Oxidative stability of meat homogenates treated with spent coffee ground extract obtained by submerged fermentation

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Objectives: The aim of the present work was to evaluate the total polyphenols content and the antioxidant activity of the aqueous ex- tract obtained from spent coffee ground fermented in submerged culture with or without mycelium from *Pleurotus ostreaus*.

Materials and Methods: A commercial supplier (CAFFENIO®) located in Hermosillo, Sonora México, donated spent coffee grounds (SCG) from dark *Coffea arabica* L. The fermentation medium used for substrate moistening was sterilized at 121°C for 20 min and composed as follows: glucose (20 g/L), yeast extract (5 g/L), KH_2PO_4 (1 g/L), $MgSO_4$ $7H_2O$ (0.5 g/L), and ascorbic acid (0.05 g/L). The pH was adjusted to 5.4. Shake flask culture was carried out in 250 mL Erlenmeyer flasks containing 100 mL of the culture medium, and the treatments were as follow: T0, only culture medium; T1, culture medium + mycelium; T2; culture medium without mycelium + 5% SCG; T3, culture medium + mycelium + 5% SCG. The flasks were incubated (under dark conditions) at 150 rpm at 29 °C for 10 d. The culture media (aqueous solution) was homogenized at 10,000 rpm for 30 s, filtered under vacuum, and lyophilized. Once obtained fermented aqueous extracts, the total phenolic (TPHC) and flavonoids content (TFC) were deter- mined by the Folin-Ciocalteu´s and Aluminum chloride complex methods, respectively. While the antioxidant activity was deter- mined through the inhibition of 2,2- diphenyl-1-picrylhydrazyl radical (DPPH•) and ferric reducing antioxidant power (FRAP). In addition, an aqueous meat homogenate from pork meat (m. *Semimembranosus*, 48 h *postmortem*; 1:10 ratio) was treated with the fermented aqueous extracts and subjected to a thermal treatment in a water bath at 65 °C for 30 min. After, lipid oxidation was evaluated through the thiobarbituric acid reactive substances method (TBARS). Data were subjected to ANOVA, and means were compared by the Tukey-Kramer test ($p<0.05$) using the statistical software NCSSv11.

Results and Discussion: Regarding the polyphenol composition, the results showed an increase ($p<0.05$) in TPHC in the aqueous extracts from samples treated with mycelium T3 and T2 (24 and 19 mg gallic acid equivalents per g of dried extract, mg GAE/g) in comparison to samples T1 and T0 (8 and 1.1 mg GAE/g). An increase ($p<0.05$) was also observed for TFC in T3 and T2 samples (9 and 4 mg quercetin equivalents per g, mg Qc/g, respectively) with respect to T1 and T0 (4 and 0.7 mg Qc/g, respectively). TPHC method is based on the electron transfer from the hydroxyl group of the phenolic compound to phosphomolybdic/phosphotungstic acid complexes, while the TFC method depend on the complex formation between the aluminum ion and the carbonyl or hydroxyl group of the antioxidant. Respect antioxidant tests, the results showed an increase ($p<0.05$) in DPPH• inhibition in the aqueous extracts from samples treated with mycelium T3 and T2 (43 and 30%, respectively) in comparison to samples T1 and T0 (55 and 27%, respectively). An increase ($p<0.05$) was also observed for FRAP values in T3 and T1 samples (16 and 9 mg Fe^{2+}/g, respectively), with respect to T2 and T0 (3 mg Fe^{2+}/g, for both). In addition, TBARS values decrease in the meat extract treated with T3 and T1 (0.029 and 0.062 mg MDA/ kg, respectively) with respect to T2 and T0 (0.077 and 0.087 mg MDA/kg, respectively).

The DPPH• method is based on the H atom transfer from the hydroxyl group from the antioxidant to neutralize the radical. In contrast, the FRAP method involves reducing the hydroxyl group from the antioxidant of the ion Fe(III) to Fe(II), using tripyridyltriazine as a binding complex.

Natural bioactive compounds have been extracted through several extraction methods like conventional (e.g., maceration and hydrodistillation), unconventional (e.g., ultrasound, microwave, etc.) and biotechnological (e.g., microbial and fungal fermentation). In agreement with our results, it has been demonstrated that fungal fermentation enhances phenolic content and antioxidant proper- ties.

Conclusions: The results obtained in this study showed that the aqueous extract obtained by submerged culture fermentation using the mycelium of *P. ostreatus* and spent coffee ground as a substrate showed an increase in the content of polyphenols (total phenols and flavonoids). As well as better antioxidant activity by inhibiting the formation of free radicals and reducing power, together to a reduction in lipid oxidation. Therefore, the extraction assisted by fungal fermentation can be a potential strategy for obtaining new food additives from agro-industrial residues.

Key words: Coffee residues, Fungal fermentation, Chemical composition, Antioxidant, *Pleurotus* spp.