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Article

Effect of spent coffee grounds aqueous extract as an antioxidant in raw pork patties during refrigerated storage

Juan Luis Murillo Hernández [a]

Rey David Vargas Sánchez [b]

Brisa del Mar Torres Martínez [b]

Nelson Huerta Leidenz [c]

Gastón Ramón Torrescano Urrutia [b]

Armida Sánchez Escalante [b*]

[a] Instituto Tecnológico de Estudios Superiores de Zamora. Ingeniería en Industrias Alimentarias. Michoacán, México.

[b] Centro de Investigación en Alimentación y Desarrollo (CIAD). Coordinación de Tecnología de Alimentos de Origen Animal (CTAOA). Carretera a La Victoria Km. 0.6, 83148 Sonora, México.

[c] Texas Tech University. Department of Animal and Food Sciences. Texas, USA.

*Corresponding author: armida-sanchez@ciad.mx

Abstract:

The effect of spent coffee grounds (SCG) aqueous extract and butylated hydroxytoluene (BHT) on color deterioration, lipid oxidation, and antioxidant status of uncooked pork patties during refrigerated storage (4 °C/9 days, under dark) was investigated. Polyphenols content and antiradical activity of SCG extract were evaluated. Pork patties were evaluated for pH, color parameters and lipid oxidation (LOX), as well as total antioxidant activity of meat. Results showed that SCG extract is an important source of polyphenols and exerts antioxidant activity. Their inclusion in meat samples mitigated undesirable changes in pH, color, and

LOX values and increased the antioxidant stability during storage (P<0.05). In conclusion, using SCG extract as a natural antioxidant can improve raw pork patties' quality and shelf life.

Keywords: Natural extract, Coffee residues, Antioxidant activity, Pro-oxidation, Meat quality.

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Introduction

Lipid oxidation (LOX) of meats leads to the development of undesirable compounds that undermine nutrient composition (e.g., essential amino acids and fatty acids) and sensory attributes (color, odor, flavor, and texture), thus compromising the purchase intentions and acceptability of ultimate consumers. Therefore, to reduce LOX process in meat and meat products, synthetic antioxidants (e.g., butylated hydroxytoluene and butylated hydroxyanisole) have been used extensively. Nevertheless, increasing concerns about the health risks posed by synthetic antioxidants are forcing their replacement for natural antioxidant compounds[1].

Natural antioxidant compounds have been extracted from each anatomical structure of plants, such as flowers, fruits, leaves, among others. However, by-products derived from the fruit processing industry have also been considered an important source of polyphenols with this property[2]. The insoluble residue obtained after filtering the drink is a by-product commonly known as spent coffee grounds (SCG). SCG is usually discarded when it is not used as a fertilizer[3].

Instead of treating SCG as a waste, other processing industries have taken advantage of this raw material as a substrate for fungal growth[4] and as a food additive for bakeries[5]. In this context, roasted ground coffee residue extract added at 15 % to salted mackerels has decreased LOX during 15 d of refrigerated storage[6]. SCG has also been proposed as an ingredient for the meat industry to reduce LOX[1]. However, the assessment of SCG and their extracts as an antioxidant additive for developing novel meat products to enhance shelf life needs further examination.

Therefore, the inclusion of SCG aqueous extract as a functional ingredient to enhance antioxidant status of raw pork patties during refrigerated storage was investigated.

Material and methods

Polyphenols extraction

SCG was procured (Caffenio®, dark *Coffea arabica* L.) and subjected to thermal sterilization. Polyphenols compounds from SCG were extracted with water as a solvent by an ultrasound-assisted method (42 KHz/25 °C/30 min), using a 1:10 SCG-solvent ratio (ultrasound bath, Bransonic 3800; Jeju, Korea). The resultant mixture was filtered (Whatman 1 filter paper) under vacuum (vacuum pump, MVP 6; Jeju, Korea), evaporated at 100 rpm/60 °C (Yamato RE301BW; Tokyo, Japan), and dried (Yamato DC401; Tokyo, Japan). The resulting SCG extract was stored at -20 °C/under dark conditions[7].

Polyphenols content

Chlorogenic acid content (CAC) was determined, as reported previously[8]. SCG extract (100 µl, 500 µg/mL) was mixed with 200 µL of urea (0.17 M) and 200 µL of glacial acetic acid (0.1 mol/L), then 500 µL of dH_2O were added. The resultant mixture was homogenized with 500 µL of $NaNO_2$ (0.14 mol/L) and 500 µL of NaOH (0.5 mol/L) and centrifuged (2,250 xg/4 °C, 10 min). At 510 nm was measured the absorbance and results displayed as mg of chlorogenic acid equivalents (CAE)/per gram of extract.

Total phenol content (TPC) was determined by the Folin-Ciocalteu procedure[9]. SCG extract (10 µL, 500 µg/mL) was mixed with 80 µL of dH_2O and 60 µL of Na_2CO_3 (7 %, w/v), then 40 µL of Folin-Ciocalteu reagent (2 M) was added. The resultant mixture was homogenized with 80 µL of dH_2O and incubated (25 °C/1 h, under dark). At 750 nm was measured the absorbance and results displayed as milligrams of gallic acid equivalents (GAE)/g.

Total flavonoids content (TFC) was determined by the $NaNO_2$-$Al(NO_3)_3$-NaOH procedure[10]. SCG extract (500 µL, 500 µg/mL) was homogenized with 1 mL of $NaNO_2$ (5 %, w/v), 10 mL of NaOH (1 mol/L), and 1 mL of $AlCl_3$ (10 %, w/v). Then 25 mL of ethanol (70 %, v/v) was added. The resultant solution was incubated (25 °C/15 min, under

dark). At 510 nm was measured the absorbance and results displayed as mg of rutin equivalents (RE)/g.

Total tannins content (TTC) was determined by the vanillin procedure[11]. SCG extract (0.2 g) was mixed with 10 mL of methanol and centrifuged (10,000 xg/4 °C, 20 min). Then 180 µL of the supernatant was mixed with 900 µL of vanillin (1 %, w/v) and 900 µL of HCl (8 %, v/v) and incubated (25 °C/20 min, under dark). At 500 nm was measured the absorbance and results were displayed as mg of (+)-catechin equivalents (CE)/g.

Antioxidant assays

Free-radical scavenging activity was determined by the radical DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl) procedure[12]. Then 100 µL of SCG extract (500 µg/mL) was homogenized with 100 µL of radical solution (300 µM/kg) and incubated (25 °C/30 min, under dark). At 520 nm was measured the absorbance and results displayed as (%) inhibition: DPPH$^{\bullet}$ (%) = [(Radical absorbance at 0 min) – (Antioxidant-radical absorbance at 30 min) / (Radical absorbance at 0 min)] × 100.

Radical-cation scavenging activity was evaluated by the radical ABTS$^{\bullet+}$ (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation) procedure[13]. The radical cation (0.8 absorbance) was mixed with SCG extract (500 µg/mL) in a ratio of 99:1 and incubated (25 °C/6 min, under dark). At 734 nm was measured the absorbance and results displayed as (%) inhibition: ABTS$^{\bullet+}$ (%) = [(Radical absorbance at solution at 0 min) – (Antioxidant-radical absorbance at 6 min) / (Radical absorbance at solution)] × 100.

Reducing power was determined by the Ferric-reducing antioxidant power (FRAP) procedure[14]. Then 5 µL of SCG extract (500 µg/mL) were homogenized with 150 µL of FRAP solution [10:1:1, 300 mM/kg of buffer sodium acetate in glacial acetic acid and 10 mM/kg of TPZ reagent in 40 nM/kg of HCl and 20 mM/kg $FeCl_3$] and incubated (25 °C/8 min/under dark). At 595 nm was measured the absorbance and results displayed as mg of iron equivalent (Fe^{2+})/g.

Reducing power was also determined by the RPA procedure[14]. Then 100 µL of SCG extract (500 µg/mL) were homogenized with 300 µL of phosphate buffer (0.2 mol/L, pH 6.6) and 300 µL of $C_6FeK_4N_6$ (1 %, w/v). The resultant mixture was incubated at 50 °C for 20 min. After that, 300 µL of TCA (10 %, w/v) were added, and the samples were centrifuged at 4,200 xg/4 °C, 15 min (Sorvall ST18R, Thermo Fisher Scientific; Waltham, USA). The

supernatant was homogenized with 100 µL of dH_2O and 250 µL of $FeCl_3$ (0.1 %, w/v). At 700 nm was measured the absorbance and results displayed as absorbance.

Pork patties elaboration

Fresh pork minced meat (*Semimembranosus* muscle) was procured (Norson®) and mixed with 1.5 % salt (NaCl, w/w) and back fat (20 %, w/w). Pork patties were assessed in four treatments (in triplicate) as follows: Control (samples without antioxidant); T1 (samples with 0.05 % of SCG extract, w/w); T2 (samples with 0.1 % of SCG extract, w/w); T3 (samples with 0.02 % of BHT, w/w). Sixteen patties (40 g per patty) per treatment were elaborated, packaged in Styrofoam™ trays (expanded polystyrene), and overwrapped with polyvinyl chloride film (17,400 cm^3 O_2/m^2/23 °C, 24 h). The packaged patties were refrigerated (4 °C/9 days/under the dark), and on each sampling day, four packages per treatment were opened for due analysis.

Meat quality measurements

The proximate chemical composition (moisture, fat, protein, ash, and carbohydrate content) of the meat product was determined following standard procedures[15].

The pH of the meat product was determined by mixing the samples with dH_2O (1:10 ratio) at 4,500 rpm/5 °C, 1 min (T25, IKA®; Staufen, Germany), and using a potentiometer (pH211, Hanna; RI, USA)[15].

Thiobarbituric acid reactive substances procedure was used to measure the lipid oxidation (LOX)[16]. The meat product (1 g) was homogenized with 2,000 µL of TCA (10 %, w/v) (4,500 rpm/5 °C, 1 min) and centrifuged (2,300 xg/4 °C, 20 min). Then 200 µL of the filtered (Whatman 1 filter paper) solution was homogenized with 200 µL of TBA reagent (0.02 mol/kg) and incubated at 98 °C, 20 min. At 531 nm was measured the absorbance and results displayed as mg of malondialdehyde (MDA)/g.

The meat product color was measured spectrophotometrically (CM-508d, Konica Minolta Inc.; Tokyo, Japan). Samples were exposed to O_2 under refrigeration at 4 °C, 30 min. After that, 10 readings were performed on the samples surface to record: L*, lightness; a*, redness; b*, yellowness; C*, chromaticity; h*, hue angle[17].

The meat homogenate was obtained after pork patties were homogenized with dH_2O (1:10 ratio) at 4,500 xg/4 °C, 10 min. Thereafter, the supernatant was filtered and subjected to polyphenols content, antiradical and reducing power activity measurements.

Statistical analysis

Polyphenols and antioxidant activity data (n=6) were analyzed by a one-way ANOVA, while meat quality measurements were subjected to a two-way ANOVA. A Tukey test was performed ($P<0.05$). In addition, a multivariate analysis was used to determine the relationship among all parameters (SPSS version 21).

Results and discussion

Polyphenols content and antioxidant activity of SCG extract

The presence of polyphenols in SCG extract was demonstrated by the obtained results, including CAC (205.03 ± 4.13 mg CAE/g), TPC (562.71 ± 20.04 mg GAE/g), TFC (756.38 ± 11.82 mg RE/g), and TTC (12.50 ± 3.33 mg CE/g). In addition, mean values of antioxidant activity also showed that SCG extract displays high $DPPH^{\bullet}$ (84.95 ± 0.61 %) and $ABTS^{\bullet+}$ antiradical activity (43.93 ± 2.08 %), although the standard (BHT) showed the highest ($P<0.05$) antioxidant values respect to SCG extract (89.12 ± 2.10 % and 81.20 ± 1.15 %, respectively). Furthermore, SCG extract displays moderate FRAP (0.21 ± 0.10 mg Fe^{2+}/g) and RPA (0.11 ± 0.01 abs) values concerning BHT (0.53 ± 0.15 mg Fe^{2+}/g and 0.60 ± 0.10 abs, respectively) ($P<0.05$).

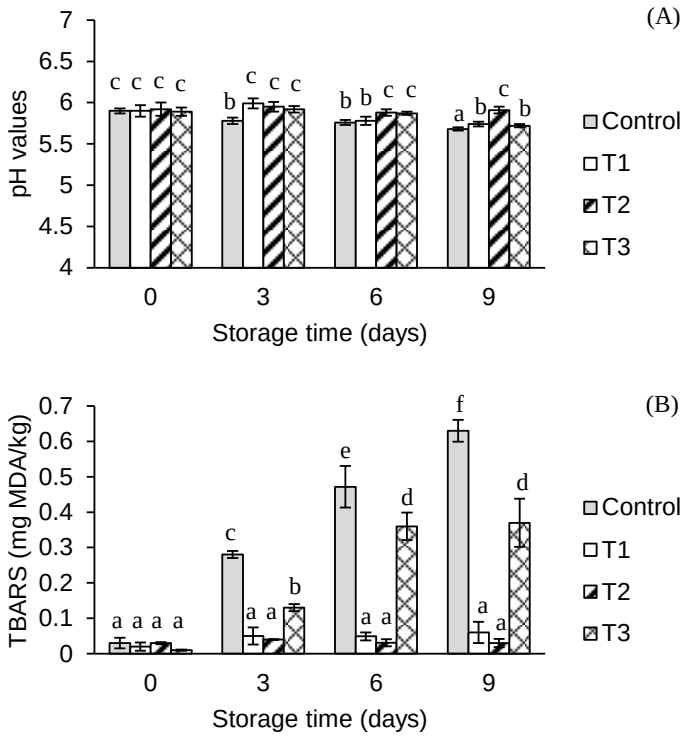
In previous works, it has been demonstrated that these agro-industrial by-products are a key source of polyphenols, including flavonoids and phenolic acids, widely correlated with their *in vitro* antioxidant effect[2]. However, the absence or presence of these components in coffee residue extracts could be associated with the variety (*C. arabica* and *C. robusta*), extraction system (solid-liquid, Soxhlet, among others), and the solvent type (water or ethanol) used for the compound extraction[18,19]. In addition, the presence of polyphenols in SCG extract is related to their antiradical effect; hence, a promissory strategy to enhance the antioxidant stability of pork meat products during storage could be the addition of SCG extract.

Pork patties quality

According to the results, the proximate composition of meat samples did not vary with the inclusion of 0.05 and 0.1 % of SCG extract (P>0.05). The average values obtained were 55.73 % (moisture), 19.5 % (protein), 22.5 % (fat), 1.8 % (ash) and 0.53 % (carbohydrates). It had been observed that adding 1 and 2 % of extracts from agro-industrial residues in formulations of pork patties did not significantly affect the original proximate composition[20].

Figure 1 illustrates raw pork patties' pH and LOX changes (A and B, respectively). The treatment × storage time interaction significantly affected (P<0.05). On the initial day of storage (d 0), the incorporation of antioxidant treatments did not affect pH and LOX values (P>0.05). However, pH values decreased, and LOX values increased during storage time (P<0.05). At d 9 (end of storage), meat samples treated with T1 and T2 exerted the highest) pH values, and the lowest LOX values (P<0.05).

Figure 1: pH (A) and LOX (B) values (Mean ± SD, n = 6) of raw pork patties during storage time



T1= pork patties with 0.05 % of SCG extract; T2= pork patties with 0.1 % of SCG extract; T3= pork patties with 0.02 % of BHT.

[abcdef] Lowercase superscripts indicate significant differences when considered treatment x storage time interaction effect (P<0.05).

Meat quality evaluation indicates *antemortem* and *postmortem* changes occurring in slaughter animals; pH and LOX are key properties in meat quality perceptions influencing the meat purchase intention associated with quality losses in meat industry products[16,21]. However, it has also been demonstrated that the inclusion of non-synthetic sources rich in polyphenols improves food quality[22].

In this investigation, initial pH values in pork patties remained within the typical range for fresh pork meat (pH 5.5-5.9). A reduction in pH values of pork patties was observed during cold storage when synthetic or natural (date pits) antioxidant extracts were added[23]. Additionally, in agreement with the results of this study, a 42 % decrease in LOX of cooked pork patties added with light and dark SCG extract (1 g/kg or 10 %), stored under freezing conditions for three months, was observed[24]. When adding 0.05 and 0.1 % of SCG ethanol extract reduced LOX in raw pork-meat system stored at 37 °C/12 h[19]. Also, a LOX reduction of ground (top round) beef added with 0.1 % of ground roasted coffee (light, medium, and dark) through storage (4 °C/6 d) has been demonstrated[25].

According to the obtained results (Table 1) the treatment × storage time interaction had a significant effect on color values (P<0.05). At d 0, the antioxidant incorporation did not affect these parameters (P>0.05). However, L*, a*, b*, and C* values were reduced during storage time, while h* values were increased (P<0.05). On d 9, meat samples treated with T1 and T2 exerted the highest L*, a*, b*, and C* values and the lowest h* values (P<0.05). Color is another key parameter in meat quality perceptions[21]. In agreement with the study, a reduction in L*, a*, and b* values through refrigerated storage (4 °C/6 d) has been reported in control meat samples compared to their counterparts treated with 0.1 % of ground roasted coffee[25].

Table 1: Colour changes of raw pork patties treated with the aqueous extract of SCG during storage time

Item	Treatment	Storage time days			
		0	**3**	**6**	**9**
L*	Control	57.14 ± 1.36^{c}	51.51 ± 1.57^{a}	51.77 ± 1.36^{a}	49.39 ± 1.35^{a}
	T1	56.83 ± 1.16^{c}	54.40 ± 1.74^{b}	54.06 ± 1.93^{b}	54.63 ± 1.56^{b}
	T2	56.97 ± 0.86^{c}	53.61 ± 0.90^{b}	53.24 ± 1.39^{b}	53.18 ± 1.98^{b}
	T3	56.35 ± 1.56^{c}	56.01 ± 0.63^{c}	54.31 ± 1.86^{b}	53.25 ± 1.31^{b}
a*	Control	10.56 ± 1.37^{c}	7.55 ± 1.39^{b}	6.27 ± 0.97^{b}	4.15 ± 0.94^{a}
	T1	9.60 ± 0.88^{c}	9.33 ± 1.14^{c}	8.28 ± 1.39^{bc}	7.25 ± 0.83^{b}
	T2	10.79 ± 1.18^{c}	10.12 ± 1.67^{c}	8.25 ± 0.74^{bc}	8.52 ± 1.01^{bc}
	T3	9.22 ± 0.68^{c}	9.10 ± 1.79^{c}	7.68 ± 0.82^{b}	4.75 ± 1.38^{a}
b*	Control	18.09 ± 1.37^{c}	15.68 ± 1.43^{b}	14.56 ± 0.95^{a}	12.28 ± 1.65^{a}
	T1	18.20 ± 0.97^{c}	16.67 ± 1.05^{b}	16.34 ± 1.01^{b}	15.84 ± 1.35^{b}
	T2	19.08 ± 1.21^{c}	17.21 ± 1.65^{bc}	16.95 ± 1.40^{b}	16.11 ± 1.50^{b}
	T3	17.62 ± 1.03^{bc}	16.16 ± 1.34^{b}	15.75 ± 0.90^{b}	14.95 ± 1.11^{a}
C*	Control	21.15 ± 1.49^{c}	17.35 ± 1.77^{b}	16.74 ± 1.29^{b}	13.54 ± 0.86^{a}
	T1	20.35 ± 1.08^{c}	18.62 ± 1.61^{bc}	18.45 ± 1.80^{bc}	16.77 ± 1.32^{b}
	T2	21.25 ± 1.29^{c}	19.45 ± 1.60^{c}	18.84 ± 1.44^{bc}	17.78 ± 1.71^{b}
	T3	19.06 ± 1.17^{c}	17.52 ± 1.69^{b}	16.51 ± 1.72^{b}	15.64 ± 1.68^{ab}
h*	Control	61.41 ± 1.16^{a}	63.62 ± 1.51^{a}	66.17 ± 1.72^{b}	72.75 ± 1.02^{c}
	T1	63.80 ± 1.60^{a}	63.10 ± 2.06^{a}	65.74 ± 1.46^{ab}	67.83 ± 1.88^{b}
	T2	62.21 ± 1.53^{a}	62.47 ± 1.90^{a}	63.01 ± 1.46^{a}	64.45 ± 1.49^{a}
	T3	62.51 ± 1.86^{a}	65.09 ± 1.84^{ab}	66.44 ± 1.51^{b}	71.73 ± 2.06^{c}

Results expressed as mean ± SD (n = 6); T1= pork patties with 0.05 % of SCG extract; T2= pork patties with 0.1 % of SCG extract; T3= pork patties with 0.02 % of BHT.

^{abc} Lowercase superscripts indicate significant differences when considered treatment x storage time effect (P<0.05).

Polyphenols content and antioxidant activity of meat homogenates

According to the obtained results (Table 2), the treatment × storage period interaction significantly affected the meat homogenates' polyphenols content (P<0.05). Non-differences (P>0.05) were detected in TTC (average value 0.87 mg CE/g), $ABTS^{\bullet+}$ (49.58 %), and RPA values (0.87 abs) during storage. At d 0, chlorogenic acid (CGA), TPC, and TFC values increased (P<0.05) in meat samples treated with SCG extract (T2>T1). Yet CGA, TPC, and TFC values significantly decreased (P<0.05) during storage period, and at day 9, the highest (P<0.05) CGA, TPC, and TFC values corresponded to T2.

Table 2: Polyphenols content of pork patties treated with the aqueous extract of SCG during storage time

Item	Day	Treatments			
		Control	**T1**	**T2**	**T3**
CGA,	0	31.01 ± 0.50^{a}	85.99 ± 1.50^{d}	110.15 ± 2.78^{e}	31.43 ± 0.50^{a}
mg CAE/g	9	31.20 ± 0.62^{a}	33.10 ± 0.42^{b}	75.72 ± 0.33^{c}	31.60 ± 0.44^{a}
TPC,	0	31.70 ± 3.01^{c}	33.17 ± 2.50^{c}	42.84 ± 2.10^{e}	38.67 ± 1.15^{d}
mg GAE/g	9	23.20 ± 1.04^{a}	21.20 ± 1.30^{a}	27.10 ± 1.23^{b}	26.62 ± 1.00^{b}
TFC,	0	34.01 ± 0.90^{d}	34.46 ± 1.50^{d}	46.20 ± 0.80^{e}	22.27 ± 1.98^{b}
mg RE/g	9	15.60 ± 1.13^{a}	20.30 ± 1.55^{b}	31.91 ± 2.56^{c}	20.16 ± 1.13^{b}
TTC,	0	1.00 ± 0.50	0.96 ± 0.15	1.06 ± 0.42	0.78 ± 0.28
mg CE/g	9	0.80 ± 0.45	0.74 ± 0.31	0.79 ± 0.30	0.84 ± 0.33

Results expressed as mean ± SD (n= 6); T1= pork patties with 0.05 % of SCG extract; T2= pork patties with 0.1 % of SCG extract; T3= pork patties with 0.02 % of BHT.

^{abcde} Lowercase superscripts indicate significant differences when considered treatment x storage time interaction effect (P<0.05).

According to the obtained results (Table 3) the treatment × storage period interaction significantly affected the meat homogenates' antioxidant status (P<0.05). Regarding the antioxidant activity, at d 0, DPPH$^{\bullet}$ and FRAP values increased (P<0.05) in meat samples due to SCG extract. However, antioxidant values were reduced (P<0.05) during storage in the Control and T3 samples. On d 9, T1 and T2 showed the highest DPPH$^{\bullet}$ and FRAP values (P<0.05).

Table 3: Antioxidant status of pork patties treated with the aqueous extract of SCG during storage time

Item	Day	Treatments			
		Control	**T1**	**T2**	**T3**
DPPH$^{\bullet}$, %	0	30.10 ± 2.70^{c}	41.97 ± 1.00^{d}	48.65 ± 1.54^{e}	43.92 ± 2.99^{d}
	9	13.50 ± 0.55^{a}	43.70 ± 1.60^{d}	45.08 ± 2.04^{de}	15.37 ± 1.02^{b}
ABTS$^{\bullet+}$, %	0	50.40 ± 1.55^{a}	51.98 ± 1.83^{a}	48.68 ± 2.75^{a}	48.52 ± 3.00^{a}
	9	49.10 ± 1.52^{a}	48.95 ± 1.45^{a}	49.25 ± 3.22^{a}	49.83 ± 2.99^{a}
FRAP,	0	4.64 ± 0.52^{a}	12.09 ± 1.13^{b}	17.03 ± 0.50^{c}	3.89 ± 0.55^{a}
mg Fe^{2+}/g	9	3.97 ± 0.50^{a}	10.62 ± 0.55^{b}	18.42 ± 1.74^{c}	4.64 ± 0.52^{a}
RPA (Abs)	0	1.10 ± 0.30	1.03 ± 0.05	0.91 ± 0.30	0.92 ± 0.25
	9	0.80 ± 0.20	0.70 ± 0.30	0.81 ± 0.30	0.70 ± 0.30

Results expressed as mean ± SD (n= 6); T1= pork patties with 0.05 % of SCG extract; T2= pork patties with 0.1 % of SCG extract; T3= pork patties with 0.02 % of BHT.

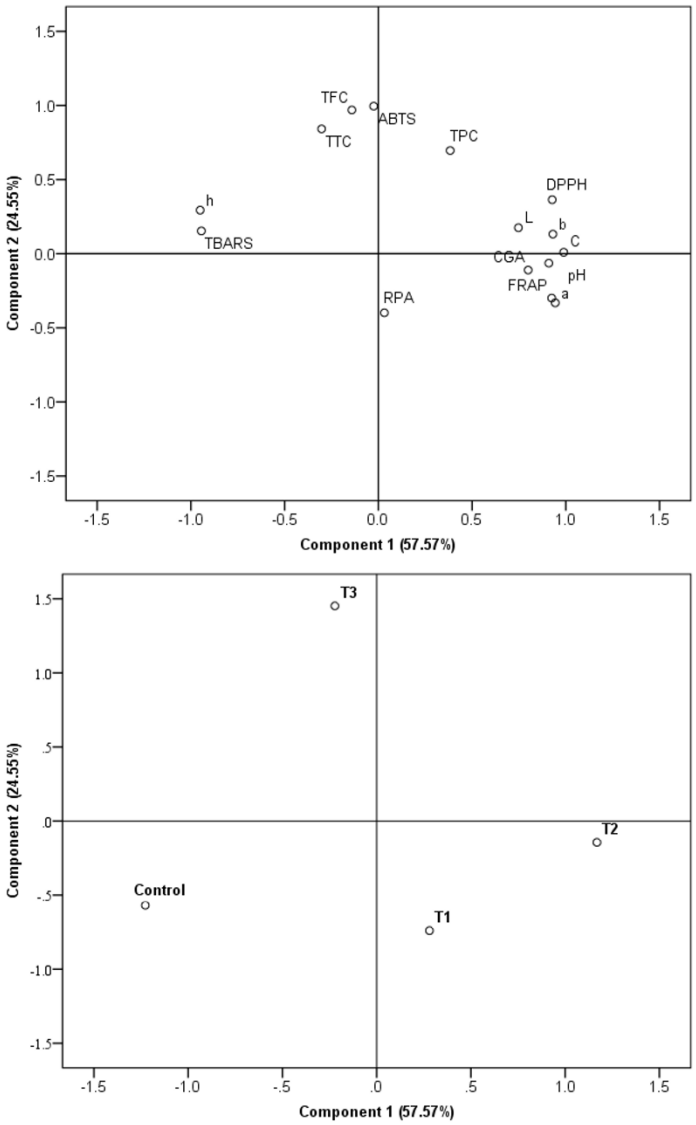
^{abcd} Lowercase superscripts indicate significant differences when considered treatment x storage time interaction effect (P<0.05).

Oxidative reactions in foods, including meat and meat products, are considered the principal non-microbial cause of quality deterioration, and it is associated with a loss of endogenous antioxidants *postmortem*. Animal species, breed, muscle type, and anatomical location can influence endogenous antioxidant content[22]. Concerning exogenous antioxidant content, the presence of phenolic compounds in meat and meat products may result from the animal´s diet[26], while the extraction and incorporation of bioactive compounds from natural sources into meat formulations can increase the antioxidant status of meat products[22]. In this context, the phenolic content and antioxidant status of raw and cooked pork patties stored (4 °C/6 d) and added with 2 % of a natural ethanol extract was increased[27].

Multivariate analysis

Figure 2 shows a principal component analysis to determine the differences among analyzed variables and treatments. The first and second components showed a 57.57 and 24.55 % variance, respectively. In this context, 82.12 % of the total variation was explained by both components. In addition, a separation of the treatments concerning the variables was observed; for example, the T2 treatment, loaded towards the right quadrant, presented the highest polyphenols and antioxidant activity content.

Figure 2: Principal component analysis of evaluated parameters and treatments



Control, samples without-antioxidant; T1= pork patties with 0.05 % of SCG extract; T2= pork patties with 0.1 % of SCG extract; T3= pork patties with 0.02 % of BHT.

Conclusions and implications

SCG extract is a novel source of antioxidant components, including polyphenols. SCG extract incorporation into pork patties elicits desirable responses in pH values, color, and lipid oxidation stabilities during storage times. Moreover, SCG extract increases polyphenols content and the antioxidant status of meat samples. SCG extract can be used in the formulation of pork patties to prevent oxidation reactions and mitigate meat quality losses during refrigerated storage.

Acknowledgements and conflict of interest

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