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Research Article

Utilization of coffee silverskin aqueous extract to improve pork meat homogenates oxidative stability

Utilización del extracto acuoso de cascarilla de café para mejorar la estabilidad oxidativa de homogenizados de carne de cerdo

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ABSTRACT

Coffee fruit processing residues have been proposed to prevent meat quality loss. This study aimed to evaluate the effectiveness of coffee silverskin aqueous extract (CSE) on the oxidative stability of a pork meat homogenate. CSE was subjected to polyphenols determination (total phenol, flavonoids, and caffeoylquinic acid contents) and antiradical and reducing power assays. In addition, raw pork meat homogenates were divided into four treatments (CN, control or without antioxidant; T1 and T2, CSE at 250 and 500 ppm, respectively; BHT, synthetic antioxidant at 500 ppm), oxidized for 1 h with potassium ferrocyanide (0, 0.5, and 1.0 %, *w/v*), and subjected to meat quality evaluation (pH, lipid oxidation, color, and metmyoglobin content). Results demonstrated that CSE is an important source of polyphenols with antioxidant activity, and their incorporation in a raw pork meat homogenate led to reduce pH values, lipid oxidation, and metmyoglobin content, as well as decreased color changes ($p < 0.05$). These results suggest that CSE has great potential as an antioxidant additive for meat products.

Keywords: coffee residues; bioactive compounds; antioxidants, meat quality.

RESUMEN

Los residuos del procesamiento del fruto de café se han propuesto como una estrategia para prevenir la pérdida de calidad de la carne. Este estudio tuvo como objetivo evaluar la efectividad del extracto acuoso de cascarilla de café (CSE) sobre la estabilidad oxidativa en un sistema de homogenizados de carne cruda de cerdo. El CSE se sometió a la determinación de polifenoles (fenoles, flavonoides y ácido cafeoilquínico), así como a ensayos de actividad antirradical y poder reductor. Además, los homogenizados de carne cruda de cerdo se dividieron en cuatro tratamientos (CN, control o sin antioxidante; T1 y T2, CSE a 250 y 500 ppm, respectivamente; BHT, antioxidante sintético a 500 ppm), oxidados por 1 h con ferrocianuro de potasio (0, 0.5 y 1.0 %, *p/v*), y fueron sometidos a evaluación de calidad de la carne (pH, oxidación de lípidos, color y contenido de metamioglobina). Los resultados demostraron que los CSE son una fuente importante de polifenoles con actividad antioxidante, y su incorporación en un homogenizado de carne de cerdo cruda condujo a una reducción de los valores de pH, oxidación de lípidos y contenido de metamioglobina, así como a una disminución de los cambios de color ($p < 0.05$). Estos resultados sugieren que el CSE tiene un gran potencial como aditivo antioxidante para productos cárnicos.

Palabras clave: residuos del café; compuestos bioactivos; antioxidantes; calidad de la carne.

INTRODUCTION

In Mexico, pork production was around 1.5 metric tons (Mt), with a *per capita* consumption of 20.8 kg in 2021 and a total domestic consumption of 2.5 Mt. Furthermore, Mexico imports approximately 1.2 Mt, mainly from the USA market, and exports approximately 0.3 Mt to the Asiatic market, including China and Japan. This country's leading pork producing states were Jalisco, Sonora, and Puebla, with a participation of 22.6, 18, and 10.8%, respectively, concerning the total production (USDA, 2023). According to Mexican regulations, different products can be obtained from meat processing, such as cured, aged, dried, raw/cooked ready, or not for consumption (NOM, 2018).

Moreover, regardless of the type of pork meat products, lipid-protein oxidation is considered a primary degradation of quality loss in these products. Oxidative deterioration induced by intrinsic factors, including a high concentration of unsaturated and polyunsaturated lipids, heme pigments, and oxidizing agents among others, can result in nutrient losses, organoleptically undesirable changes, formation of toxic compounds, and consequently, loss of acceptability by consumers (Hadidi *et al*., 2022). In this regard, antioxidant agents are incorporated into different meat products to enhance oxidative stability; however, synthetic antioxidants have been associated with adverse effects on human health. Although these agents are widely used due to low cost, effectiveness and stability, natural antioxidants from plant by-products are considered a significant option to reduce partial or complete uses (Ribeiro *et al*., 2019; Hadidi *et al*., 2022).

In this context, coffee is one of the most consumed

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beverages in the world, and to obtain it, its fruit must be subjected to a process of pulping, fermenting, washing, drying, and roasting, which generates many residues as by-products and waste at each stage (Klingel *et al*., 2020; Serna *et al*., 2022). Coffee silverskin (CSS), the major coffee-roasting residue, is an important source of dietary fiber and bioactive compounds, including caffeine, melanoidins, and phenolic compounds. Therefore, it has been incorporated as an ingredient in the formulation of bakery products to improve nutritional, physicochemical, and sensory attributes (Klingel *et al*., 2020).

In a previous investigation, the effects of CSS powder as a natural ingredient in chicken meat patties were evaluated. It was concluded that the inclusion of this natural antioxidant reduced the formation of oxidative products, thereby influencing the sensory attributes. Consequently, they suggested further research into its application in novel meat formulations (Martucelli *et al*., 2021a). Based on the above, the use of CSS as an antioxidant ingredient for improving the oxidative stability of meat products is still limited.

Therefore, this investigation aimed to evaluate the effectiveness of coffee silverskin aqueous extract on the oxidative stability of a pork meat homogenate.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used were of analytical grade. Folin-Ciocalteu, sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), sodium nitrite ($NaNO_2$), sodium phosphate monobasic and dibasic ($NaH_2PO_4 \cdot H_2O$ and Na_2HPO_4, respectively), potassium persulfate ($K_2S_2O_8$), ethanol (HPLC), butylated hydroxytoluene (BHT), gallic acid, quercetin, chlorogenic acid, iron(III) chloride 6-hydrate ($FeCl_3 \cdot 6H_2O$), iron(II) sulfate 7-hydrate ($FeSO_4 \cdot 7H_2O$), aluminum chloride ($AlCl_3$), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), hydrochloric acid (HCl), glacial acetic acid (CH_3COOH), urea (CH_4N_2O) and potassium ferrocyanide ($[Fe(CN)_6]^{4-}$), were purchased from Sigma Chemicals. While 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA) and tetramethoxypropane (TMP), were acquired from J.T. Baker®.

Coffee residues processing

A commercial supplier (CAFFENIO®, Hermosillo, Sonora, México) donated coffee silverskin flakes from dark *Coffea arabica* L. Coffee silverskin flakes were pulverized to a particle size of 20 mesh (Pulvex 200, CDMX., Mexico). The obtained material was packed under vacuum (Food Saver®, FLA., USA), sterilized at 121 °C for 20 min (Yamato SM300, TYO. Japan), and stored at room temperature (25 °C) until further use.

Coffee residues processing

Bioactive compounds from coffee silverskin were extracted with water (1:10 ratio) by maceration-assisted extraction at 150 rpm (24 °C) for 48 h, in the dark (Fisher Scientific MaxQ-5000, NEP, Canada). The resultant mixture was filtered (Whatman no. 1 filter paper), concentrated under reduced pressure at 60 °C in a rotary evaporator (RE301BW, Yamato, TYO., Japan), and lyophilized in a freeze dryer (DC401, Yamato, TYO., Japan). The coffee silverskin aqueous extract (CSE) obtained was stored in the dark at -20 °C until analysis.

Polyphenols content of CSE

Total phenolic content (TPC)

TPC was measured by the Folin-Ciocalteu method (Matić and Jakobek, 2021) with slight modifications. Briefly, 20 µL of CSE (5 mg/mL) were mixed with 160 µL of distilled water, 40 µL of Folin-Ciocalteu's reagent (2 M), and 60 µL of Na_2CO_3 solution (7 %, *w/v*). The resultant mixture was incubated at 25 °C for 1 h under dark conditions. The absorbance was measured at 750 nm in a spectrophotometer (Multiskan FC UV-Vis, Thermo Scientific, TYO., Japan), and results were expressed as mg of gallic acid equivalent/g of dried extract (mg GAE/g).

Total flavonoids content (TFC)

TFC was measured by the aluminum chloride-complex formation method (Matić and Jakobek, 2021) with slight modifications. Briefly, 20 µL of CSE (5 mg/mL) were homogenized with 130 µL of methanol and 20 µL of $AlCl_3$ (5 %, *w/v*). The resultant mixture was incubated 30 min at 25 °C under dark conditions. The absorbance was measured at 415 nm, and results were expressed as mg of quercetin equivalents/g (mg QE/g).

Caffeoylquinic acid content (CAC)

CAC was evaluated according to Griffiths *et al*. (1992) with slight modifications. Briefly, 100 µL of CSE (5 mg/mL) were mixed with 200 µL of urea (0.17 M), 200 µL of glacial acetic acid (0.1 M), and 500 µL of distilled water. The resultant mixture was homogenized with 500 µL of $NaNO_2$ (0.14 M) and 500 µL of NaOH (1 M), centrifuged at 2250× g for 10 min at 4 °C (Sorvall ST18R, Thermo Fisher Scientific, MA, USA). The absorbance was measured at 510 nm, and results were expressed as mg chlorogenic acid equivalents/g (mg CGA/g).

Antiradical activity of CSE

Free radical scavenging activity

This activity was measured by the DPPH method (Ozgen *et al*., 2006) with slight modifications. Briefly, 100 µL of CSE (5 mg/mL) were mixed with 100 µL of DPPH ethanol solution (300 µM). The resultant mixture was incubated at 25 °C for 30 min under dark conditions. BHT (0.1 mg/mL) was used as positive control. The absorbance was measured at 517 nm, and the results were expressed as a percentage of inhibition: [(Radical absorbance at 0 min) – (Radical absorbance + antioxidant at 30 min) / (Radical absorbance at 0 min)] x 100.

Radical cation scavenging activity

This activity was determined by the ABTS method (Ozgen *et al*., 2006) with slight modifications. Briefly, 20 µL of CSE (5 mg/mL) were mixed with 180 µL of ABTS solution (abs 0.7

at 730 nm). The resultant mixture was incubated at 25 °C for 8 min under dark conditions. BHT (0.1 mg/mL) was used as positive control. The absorbance was measured at 730 nm, and the results were expressed as a percentage of inhibition: [(Radical absorbance at 0 min) – (Radical absorbance + antioxidant at 30 min) / (Radical absorbance at 0 min)] x 100.

Reducing power activity of the aqueous extract
Reducing power activity
This activity was determined by the ferricyanide/Prussian blue method (Berker *et al*., 2010) with slight modifications. Briefly, 200 µL of CSE (5 mg/mL) were mixed with 300 µL of phosphate buffer (0.2 M, pH 6.6) and 500 µL of potassium ferrocyanide (1%, *w/v*) and incubated at 50 °C for 20 min, under dark conditions. The resultant mixture was homogenized with 500 µL of TCA (10%, *w/v*) and centrifuged at 2300× g at 4 °C for 10 min. Subsequently, 100 µL of the supernatant were homogenized with 100 µL of $FeCl_3$ (0.1%, *w/v*). BHT (0.1 mg/mL) was used as positive control. The absorbance was measured at 700 nm, and the results were expressed as absorbance at the same wavelength.

Ferric reducing/antioxidant power
This activity was determined by the FRAP method (Berker *et al*., 2010) with slight modifications. Briefly, 20 µL of CSE (5 mg/mL) were homogenized with 180 µL of FRAP solution [10:1:1, 300 mM buffer sodium acetate in glacial acetic acid at pH 3.6 and TPTZ (10 mM) in HCl (40 nM) and $FeCl_3$ (20 mM)]. The reaction mixture was incubated at 25 °C for 8 min under dark conditions. BHT (0.1 mg/mL) was used as positive control. The absorbance was measured at 595 nm, and the results were expressed as mg of Fe^{2+} equivalent/g (mg Fe^{2+}/g).

Meat homogenates preparation
Fresh pork meat (*Semimembranosus* muscle, at 48 h *post-mortem*) was purchased from a local processor (Norson®, Hermosillo, Mexico). Any visible extra-muscular fat was trimmed, and then the meat minced using a 4.5 mm-hole plate (meat grinder 4152, 4 Hobart Dayton, OH, USA). The minced pork meat was mixed with salt (1.5 %, *w/w*) and pork back fat (10 % in the final formulation, *w/w*). Subsequently, the obtained minced meat (1 g) was homogenized with 10 mL of distilled water at 6000 rpm at 5 °C for 1 min (Ultraturrax T25, IKA, Germany), and 100 µL of the respective antioxidants: CN, without antioxidants; T1 and T2, CSE at 250 and 500 ppm, respectively; BHT, synthetic antioxidant at 500 ppm. The mixture was oxidized with 50 µL potassium ferrocyanide at 0, 0.5, and 1.0 % (*w/v*). After that, meat homogenates were stored at 4 °C for 1 h and subjected to oxidative stability evaluation.

Oxidative stability of meat homogenates
pH values
The pH values of meat homogenates were determined using a potentiometer with automatic temperature control (pH211, Hanna Instruments Inc., RI, USA) following the procedure 981.12 (AOAC, 2020).

Thiobarbituric acid reactive substances (TBARS)
Lipid oxidation of meat homogenates was determined by the TBARS method (Pfalzgraf *et al*., 1995). Meat homogenates (10 g) were homogenized with 20 mL of TCA (10%, *w/v*) (4500 rpm at 5 °C for 1 min) and centrifuged (2500× g at 5 °C for 20 min). Subsequently, 2 mL of the filtered supernatant were mixed with 2 mL of TBA solution (20 mM) and incubated at 98 °C for 20 min. After that, the absorbance was measured at 531 nm. The results were expressed as mg of malondialdehyde/kg of pork meat (mg MDA/kg).

Color parameters values
The color parameters values of meat homogenates were evaluated as described previously (Sánchez *et al*., 2001). The color parameters were performed using a spectrophotometer (CM 508d, Konica Minolta Inc., TYO, Japan) with a D65 illuminant and a 10° observer calibrated with a white calibration cap (CM-A70). Color parameters consisted of lightness (L*), redness (a*), yellowness (b*), Chroma (C*), and hue (h*).

Metmyoglobin content (MMb)
MMb of meat homogenates was measured using a spectrophotometer as described previously (Sánchez *et al*., 2001). The maximum value of the quotient K/S525 and K/S572 at the beginning of the experiment (day 0) was fixed as 0 % MMb, while 100 % MMb was obtained after oxidizing the meat sample in potassium ferricyanide (1 %, *w/v*).

Statistical analysis
Data obtained from all measurements were expressed as mean ± standard deviation (SD) of at least three independent trials. Polyphenols content and antioxidant activity data were subjected to a t-test. In addition, data from meat quality assays were subjected to a two-way analysis of variance, using the treatments (CN, T1, T2, and BHT) and level of oxidized agent (0, 0.5, and 1.0 %) as the fixed effect, including the interaction. A Tukey-Kramer test was conducted for means comparison ($p < 0.05$). Also, a principal components analysis was performed to determine the relationship between the analyzed parameters and treatments (SPSS, version 21).

RESULTS AND DISCUSSION

Polyphenols content of CSE

Table 1 reports the polyphenols content results of CSE. The results showed the presence of phenolic, flavonoids, caffeoylquinic acid, in this extract.

According to the literature, coffee silverskin is an important source of antioxidant components like alkaloids (caffeine), vitamins (tocopherols *α*, *β*, *γ*, and *δ*), phenolic components (chlorogenic acid and their derivates, including 5-*O*-, 3-*O*- and 4-*O*-caffeoylquinic acids), and carbohydrates associated with polysaccharides (Bessada *et al*., 2018). In agreement with our study, it has been demonstrated that CSE obtained by maceration-assisted extraction is a source of polyphenols, including phenolics, flavonoids, and caffeoylquinic acid; however, the results of the cited study were

Table 1. Polyphenols content and antioxidant activity of CSE.
Tabla 1. Contenido de polifenoles y actividad antioxidante del CSE.

Item	Results
Polyphenols	
TPC (mg GAE/g)	103.16 ± 3.48
TFC (mg QE/g)	43.62 ± 1.86
CAC (mg CGA/g)	23.19 ± 0.35
Free radical scavenging activity (% inhibition)	
CSE	42.76 ± 4.02
BHT	56.84 ± 3.90
p-value	< 0.001
Radical cation scavenging activity (% inhibition)	
CSE	12.82 ± 1.07
BHT	47.12 ± 2.61
p-value	< 0.001
Reducing power ability (Abs 700 nm)	
CSE	0.43 ± 0.01
BHT	1.47 ± 0.04
p-value	< 0.001
Ferric reducing antioxidant power (mg Fe^{2+}/g)	
CSE	1.23 ± 0.18
BHT	1.39 ± 0.09
p-value	< 0.001

Values expressed as mean ± SD of at least three independent experiments. CSE, coffee silverskin aqueous extract; BHT, butylated hydroxytoluene; TPC, total phenolic content; TFC, total flavonoids content; CAC, caffeoylquinic acid content.
Valores expresados como media ± DE de al menos tres experimentos independientes. CSE, extracto acuoso de piel plateada de café; BHT, butilhidroxitolueno; TPC, contenido total de fenoles totales; TFC, contenido total de flavonoides; CAC, contenido de ácido cafeoilquínico.

greater than those obtained in our study, which is associated with the extraction method used, i.e., ultrasound-assisted extraction (Rodrigues *et al*., 2015; Vimercati *et al*., 2022; Vargas *et al*., 2023). In contrast with our study, lower TPC and CAC values (approx. 5 mg GAE/g and 0.4 mg CGA/g, respectively) were reported in the extract obtained from coffee silverskin (Martuscelli *et al*., 2021b).

Antioxidant activity of CSE

Table 1 also reports the antioxidant activity results of CSE. The results showed that the synthetic antioxidant (BHT) showed 35 % greater ($p < 0.05$) free radical scavenging activity compared to CSE. Although both antioxidants showed values greater than 40 % of inhibition. In addition, BHT showed 72.8 % greater radical cation scavenging activity compared to CSE ($p < 0.05$). Regarding reducing power activity, BHT showed FRAP values 11.5 % greater ($p < 0.05$) than CSE, both antioxidants report values greater than 1.0 mg Fe^{2+}/g. Also, BHT showed 70.7 % greater ($p < 0.05$) reducing power ability than CSE.

In agreement with our study, it has been evidenced that CSE exerts *in vitro* free radical and radical cation scavenging activity, as well as reducing power activity; however, the results of the cited work were greater than those obtained in our investigation, which is associated with the extraction method used, i.e., ultrasound-assisted extraction (Rodrigues *et al*., 2015; Vimercati *et al*., 2022; Vargas *et al*., 2023). The hydrogen atom transfer (HAT) is the principal mechanism through which polyphenols exert their antioxidant activity, and according to a theoretical investigation, the capacity of caffeoylquinic acid to transfer hydrogen atoms is higher when compared to the caffeic, rosmarinic, ferulic, sinapic, ellagic, vanillic, syringic, *p*-coumaric, and *p*-hydroxybenzoic acids (Sabiq *et al*., 2016).

Oxidative stability of meat homogenates

pH values

Figure 1 illustrates the results of the treatment and the induced pro-oxidation effect, using potassium ferrocyanide in pH changes of pork meat homogenates, which were incorporated with CSE and BHT. The results showed an effect of treatment and pro-oxidation interaction ($p < 0.05$) on this parameter. At 0% of oxidizing, BHT samples showed the higher ($p < 0.05$) pH values for meat homogenates (6.01) respect to CN, T1 and T2 whose values ranged between 5.90-5.97. At 1.0 % of the oxidizing agent, T1 and T2 showed lower ($p < 0.05$) pH values than other treatments.

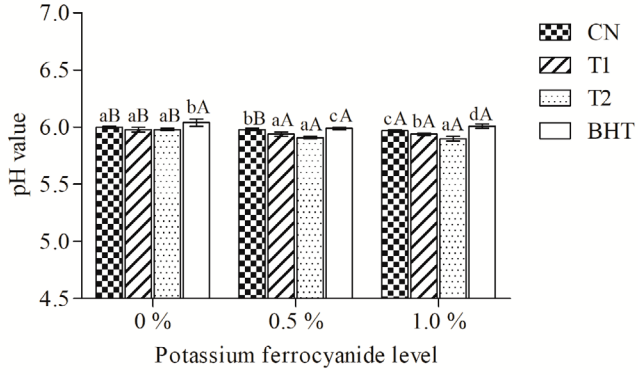

Figure 1. Effect of treatment and pro-oxidation on pork meat homogenates pH values. Lowercase letters indicate significant differences between treatments; capital letters indicate significant differences in each treatment at different pro-oxidation levels ($p < 0.05$).
Figura 1. Efecto del tratamiento y pro-oxidación sobre los valores de pH de homogenizados de carne de cerdo. Las letras minúsculas indican diferencias significativas entre tratamientos; las letras mayúsculas indican diferencias significativas en cada tratamiento a diferentes niveles de pro-oxidación ($p < 0.05$).

A previous investigation evaluated the influence of the anticaking agent potassium ferrocyanide (80, 2500, and 17500 ppm) on the oxidative stability of frozen minced pork meat, and results showed that pH values were not affected by pro-oxidant incorporation (Hansen *et al*., 1996). In addition, it has been reported that including coffee husk aqueous-ethanol extract (100 and 200 ppm) in raw frozen chicken patties did not affect initial pH values for CN samples (De Farias Marques *et al*., 2022). However, regardless of the antioxidant and pro-oxidant agent addition, in our study, pH values remained within the acceptable range (> 5.5 - 6.1) reported for pork meat (Kim *et al*., 2016a). In agreement with

our study, it has been evidenced that initial pH values of raw and cooked chicken patties were not affected by 1.5 and 3.0 % of coffee silverskin powder addition (Martuscelli *et al*., 2021a). Also, initial pH values of raw and cooked pork patties were not affected by non-compliant green coffee beans extract at 0.15, 0.30, and 0.60 % (Bergamaschi *et al*., 2023).

TBARS values

The incorporation of extracts obtained from agro-industrial residues into meat products is a promissory strategy to improve oxidative stability (Hadidi *et al*., 2022). pH is an intrinsic meat quality parameter involved in lipid oxidation chain reactions (Chaijan, 2008). Oxidative stability decrease has been associated with several oxidant products, including transition metals and salt, which affect their physicochemical properties and promote lipid and protein oxidation (Min *et al*., 2010). In this context, potassium ferrocyanide, a non-physiological iron compound used as an anti-caking agent in salt for human consumption, has also been related to pro-oxidant effects in meat systems (Hansen *et al*., 1996; Van Nguyen *et al*., 2012).

Figure 2 reports the results of the treatment and pro-oxidation effect on lipid oxidation of pork meat homogenates incorporated with CSE and BHT. The results showed an effect of treatment and pro-oxidation interaction ($p < 0.05$) on this parameter. At 0 % of oxidizing, no differences ($p > 0.05$) were found between treatments on TBARS values for meat homogenates; however, these values increased ($p < 0.05$) by the pro-oxidation effect. At 1.0 % of oxidizing agents, T1, T2, and BHT showed the lowest ($p < 0.05$) TBARS values compared to samples from CN.

In a previous study, the influence of potassium ferrocyanide at concentrations of 80, 2500, and 17500 ppm on lipid oxidation in frozen minced pork meat was evaluated. The results indicated that TBARS values remained unaffected at 0 d of storage. However, these values exhibited an increased at 56 d of storage, with a concentration-dependent effect (Hansen *et al*., 1996). In another work, it was evidenced that the incorporation of Fe^{2+} and Fe^{2+} + NaCl increased MDA formation in raw beef patties, 45.1 and 51.2% of inhibition respect CN samples (Min *et al*., 2010). Also, it has been demonstrated that incorporation of potassium ferrocyanide (2.5, 7.5, and 100 ppm) to cod fillets (*Gadus morhua*) accelerated in concentration-dependent lipid oxidation during the salting process, as well as during storage for 6 months (Van Nguyen *et al*., 2012).

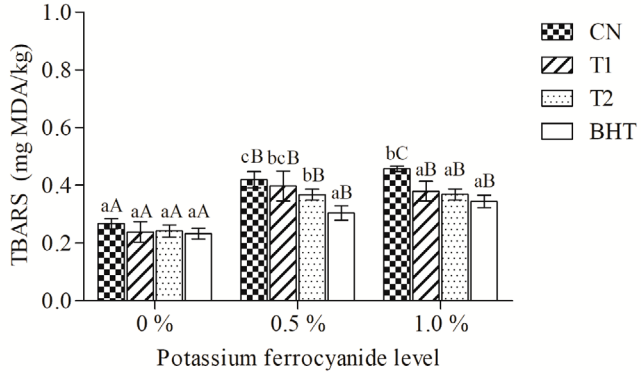

Figure 2. Effect of treatment and pro-oxidation on pork meat homogenates lipid oxidation values. Lowercase letters indicate significant differences between treatments; capital letters indicate significant differences in each treatment at different pro-oxidation levels ($p < 0.05$).

Figura 2. Efecto del tratamiento y pro-oxidación sobre los valores de oxidación de lípidos de homogenizados de carne de cerdo. Las letras minúsculas indican diferencias significativas entre tratamientos; las letras mayúsculas indican diferencias significativas en cada tratamiento a diferentes niveles de pro-oxidación ($p < 0.05$).

In agreement with our study, the incorporation of spent coffee ground aqueous extract (500 and 1000 ppm) to raw pork meat homogenates reduces MDA formation, approx. 39.5 and 46.9% of inhibition respect to CN, after being subjected to thermal treatment at 37 °C for 12 h (Kim *et al*., 2016b). Also, it has been reported that incorporation of coffee husk aqueous-ethanol extract (100 and 200 ppm) to raw and cooked frozen chicken patties during storage (- 18 °C for 45 d), enhances antioxidant stability through MDA reduction, approx. 90 and 66 % of inhibition, respectively, respect to CN (de Farias Marques *et al*., 2022). In another study, it was demonstrated that incorporation of CSE (500 ppm) into raw pork meat homogenates reduces MDA formation, approx. 55 % of inhibition respect to CN, after being subjected to thermal treatment at 37 °C for 8 h (Vargas *et al*., 2023). Additionally, although the oxidation of meat products can begin from their processing and storage, it has been reported that meat products treated with antioxidants, and whose values did not exceed 0.6 mg MDA/kg, do not present rancid aroma (Georgentelis *et al*., 2007).

Color parameter values

Lipid oxidation is a primary factor that negatively influences meat discoloration, reducing consumer acceptance (Chaijan, 2008). Table 2 reports the results of the treatment and pro-oxidation effect on color parameter values of pork meat homogenates incorporated with CSE and BHT. The results showed an effect of treatment and pro-oxidation interaction ($p < 0.05$) on evaluated color parameters, except for L* values ($p > 0.05$). At 0 % of an oxidizing agent, no differences ($p > 0.05$) were found between treatments on a*, b*, C*, and h* values for meat homogenates. In addition, a*, b*, and C* values decreased by the pro-oxidation effect, while h* values increased ($p < 0.05$). At 1.0 % of the oxidizing agent, T1, T2, and BHT showed higher ($p < 0.05$) a* and C* values respect to CN, which reveals the protective effect of the antioxidants used on color changes of the product. However, non-differences were found in b* values ($p > 0.05$). Furthermore, T1 and T2 showed the lowest ($p < 0.05$) h* values.

In a previous investigation, the influence of potassium ferrocyanide (80, 2500, and 17500 ppm) was determined, on the color of frozen minced pork meat, and results showed that a* values decreased at 0 and 56 d of storage in a concentration-dependent manner (Hansen *et al*., 1996). In addition,

Table 2. Effect of treatment and pro-oxidation on pork meat homogenates color parameter values.
Tabla 2. Efecto del tratamiento y pro-oxidación sobre los valores de los parámetros de color de homogenizados de carne de cerdo.

Item	Treatment	Potassium ferrocyanide level		
		0 %	0.5 %	1.0 %
L* values	CN	39.12 ± 0.39	39.63 ± 0.64	41.12 ± 1.26
	T1	39.84 ± 0.78	39.68 ± 1.29	41.17 ± 0.50
	T2	39.04 ± 0.18	40.96 ± 0.26	40.94 ± 0.09
	BHT	39.26 ± 0.46	41.38 ± 0.76	41.45 ± 1.05
a* values	CN	6.99 ± 0.15 ^{aC}	5.27 ± 0.24 ^{aB}	3.46 ± 0.03 ^{aA}
	T1	7.31 ± 0.26 ^{aB}	5.21 ± 0.16 ^{aA}	4.83 ± 0.34 ^{bA}
	T2	7.35 ± 0.06 ^{aB}	5.32 ± 0.13 ^{aA}	5.57 ± 0.09 ^{bA}
	BHT	6.91 ± 0.10 ^{aB}	5.01 ± 0.13 ^{aA}	4.57 ± 0.36 ^{bA}
b* values	CN	8.76 ± 0.19 ^{aB}	8.66 ± 0.48 ^{aB}	7.34 ± 0.25 ^{aA}
	T1	9.55 ± 0.14 ^{aB}	8.51 ± 0.32 ^{aA}	6.99 ± 0.81 ^{aA}
	T2	9.33 ± 0.10 ^{aB}	8.34 ± 0.23 ^{aA}	7.91 ± 0.09 ^{aA}
	BHT	9.36 ± 0.74 ^{aA}	9.11 ± 0.33 ^{aA}	8.85 ± 0.85 ^{aA}
C* values	CN	11.21 ± 0.13 ^{aC}	10.13 ± 0.53 ^{aB}	8.11 ± 0.24 ^{aA}
	T1	12.03 ± 0.25 ^{aB}	9.98 ± 0.35 ^{aA}	8.50 ± 0.86 ^{abA}
	T2	11.88 ± 0.10 ^{aB}	9.89 ± 0.26 ^{aA}	9.67 ± 0.11 ^{bA}
	BHT	12.30 ± 1.14 ^{aB}	10.40 ± 0.35 ^{aA}	9.96 ± 0.19 ^{bA}
h* values	CN	51.39 ± 1.04 ^{aA}	58.65 ± 0.56 ^{bB}	64.72 ± 0.61 ^{cC}
	T1	52.59 ± 0.75 ^{aA}	55.24 ± 1.20 ^{aB}	58.50 ± 0.62 ^{aC}
	T2	51.72 ± 0.22 ^{aA}	54.83 ± 0.32 ^{aB}	57.44 ± 0.16 ^{aC}
	BHT	53.16 ± 0.59 ^{aA}	61.18 ± 0.42 ^{cB}	62.64 ± 0.57 ^{bC}

Values expressed as mean ± SD of at least three independent experiments. CN, control; T1, T2, CSE at 250 and 500 ppm, respectively; BHT, butylated hydroxytoluene at 500 ppm. Lowercase letters indicate significant differences between treatments; capital letters indicate significant differences in each treatment at different pro-oxidation levels ($p < 0.05$).
Valores expresados como media ± DE de al menos tres experimentos independientes. CN, control; T1, T2, CSE a 250 y 500 ppm, respectivamente; BHT, butilhidroxitolueno a 500 ppm. Las letras minúsculas indican diferencias significativas entre tratamientos; las letras mayúsculas indican diferencias significativas en cada tratamiento a diferentes niveles de pro-oxidación ($p < 0.05$).

it has been reported that the incorporation of coffee husk aqueous-ethanol extract (100 and 200 ppm) to raw frozen chicken patties during storage (-18 °C for 45 d) did not affect L* values during the storage period; however, the addition of this extract promotes an increase of a* and b* values (de Farias Marques *et al*., 2022). While the incorporation of potassium ferrocyanide (2.5, 7.5, and 100 ppm) to cod fillets exerts an effect on color parameters (L*, a*, and b*), which was associated with lipid oxidation during the salting process (Van Nguyen *et al*., 2012). In agreement with our study, L*, a*, and b* values of raw and cooked pork patties were not affected by non-compliant green coffee beans extract at 0.15, 0.30, and 0.60 % (Bergamaschi *et al*., 2023). Also, a* value of raw meat balls was not affected by green coffee powder addition at 250 ppm during storage (Mostafa and El Azab, 2022).

MMb values

Lipid and myoglobin oxidation in meat occurs simultaneously, and each process enhances the other. This is because aldehydes affect myoglobin´s redox stability, leading to meat discoloration (Chaijan, 2008). Figure 3 reports the results of the treatment and pro-oxidation effect on the MMb formation of pork meat homogenates incorporated with CSE and BHT. The results showed an effect of treatment and pro-oxidation interaction ($p < 0.05$) on this parameter. At 0 % of oxidizing, T2 showed the lowest ($p < 0.05$) MMb values for meat homogenates. At 1.0 % of the oxidizing agent, T2 showed the lowest ($p < 0.05$) MMb values compared to samples from CN.

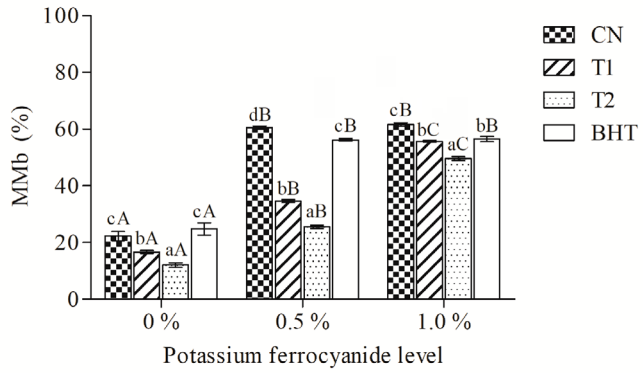

Figure 3. Effect of treatment and pro-oxidation on pork meat homogenates metmyoglobin content. Lowercase letters indicate significant differences between treatments; capital letters indicate significant differences in each treatment at different pro-oxidation levels ($p < 0.05$).
Figura 3. Efecto del tratamiento y pro-oxidación sobre el contenido de metamioglobina de homogenizados de carne de cerdo. Las letras minúsculas indican diferencias significativas entre tratamientos; las letras mayúsculas indican diferencias significativas en cada tratamiento a diferentes niveles de pro-oxidación ($p < 0.05$).

In a previous study, it has been reported that high concentrations of potassium ferrocyanide result in ferricyanide formation, which improves an immediate oxidative discoloration reaction of pork meat, i.e., $MbO_2 \rightarrow MMb + O_2$ (Hansen *et al*., 1996). In addition, another work demonstrated that the incorporation of Fe^{2+} and Fe^{2+} +NaCl increased MMb formation of beef patties, 68.3 and 68.7% of inhibition respect to CN (Min *et al*., 2010). In agreement with our study, the incorporation of spent coffee grounds to frozen cooked pork patties (0.1 %) reduced MMb values (Jully *et al*., 2016).

Principal components analysis

A principal components analysis was conducted to determine the differences between analyzed parameters and treatments (Figure 4).

The first and second components showed a variance of 56.98 and 38.58 %, respectively; thus, the two components explained an accumulation of 95.56 % of the total variation. The results indicate a significant separation of the analyzed data from antioxidant treatments compared to CN ($p < 0.05$). The above suggests that multivariate analysis is an excellent predictor for oxidative stability of meat quality (Sánchez *et al*., 2001).

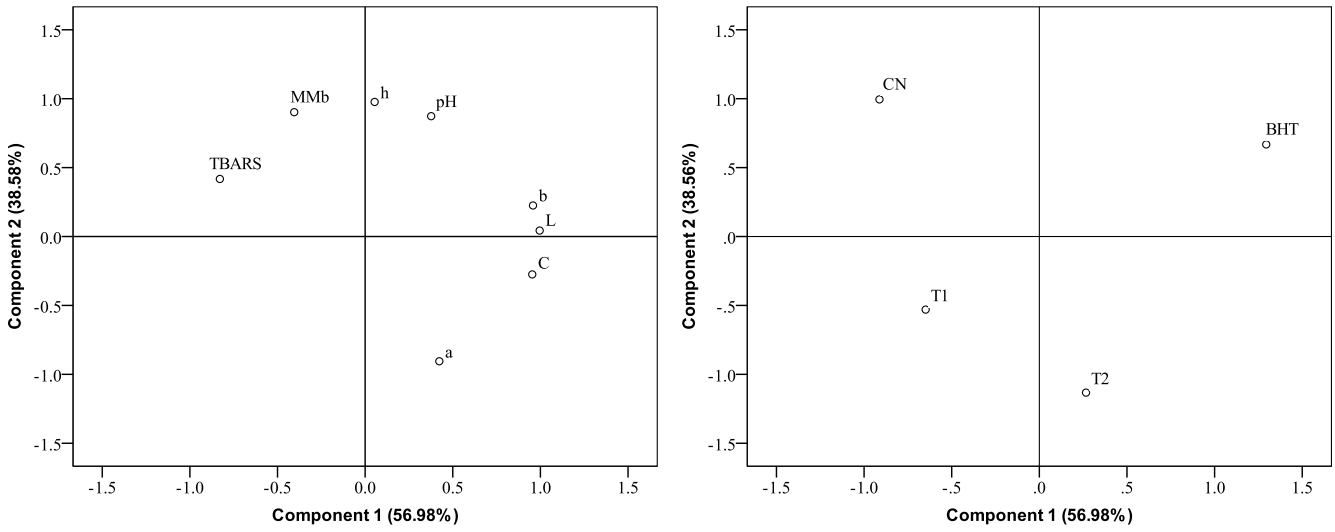

Figure 4. Principal components analysis of evaluated parameters and treatments.
Figura 4. Análisis de componentes principales de los parámetros evaluados y tratamientos.

CONCLUSIONS

In this investigation, the current findings revealed that CSE is an important source of polyphenols, including phenolic components, flavonoids, caffeoylquinic acid, and carbohydrates. In terms of antioxidant activity, this extract exerts antiradical and reducing power activity. Furthermore, incorporating CSE into pork meat homogenates led to reduce pH values, lipid oxidation, and metmyoglobin content, as well as decreased color changes when exposed to an oxidizing agent in comparison to controls. Therefore, our research highlights the significant potential of coffee residues as a valuable source of antioxidant additives for meat industry.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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