaining new additives for the pharmaceutical or food industry (Ozuna *et al*., 2020).

Fermentation in solid or liquid/submerged culture of various substrates (agro-industrial waste), using bacteria and fungi, is reported to be a useful tool for the production (secondary metabolic pathway) and procurement of antioxidant compounds (extracellular enzymatic release), compared to conventional methods that use organic solvents that do not allow the complete release of bioactive compounds bound to plant tissues (Dey *et al*., 2016). In addition, some of these solvents cannot be used in food production. Fermentation in submerged culture presents potential advantages over solid-state culture by allowing uniformity in the dispersion of the inoculum in the substrate and a considerable reduction in time. However, this will depend on the species of microorganisms used, culture conditions (pH, temperature, aeration, among others), and substrate (Dey *et al*., 2016; Xu *et al*., 2015).

In this context, Zerva *et al*. (2021) reported obtaining aqueous-methanolic extracts (1:1) with the capacity to inhibit free radicals ($DPPH^•$) from agro-industrial wastes (oil mill wastewater and corn cobs) fermented in a submerged culture with *Pleurotus citrinopileatus*. Ogidi *et al*. (2020) reported the antiradical capacity ($DPPH^•$, $HO^•$, and $NO^•$) of polysaccharides obtained from fruit skins (pineapple, banana, and mango) and peels (walnut and peanut) fermented in a submerged culture with *P. pulmonarius*. However, information on the recovery of antioxidant compounds from spent coffee beans by fermentation in a submerged culture using *Pleurotus* spp. is still limited. Therefore, the objective of the present work was to evaluate the metabolite content and antioxidant activity of the aqueous extract of spent coffee beans fermented in a submerged culture using the mycelium of *P. pulmonarius*.

MATERIALS AND METHODS

Plant material

Spent coffee bean residues were collected from local commercial suppliers (CAFFENIO®; Hermosillo, Mexico) and dried at 60 °C in a drying oven (Yamato DX402; Tokyo, Japan) to a moisture content of 10 %. Subsequently, these residues were sterilized at 121 °C for 20 min in an autoclave (Yamato SM300; Tokyo, Japan).

Mycelium collection and fermentation medium

The strain of *P. pulmonarius* (IE-115) used belongs to the fungal collection of the Coordination of Food Technology of Plant Origin (CIAD A.C., Hermosillo, Mexico). The strain was cultivated on Petri dishes with potato dextrose agar at 25 °C for 5 d in an incubator (Yamato IC602; Tokyo, Japan), ensuring that the mycelium covered the entire surface of the plate. The fermentation medium for substrate wetting was sterilized at 121 °C for 20 min using the following composition: glucose (20 g L^{-1}), yeast extract (5 g L^{-1}), potassium phosphate (1 g L^{-1}), magnesium sulfate (0.5 g L^{-1}), and ascorbic acid (0.05 g L^{-1}). The pH was adjusted to 5.4 by the addition of hydrochloric acid (0.1 N) or sodium hydroxide (2.5 M).

The fermentation process was carried out in 250 mL Erlenmeyer flasks with 100 mL of fermentation medium and different proportions of spent coffee beans (0, 5, and 10 %, w/v), which were fermented with or without the addition of 1.5 mg of *P. pulmonarius* mycelium, which was collected aseptically from the surface of the plates with culture. Flasks were incubated at 28 °C and 150 rpm in a rotary incubator (MaxQTM 5000, Fisher Scientific; Nepean, Canada) for 10 d in the dark (Vargas-Sánchez *et al*., 2023).

Extract preparation

The fermented culture medium was homogenized at 10 000 rpm for 30 s (Ultraturrax T25, IKA®; Staufen, Germany), filtered using Whatman 1 filter paper under vacuum (MVP 6 pump, Soosung Vacuum Co. Ldt.; Jeju, South Korea), and dried (Yamato DC401 freeze dryer; Tokyo, Japan). The resulting aqueous extract was stored at -20 °C in the dark until analysis (Liu *et al*., 2018).

Qualitative metabolite profile

The metabolites in the aqueous extract were analyzed qualitatively using standard methods (Griffiths *et al*., 1992; Samejo *et al*., 2013). To extract the metabolites, 0.5 g of the aqueous extract was homogenized with 10 mL of distilled water at 10 000 rpm for 1 min (vortex mixer, Fisher Scientific; CA, USA) and filtered through Whatman 1 filter paper (stock solution).

For carbohydrate analysis (phenol-sulfuric acid test), 2 mL of the stock solution was mixed with 1 mL of aqueous phenol (1 %, v/v) and 1 mL of concentrated sulfuric acid. After mixing, it was incubated at 100 °C for 5 min in the dark. The formation of a brown precipitate indicated a positive result. For phenol analysis (ferric chloride test),

the stock solution (2 mL), previously incubated at 100 °C for 10 min (Yamato BM510 water bath; Tokyo, Japan) and filtered, was mixed with 2 mL of iron chloride solution (0.1 %, w/v). The formation of a blue-black precipitate indicated a positive result.
For flavonoid analysis (Shinoda test), a few pieces of magnesium tape and 0.1 mL of concentrated hydrochloric acid were carefully added to 1 mL of the stock solution. The appearance of a red color indicated a positive result. Finally, for chlorogenic acid analysis (sodium nitrite test), 1 mL of the stock solution was mixed with 1 mL of urea (0.17 M), 1 mL of glacial acetic acid (0.1 M), and 2.5 mL of distilled water. Subsequently, 2.5 mL of sodium nitrite (0.14 M) and 2.5 mL of sodium hydroxide (0.5 M) were added. The appearance of a red color indicated a positive result.

Total metabolite content

Total carbohydrate content was determined using the phenol-sulfuric acid method (Albalasmeh *et al*., 2013). The aqueous extract (50 μL, 5 mg mL^{-1}) was placed in a 96-well microplate and homogenized with 25 μL of aqueous phenol solution (5 %, v/v) and 125 μL of concentrated sulfuric acid. The reaction mixture was incubated at room temperature (25 °C) for 10 min in the dark. Subsequently, absorbance was measured at 490 nm in a spectrophotometer (Multiskan FC UV-Vis, Thermo Scientific; Vantaa, Finland). The results were expressed as mg glucose equivalents per g of dry extract (mg GE g^{-1}).
The Folin-Ciocalteu technique was used to measure total phenol content (Ainsworth and Gillespie, 2007). The aqueous extract (20 μL, 5 mg mL^{-1}) was homogenized with 160 μL of distilled water, 60 μL of sodium carbonate (7 %, w/v), and 40 μL of Folin-Ciocalteu reagent (2 M). The reaction mixture was incubated at 25 °C for 1 h in the dark. Subsequently, the absorbance was measured at 750 nm. The results were expressed as mg of gallic acid equivalents per g of dry extract (mg GAE g^{-1}).
The total flavonoid content was determined using the procedure described by Zhishen *et al*. (1999). An aliquot of the aqueous extract (500 μL, 5 mg mL^{-1}) was mixed with 1 mL of sodium nitrite (5 %, w/v), 1 mL of aluminum chloride (10 %, w/v), and 10 mL of sodium hydroxide (1 M). The resulting mixture was adjusted to 25 mL with 70 % ethanol and incubated at 25 °C for 15 min in the dark. The absorbance was measured at 510 nm, and the results were expressed as mg of quercetin equivalents per g of dry extract (mg EQ g^{-1}).
Total caffeoylquinic acid content was determined by the method previously described (Griffiths *et al*., 1992). The aqueous extract (100 μL, 5 mg mL^{-1}) was homogenized with 200 μL of urea (0.17 M), 200 μL of glacial acetic acid (0.1 M), and 500 μL of sodium hydroxide (0.5 M). The reaction mixture was centrifuged at 2250 ×g at 4 °C for 10 min (Sorvall ST18R, Thermo Scientific; Waltham, USA). Subsequently, the absorbance was measured at 510 nm. The results were expressed as mg of chlorogenic acid equivalents per g of dry extract (mg CAE g^{-1}).

Antioxidant activity

Free radical inhibition was determined by the method described by Molyneux (2004). The aqueous extract (100 µL, 100 µg mL^{-1}) was homogenized with 100 µL of 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) solution (300 µM) and incubated at 25 °C for 30 min in the dark. Absorbance was measured at 517 nm. Ascorbic acid (70 µM) was used as a standard. The ability of the extract to inhibit the radical was calculated as follows:

$$DPPH \bullet (\%) = AbsA - \frac{Abs\ B}{Abs\ A} \times 100$$

where Abs A is the absorbance of the control (t = 0) and Abs B is the absorbance of the antioxidant (t = 30 min).

Cation radical inhibition was determined by the method described by Re *et al*. (1999). Prior to analysis, equal parts of ethanolic solution of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) (7 mM) and potassium persulfate (2.45 M) were homogenized at 10 000 rpm for 1 min. The resulting solution was stored at 25 °C for 16 h in the dark. The radical formed was adjusted to an absorbance of 0.8 with ethanol, mixed with the aqueous extract (100 µg mL^{-1}) in a 99:1 ratio, and incubated for 10 min. The absorbance was measured at 734 nm, using ascorbic acid (70 µM) as a standard. The ability of the extract to inhibit the cation radical was calculated as follows:

$$ABTS^{\bullet+} (\%) = AbsA - \frac{Abs\ B}{Abs\ A} \times 100$$

where Abs A is the absorbance of the control (t = 0) and Abs B is the absorbance of the antioxidant (t = 10 min).

The reducing power was determined using the ferricyanide/Prussian blue method (Berker *et al*., 2010). An aliquot of aqueous extract (100 µL, 100 µg mL^{-1}) was homogenized with 300 µL of phosphate buffer (0.2 M, pH 6.6) and 300 µL of potassium ferricyanide (1 %, w/v). The resulting solution was incubated at 50 °C for 20 min in a water bath in the dark. After cooling at 25 °C for 10 min, the mixture was homogenized with 300 µL of trichloroacetic acid (10 %, w/v) and centrifuged at 4200 ×g at 4 °C for 10 min. Then, 100 µL of the supernatant was mixed with 100 µL of distilled water and 250 µL of ferric chloride (0.1 %, w/v). The absorbance was measured at 700 nm, and the results were expressed as an increase in absorbance at the same wavelength.

Ferric-reducing antioxidant power was determined using the method described by Berker *et al*. (2010). An aliquot of aqueous extract (20 µL, 100 µg mL^{-1}) was homogenized with 150 µL of Ferric Reducing Antioxidant Power (FRAP) solution (10:1:1, 300 mM

of sodium acetate buffer in glacial acetic acid at pH 3.6 and 4,4,6-tripyridyl-S-triazine in 40 nM of hydrochloric acid and 20 mM of iron chloride). 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 mg iron ion equivalents per g of dry extract (mg Fe^{2+} g^{-1}).
Lipid oxidation was determined using the Thiobarbituric Acid Reactive Substances (TBARS) assay (Kim *et al*., 2016) with slight modifications. Pork (semimembranosus muscle, 48 h *postmortem*) was homogenized with 10 mL of water and 1 mL of each aqueous extract at 500 ppm at 4500 rpm at 4 °C for 1 min. The homogenate obtained was mixed with a 1:2 ratio of trichloroacetic acid solution (10 %, w/v). Subsequently, the sample was centrifuged at 2300 ×g at 4 °C for 10 min and filtered through Whatman paper 1. The resulting solution was homogenized with 2-thiobarbituric acid solution (0.02 M) at a 1:1 ratio and placed in a water bath (97 °C for 20 min). The absorbance was measured at 531 nm, expressing the results as mg of malondialdehyde per kg of meat (mg MDA kg^{-1}).

Statistical analysis

Values were expressed as the mean ± standard deviation. All data were obtained from three independent experimental trials (with three replicates). The data on metabolite content and antioxidant activity were subjected to a two-way analysis of variance (ANOVA), considering as factors the level of addition of spent coffee beans to the culture medium and the use or non-use of the fungal mycelium. Additionally, a Tukey-Kramer multiple comparison test was performed to determine the statistical differences between treatments ($p \leq 0.05$). To evaluate the relationship between the variables analyzed, a principal component analysis (SPSS version 21, IBM Statistics) was performed.

RESULTS AND DISCUSSION

The metabolite content of aqueous extracts was affected by the level of addition of spent coffee beans fermented with *P. pulmonarius* mycelium (Table 1). The highest content of carbohydrates (> 100 mg g^{-1}), phenols (> 20 mg g^{-1}), flavonoids (> 10 mg g^{-1}), and caffeoylquinic acid (> 300 mg g^{-1}) was presented by the aqueous extract obtained from the culture medium added with 10 % spent coffee beans fermented with mycelium, compared to the extracts obtained from fermented samples without the addition of residue and mycelium.
In a study by Xu *et al*. (2014), the ability of *Inonotus obliquus* mycelium to degrade peanut shell biomass (substrate/carbon source) and the recovery of bioactive compounds during submerged culture fermentation were evaluated. These authors demonstrated that more than 60 % of the cellulose, hemicellulose, and lignin in the shell were degraded after 12 d of fermentation. They also reported an increase in the content of polysaccharides and total phenols when using this residue as a substrate and *I. obliquus* mycelium. The increase in total phenol content was attributed to the degradation of

Table 1. Total metabolite composition of aqueous extracts of spent coffee beans fermented in a submerged culture with and without *Pleurotus pulmonarius* mycelium.

Parameters	Residue	Quality profile		Quantity profile	
		Without mycelium	With mycelium	Without mycelium	With mycelium
Carbohydrates (mg GE g^{-1})	0 %	+	+	21.19 ± 0.97 a	30.81 ± 2.35 c
	5 %	+	++	23.86 ± 0.31 b	92.40 ± 2.19 d
	10 %	+	+++	23.85 ± 0.32 b	103.54 ± 3.30 e
Phenols (mg GAE g^{-1})	0 %	-	+	0.91 ± 0.19 a	7.82 ± 0.09 b
	5 %	+	++	7.69 ± 1.35 b	17.63 ± 2.01 c
	10 %	+	+++	7.86 ± 1.53 b	22.03 ± 1.69 d
Flavonoids (mg EQ g^{-1})	0 %	-	+	0.72 ± 0.02 a	4.50 ± 0.12 b
	5 %	+	++	4.03 ± 0.75 b	10.25 ± 0.24 c
	10 %	+	++	3.72 ± 0.90 b	11.81 ± 0.39 d
Caffeoylquinic acid (mg CAE g^{-1})	0 %	-	+	1.65 ± 0.04 a	30.71 ± 3.82 b
	5 %	+	++	51.99 ± 2.70 c	216.49 ± 3.64 e
	10 %	+	+++	88.22 ± 2.70 d	333.15 ± 4.76 f

(-): absent; (+): slight presence; (++): moderate presence; (+++): high presence; 0 %: culture medium without addition of spent coffee beans; 5 %: culture medium with 5 % spent coffee beans; 10 %: culture medium with 10 % spent coffee beans. EG: glucose equivalents; GAE: gallic acid equivalents; EQ: quercetin equivalents; CAE: chlorogenic acid equivalents. Metabolite content values are expressed as mean ± standard deviation. Means with different superscripts (a-f) between samples indicate significant differences ($p \leq 0.05$).

lignocellulose because it showed a stimulatory effect on the yield of extracellular and intracellular flavonoids such as epigallocatechin-3-gallate, epicatechin-3-gallate, feligidrin G, davallialactone, and inoscavin B. However, the content of phenolic acids, such as gallic and ferulic, was reduced by the effect of lignocellulose degradation.

Likewise, in a study conducted by Choi *et al*. (2010), the optimal conditions to produce *Cordypces sinensis* by fermentation in a submerged medium for 7 d using citrus peel as a substrate were evaluated. The results showed an increase in the total content of polysaccharides, phenols, and flavonoids after fermenting citrus peel with *C. sinensis*. In another study, Vargas-Sánchez *et al*. (2023) evaluated the qualitative profile and metabolite content of the aqueous extract of spent coffee beans fermented in a submerged culture using the mycelium of *P. ostreatus*. According to their results, an increase in the content of carbohydrates, phenols, flavonoids, and caffeoylquinic acid was reported after the fermentation process (10 d) in a submerged culture with the use of mycelium from this species.

Based on the studies described above, the increase in the content of these metabolites may be associated with the degradation of the lignocellulosic material of agro-industrial wastes during the fermentation process. The increased release of these compounds is associated with their bioactivity, which is mainly antioxidant and antimicrobial (Xu

et al., 2014; Ogidi *et al*., 2020). The evaluation of antioxidant activity makes it possible to determine the ability of certain compounds to scavenge or reduce free radicals, as well as to yield electrons to reduce an oxidizing agent. This evaluation can be carried out by different methods or reaction mechanisms, such as oxygen radical scavenging capacity, hydroxyl radical prevention capacity, ferric ion reducing power, inhibition of peroxyl and hydroxyl radicals, as well as inhibition of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals (Echegaray *et al*., 2021).

The antioxidant activity of the extracts was affected by the level of addition of spent coffee beans fermented with *P. pulmonarius* mycelium (Table 2). The highest antiradical activity was presented by the aqueous extract obtained from the culture medium added with 5 and 10 % of spent coffee beans fermented with mycelium, compared to the extracts obtained from the samples in which no mycelium and spent coffee beans were used. On the other hand, the highest reducing power (> 1.5 absorbance and 30 mg Fe^{2+} g^{-1}) was presented by the aqueous extract obtained from the culture medium added with 10 % of spent coffee beans fermented with *P. pulmonarius* mycelium, in relation to the extracts obtained from samples fermented without the addition of residue and mycelium.

Table 2. Antioxidant activity of aqueous extract of spent coffee beans fermented in a submerged culture with and without *Pleurotus pulmonarius* mycelium.

Parameters	Residue	Without mycelium	With mycelium
$DPPH^{\bullet}$ (% inhibition)	0 %	28.57 ± 0.32 a	44.26 ± 1.48 b
	5 %	58.06 ± 0.32 c	64.30 ± 0.69 e
	10 %	61.39 ± 0.94 d	64.74 ± 0.93 e
$ABTS^{\bullet+}$ (% inhibition)	0 %	49.64 ± 1.18 a	60.47 ± 2.37 b
	5 %	88.29 ± 0.79 c	89.58 ± 0.64 c
	10 %	88.35 ± 0.89 c	88.77 ± 0.88 c
Reducing power (absorbance)	0 %	0.42 ± 0.01 a	1.31 ± 0.01 c
	5 %	0.51 ± 0.01 b	1.31 ± 0.01 c
	10 %	0.51 ± 0.01 b	1.54 ± 0.04 d
FRAP (mg Fe^{2+} g^{-1})	0 %	3.13 ± 0.10 a	3.19 ± 0.92 a
	5 %	9.97 ± 0.10 b	12.44 ± 0.66 c
	10 %	11.73 ± 0.82 c	30.04 ± 1.63 d

Values are expressed as mean ± standard deviation. 0 %: culture medium without addition of spent coffee beans; 5 %: culture medium with 5 % spent coffee beans; 10 %: culture medium with 10 % spent coffee beans. FRAP: ferric reducing antioxidant power; $DPPH^{\bullet}$: 2,2-diphenyl-1-picrylhydrazyl; $ABTS^{\bullet+}$: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). Means with different superscripts (a-e) between samples indicate significant differences ($p \leq 0.05$).

In this regard, Ogidi *et al*. (2020) extracted antioxidant compounds by assisted extraction with fungal fermentation for 7 d, using *P. pulmonarius* mycelium and different agro-industrial residues as carbon sources (banana peel, pineapple, mango, peanut, walnut, and coconut fiber). These authors reported activity against free radicals ($DPPH^{\bullet}$), hydroxyl ($HO^{\bullet}$), and nitric oxide ($NO^{\bullet}$) (> 70 % inhibition) in the extracts after fermentation in a submerged medium with mycelium, comparable to the evaluated commercial antioxidant butylated hydroxytoluene (BHT). In addition, these extracts showed a reduction capacity (> 70 %) to chelate metal ions such as iron (Fe^{+2}), as did the commercial antioxidant.

In another study, Xu *et al.* (2014) reported increased activity against $DPPH^{\bullet}$ and $HO^{\bullet}$ radicals (> 40 and 50 % inhibition, respectively) by subjecting peanut shells to fermentation for 12 d in a submerged culture with *I. obliquus*, which was associated with an increase in flavonoid content (epigallocatechin-3-gallate and epicatechin-3-gallate) during fermentation with the mycelium. Meanwhile, Choi *et al*. (2010) demonstrated an increase in $ABTS^{\bullet+}$ antiradical activity (approximately 400 mg of ascorbic acid equivalents per 100 g) by fermenting citrus peel in a submerged culture for 7 d using *C. sinensis* mycelium. In addition, increased activity has been demonstrated against $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals (> 40 and 50 % inhibition, respectively), reducing power (0.4–0.9 absorbance), and metal ion chelation (> 60 %) of the extract obtained by fermentation in a submerged culture for 5 d, using the mycelium of *P. ostreatus*, whose bioactivity was associated with metabolite formation (Vamanu, 2012).

In turn, Vargas-Sánchez *et al*. (2023) evaluated the antioxidant activity of the aqueous extract of spent coffee beans fermented in a submerged culture using the mycelium of *P. ostreatus*. In that study, an increase in the inhibition values of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals, as well as reducing power and FRAP values, was achieved after the fermentation process (10 d) in a submerged culture with the use of mycelium from this species. Also, the activity shown in the controls is reported to be attributed to the use of ascorbic acid in the formulation of the culture medium. On the other hand, it has been demonstrated that food matrices, such as meat and meat products, are susceptible to oxidative deterioration due to their main components, such as proteins and lipids. These components can serve as targets for different radicals such as hydroxyl ($HO^{\bullet}$), peroxyl ($ROO^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$), and alkoxy ($RO^{\bullet}$), which favor the oxidative process of proteins and lipids (Echegaray *et al*., 2021). In this context, the formation of thiobarbituric acid-reactive substances is a widely used method to measure the formation of lipid oxidation by-products, such as MDA, and consequently, to measure the oxidative stability of extracts from natural sources in foods (Kim *et al*., 2016; Shah *et al*., 2014).

Based on the above, the oxidative stability of meat homogenates subjected to heat treatment was affected by time and the inclusion of aqueous extracts obtained from spent coffee beans fermented in a submerged culture with or without *P. pulmonarius* mycelium (Figure 1).

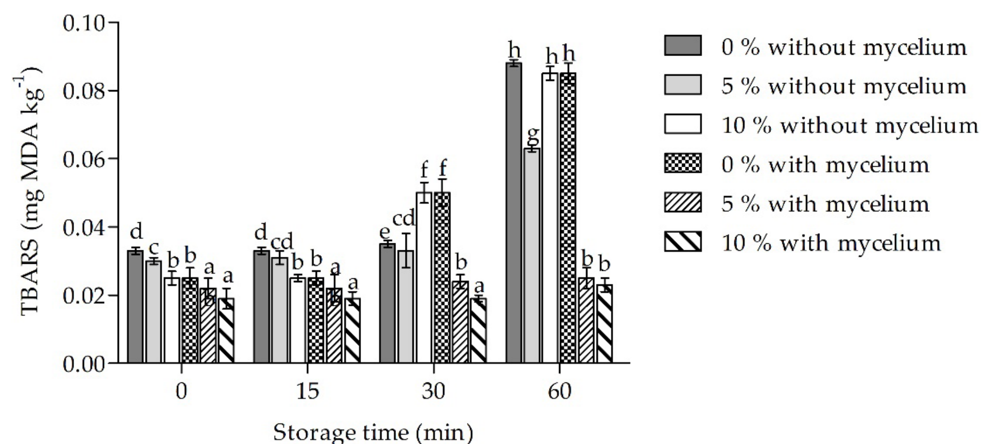

Figure 1. Oxidative stability of meat homogenate added with aqueous extract of spent coffee beans fermented in a submerged culture with and without *Pleurotus pulmonarius* mycelium.

At the beginning of storage, the results showed that the lowest lipid oxidation values (< 0.02 mg MDA kg^{-1}) were presented by the aqueous extracts obtained from the culture medium added with 5 and 10 % of spent coffee beans fermented with mycelium of *P. pulmonarius*, in comparison to the aqueous extracts obtained from fermented samples without the addition of residue and mycelium ($p \leq 0.05$). Lipid oxidation values increased during storage time ($p \leq 0.05$), with no changes observed in the oxidation values of meat extracts treated with an aqueous extract of spent coffee beans fermented with mycelium. At the end of storage (60 min), a reduction of lipid oxidation (> 70 % inhibition) was observed in the meat extract treated with aqueous extracts obtained from the culture medium added with 5 and 10 % spent coffee beans fermented with mycelium, compared to the rest of the treatments ($p \leq 0.05$).

Information related to the use of extracts obtained by fungal fermentation to increase oxidative stability in foods is limited. However, the antioxidant effect of extracts obtained from spent coffee beans on raw and cooked chicken meat during storage has been demonstrated (Kim *et al*., 2016). In a recent study, Vargas-Sánchez *et al*. (2023) evaluated the oxidative stability of pork meat treated with the aqueous extract of spent coffee beans fermented in a submerged culture using *P. ostreatus* mycelium. They proved that lipid oxidation values showed a reduction in meat samples treated with aqueous extracts fermented using the mycelium of this species.

Additionally, the principal component analysis showed that 91.3 % of the variation among treatments and parameters evaluated was due to two main components (76.7 and 14.6 %, respectively) (Figure 2).

The results showed a separation of treatments of fermented samples with and without *P. pulmonarius* mycelium compared to chemical composition and antioxidant activity, in dependence on the level of addition of spent coffee beans ($p \leq 0.05$) during fermentation.

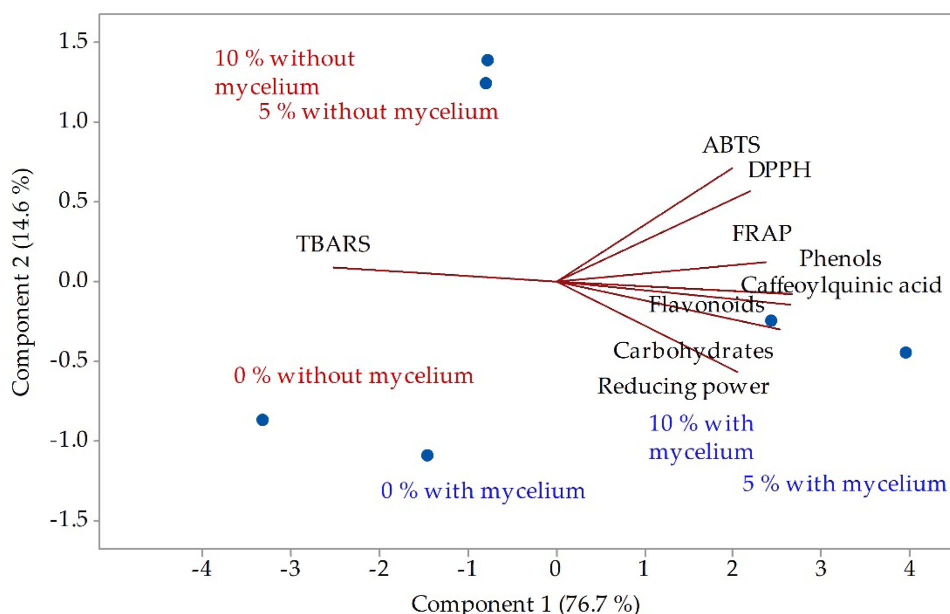

Figure 2. Principal component analysis of the parameters evaluated. TBARS: thiobarbituric acid reactive substances; DPPH: 2,2-diphenyl-1-picrylhydrazyl radicals; ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radicals; FRAP: ferric reducing antioxidant power.

CONCLUSIONS

The aqueous extract obtained by submerged culture fermentation using *Pleurotus pulmonarius* mycelium and spent coffee beans as substrate showed an increase in the content of metabolites (carbohydrates, phenols, flavonoids, and caffeoylquinic acid), as well as greater antioxidant activity by inhibiting the formation of free radicals and lipid oxidation while increasing the reducing power, compared to the fermented aqueous extracts without mycelium.

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