Walnut (*Juglans regia* L.) leaf powder as a natural antioxidant in cooked pork patties

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**ABSTRACT**

The addition of walnut leaf powder (WLP) at 0.2% and 0.5% as a natural antioxidant in salted pork meat patties during refrigerated storage for 15 days was investigated, and its efficiency was evaluated against butylated hydroxytoluene (BHT). Proximate composition, cooking yield, pH, total phenolic content, free radical scavenging activity, lipid peroxidation expressed as thiobarbituric acid reactive substances (TBARS) and sensory analyses were conducted on manufactured patties. The pork patties containing 0.5% WLP had lower TBARS values and higher antioxidant activity compared to the BHT added and control samples during the 15 days of refrigerated storage. Addition of WLP significantly (*P* < .05) reduced lightness and redness but increased the hue angle of pork patties. In the sensory evaluation, WLP produced satisfactory results concerning the appearance, flavor and overall acceptability. The results indicated the potential of walnut leaf powder to retard lipid oxidation and to enhance the quality of cooked ground pork meat.

**Polvo de hojas de nogal (*Juglans regia* L.) como antioxidante natural en medallones de cerdo cocidos**

**RESUMEN**

El presente estudio investigó el desempeño del polvo de hoja de nogal (WLP) como antioxidante natural cuando es adicionado a 0.2 y 0.5% en medallones de carne de cerdo salada mantenida en almacenamiento refrigerado durante 15 días. Además, se evaluó su eficacia contra el butilhidroxitolueno (BHT). Al respecto, se analizó la composición proximal, el rendimiento de cocción, el pH, el contenido fenólico total, la actividad de eliminación de radicales libres, la peroxidación lipídica expresada como sustancias reactivas al ácido tiobarbitúrico (TBARS) y se realizaron análisis sensoriales en medallones preparados de manera industrial. Durante los 15 días de almacenamiento refrigerado, los medallones de cerdo que contenían 0.5% de WLP mostraron valores de TBARS más bajos y mayor actividad antioxidante en comparación con las muestras de BHT agregadas y de control. La adición de WLP redujo significativamente (*P* < .05) la luminosidad y el enrojecimiento, pero aumentó el ángulo de tono de los medallones de cerdo. A partir de la evaluación sensorial, se constató que el WLP produjo resultados satisfactorios en la apariencia, el sabor y la aceptabilidad general de los medallones. Los resultados obtenidos indican que es posible usar polvo hoja de nogal para retardar la oxidación de los lípidos y mejorar la calidad de la carne de cerdo molido y cocida.

**Introduction**

Consumption of convenience food such as precooked meat products has increased as a result of changing eating habits arising from the development of society. Ground meat products, such as meatballs, burgers and meat patties, are widely produced and consumed, but they are frequently exposed to some quality losses such as oxidative changes and moisture loss causing freezer burn (Turgut, İşikç, & Soyer, 2017). The food industry has, therefore, had to develop innovative strategies to improve the shelf life and safety of both conventional and organic meat products (Choe et al., 2011; Mancini et al., 2015).

Lipid oxidation is a major cause for the deterioration of muscle-based foods, lowering their nutritional and functional properties and affecting their sensory characteristics, like flavor, color, and texture (Das, Rajkumar, Verma, & Swarup, 2012). Lipid oxidation determines rancidity and off-flavors and reduces the shelf-life of meat and meat products as it results in the formation of toxic compounds including reactive oxygen species, free radicals, hydroperoxides and malonaldehyde which are believed to be associated with carcinogenesis, mutagenesis, inflammation, DNA changes, aging, and cardiovascular diseases. Processing conditions such as mincing, salt addition, freezing rate and storage time increase the intensity of oxidative reactions (Naveena, Sen, Vaithiyanathan, Babji, & Kondaiah, 2008; Turgut et al., 2017). Moreover, additional oxidation is induced by cooking in relation to raw meat (Selani et al., 2011).

In order to retard the formation of toxic oxidation products, maintain nutritional quality, improve color stability and increase the shelf life of food products, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) have been widely used. They are powerful and...

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**KEYWORDS**

Pork meat; walnut leaves; lipid peroxidation; polyphenols; antioxidant activity

**PALABRAS CLAVE**

carne de cerdo; hojas de nogal; peroxidación lipídica; polifenoles; actividad antioxidante

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inexpensive but suffer from a negative consumer image because they are reported to have toxic effects and to be carcinogenic.

In recent years, the interest in products with natural antioxidants has increased (Mancini et al., 2015). Hence, research into safer and more effective natural antioxidants has intensified, and several natural sources have been examined as alternatives to synthetic antioxidants in food products. Plants which are naturally rich in antioxidants, e.g., polyphenols, have attracted considerable interest because of their presumed safety, bioactivity and potential nutritional and therapeutic value. Examples of natural products added as natural antioxidant in precooked meat products included green tea and grape seed extracts in beef patties (Bañón, Díaz, Rodríguez, Garrido, & Price, 2007), pomegranate juice and rind powder in cooked chicken patties (Naveena et al., 2008), turmeric powder in rabbit burgers (Mancini et al., 2015), pomegranate peel extract in beef meatballs (Turgut et al., 2017), lotus leaf and barley leaf powder in cooked ground pork (Choe et al., 2011), *Morina oleifera* leaves extract in cooked goat meat patties (Das et al., 2012), and pistachio seed hull extracts in chicken burgers (Al-Juhaimi et al., 2017).

Walnut leaves have been used in traditional medicine for the treatment of many diseases including skin inflammations, venous insufficiency, haemorrhoidal symptomatology, hyperhidrosis, and ulcers. Moreover, research in pharmacology and therapeutics have shown that walnut leaves have hypoglycaemic, anthelmintic, anti-septic, deprotoxic, and antihypertensive effects (Almeida, Fernandes, Lima, Costa, & Bahia, 2008). Many studies revealed that walnut leaves are a rich source of phenolic compounds (Nour, Trandafir, & Cosmulescu, 2013) which appear to be responsible for some of their therapeutic properties as they show a broad spectrum of pharmacological activity, including antiallergic, anti-inflammatory, antiviral, antiproliferative and anticarcinogenic activities (Yao et al., 2004). Phenolic acids, flavonoids, and naphthoquinones are the primary phenolic compounds in walnut leaves. Several studies have investigated the phenolic composition of walnut leaves in correlation with their antioxidant activity (Almeida et al., 2008; Carvalho et al., 2010; Nour, Trandafir, & Cosmulescu, 2016; Santos et al., 2013), but no study evaluated the effects of their use as natural antioxidant in precooked ground meat products. The aim of this study was to investigate the effects of the addition of different levels of walnut leaf powder (WLP) on lipid oxidation, instrumental color, pH, and sensory properties of cooked pork patties during 15 days of refrigerated storage.

**Materials and methods**

**Vegetable material. Preparation of walnut leaf powder**

Fresh walnut leaves were harvested in the middle of July from different parts of three walnut trees grown in the experimental orchard of the University of Craiova located at Râmnicu Vâlcea (Romania) research station (45°07′N/24°22′E). After collection, the leaves were immediately transferred to the laboratory, removed from the stems, air dried in the shade (final moisture content = 5.93%) and stored at ambient temperature in the dark. The dried leaves were milled in a coffee grinder (Bosch MKM6000, Germany) and sieved through a 1 mm stainless steel sieve to obtain the fine fraction. The powder was then packaged and stored at room temperature in closed containers in the dark until use.

**Preparation of pork patties**

Fresh pork meat and back fat were purchased from a local meat supplier. All separable fat and visible connective tissues were removed from the meat. The pork patties were made according to the following recipe: 73.5% lean pork meat, 20% pork back fat, 5% ice and 1.5% salt. The meat and fat were chopped through a 3 mm plate, then the ice and salt were added. The mixture was divided into four experimental variants: (I) C0 – control, meat without antioxidant; (II) C1 – meat + 0.1% BHT; P1 – meat + 0.2% WLP; P2 – meat + 0.5% WLP. Samples were hand mixed for 10 min. Then, the mixed meat was weighed (50 g), and the samples were shaped in a Petri dish (565 mm diameter and 180 mm depth) resulting in 80 patties for each experimental variant. The samples were placed in an electric oven (Beko, BIM24300GPS, Turkey) preheated for 15 min at 180°C and allowed to cook for 10 min until the meat reached 75 ± 1°C in the center. After cooling to room temperature, the patties were aerobically packed in polyethylene bags and analyzed for sensory attributes, color, total phenolic content, pH, antioxidant activity, and thiobarbituric acid reactive substances (TBARS) value. The samples were stored at 4 ± 1°C for 15 days and analyzed at 5-day intervals (0, 5th, 10th and 15th day). The cooking yield was determined by dividing the mass of the cooked product by the mass of the raw meat mixture and expressed as a percentage.

**Proximate composition**

The samples were analyzed for moisture, fat and protein content. Moisture content was determined based on moisture loss after 12 h at 105°C in a drying oven (Memmert ULM500, Germany) according to SR ISO 1442/2010. The fat content was determined by the Soxhlet method (SR ISO 1444/2008) using a Soxhlet automatic extraction system (SER 148/3, Velp Scientific, Italy), and the protein content was determined by the Kjeldahl method (SR ISO 937/2007) using an automated nitrogen analyzer (UDK 149 Velp Scientific, Italy).

**pH**

The pH value was determined on the sample extracts with a digital pH meter (Hanna HI225, Italy). The extracts were obtained by homogenizing 10 g of the sample in 50 ml of distilled water using a vertical homogenizer (Braun MQS137BK, 750W).

**Extraction of meat samples**

Samples were weighed (10 g) in centrifuge tubes and first extracted with 40 ml of methanol/water (50:50, v/v). The mixture was vigorously shaken in a vortex for 1 min and left for extraction for 60 min at room temperature. The tubes were centrifuged at 6000 rpm for 15 min, and the supernatant was collected. Next, 40 ml of acetone/water (70:30, v/v) was added to the residue. The mixture was vortexed for 1 min, left for extraction without shaking for 60 min at room temperature, and centrifuged at 6000 rpm for 15 min. Methanol and acetone extracts were combined to make up 100 ml with distilled water and used to determine antioxidant activity and total phenolic content.
Total phenolic content

The total phenolic content of patties was determined by the Folin-Ciocalteu method as described by Singleton, Orthofer, and Lamuela-Raventós (1999). Gallic acid was employed as a calibration standard, and results were expressed as mg of gallic acid equivalents (GAE)/100 g.

ABTS antioxidant activity

The antioxidant activity of the samples was measured using an ABTS (2,2-azinobis-3-ethylbenzthiazoline-6-sulfonic acid) procedure described by Re et al. (1999). The ABTS cation radical solution (ABTS +) was prepared using 5 mL of a 7.0 mM ABTS solution and 88 μL of a 145 mM potassium persulfate solution. The ABTS+ solution was diluted with 80% ethanol to an absorbance of 0.700 ± 0.005 at 734 nm. Twelve milliliters of diluted ABTS+ solution was added to 120 μL of sample extract and vigorously mixed in a Vortex. After 6 min, the absorbance at 734 nm was read using ethyl alcohol as blank. The calibration curve was constructed using ethanol solutions with known concentrations of Trolox (100–2000 μM Trolox/L), and the results were expressed as μM Trolox/100 g.

Thiobarbituric acid reactive substances (TBARS) value

TBARS of pork patties were determined using the extraction method described by Witte, Krause, and Bailey (1970) with minor changes. Briefly, the sample (5 g) was extracted in 12.5 ml of 20% trichloroacetic acid with vigorous stirring then transferred to a 25-mL volumetric flask and diluted up to the volume with cold distilled water. Five mL of extract was mixed with 5 mL of 0.02 M 2-thiobarbituric acid and heated at 100°C for 35 min. After cooling, the absorbance was recorded at 532 nm with a Varian Cary 50 UV spectrophotometer (Varian Co., USA). The calibration curve of 1,1,3,3-tetraethoxypropane (Sigma-Aldrich) standard solutions was used to determine the concentrations of TBA reactive substances in samples. TBARS values were expressed as mg of malondialdehyde (MDA)/kg of meat sample.

Instrumental color evaluation

Color changes during storage were monitored by measuring the L*, a* and b* values of the CIELab system using a PCE-CSM1 colorimeter calibrated against a white standard. The analysis was performed on three samples from each treatment with four readings on each sample. The hue angle (h) was calculated as arctan (-b*/a*) while chroma (C) was calculated as (a*² + b*²)¹/².

Sensory evaluation

Samples were evaluated initially as well as after 5, 10 and 15 days of refrigerated storage, in terms of appearance, color, flavor and overall acceptability. Initially, samples were cooled to room temperature, cut and served randomly to panelists. Sensory characteristics of the samples were evaluated using a 10-point hedonic scale where 1 = extremely dislike and 10 = extremely like. Water was served between samples for cleansing the palate. The panel consisted of 10–12 trained members from the University of Craiova staff and students of food science.

Statistical analysis

Three replications of the study were performed, and measurements of all parameters were taken in duplicate. The data were reported as mean ± standard deviation. Mean values for various parameters were calculated and compared by analysis of variance using the Statgraphics Centurion XVI software (StatPoint Technologies, VA, USA). Fisher LSD (least significant difference) test was applied for determining group differences at the 95% significance level.

Results and discussion

Proximate composition and cooking yield

The proximate composition and cooking yield of cooked pork patties are shown in Table 1.

| Treatment | Moisture (%) | Protein (%) | Fat (%) | Cooking yield (%) |
|-----------|--------------|-------------|---------|-------------------|
| C0        | 51.77 ± 0.59a | 22.14 ± 0.30a | 17.03 ± 0.22a | 78.83 ± 0.55a |
| C1        | 52.26 ± 0.38ab | 21.97 ± 0.46ab | 16.86 ± 0.38ab | 79.61 ± 0.84ab |
| P1        | 53.20 ± 0.47ab | 21.68 ± 0.22ab | 16.52 ± 0.34ab | 81.23 ± 0.46ab |
| P2        | 53.65 ± 0.63b  | 21.47 ± 0.35b  | 16.35 ± 0.28b  | 82.02 ± 0.71b  |

* Data represent mean ± standard deviation of three replicates. Different superscript letters indicate significant differences due to treatment (P < 0.05)
1 C0: control, meat without antioxidant; C1: meat + 0.1% BHT; P1: meat + 0.2% WLP; P2: meat + 0.5% WLP.
2 Los datos representan la media ± desviación estándar de tres repeticiones. Las diferentes letras en superíndice indican diferencias significativas debido al tratamiento (P < 0.05).
3 C0: control, carne sin antioxidante; C1: carne + 0.1% BHT; P1: carne + 0.2% WLP; P2: carne + 0.5% WLP.

Lipid oxidation

The efficacy of walnut leaf powder in retarding lipid oxidation of cooked pork patties was estimated by the thiobarbituric acid reaction substances (TBARS) assay which measures the amount of secondary products of lipid oxidation such as aldehydes, carbonyls, and hydrocarbons, reacting with thiobarbituric acid (Teets, Sundararaman, & Were, 2008). TBARS values (mg MDA/kg) of the pork patty samples during refrigerated storage for 15 days are shown in Table 2.

The results showed that lipid oxidation, as measured by TBARS values, was significantly delayed (P < .05) in pork patties during refrigerated storage by the addition of WLP compared to the control. According to Al-Kahtani et al.
meat products appropriate for consumption should present lipid oxidation values below 3 mg MDA/kg. In the present experiment, only the control without antioxidant (C0) had TBARS values above 3 mg MDA/kg, indicating the advanced oxidation of these samples.

After cooking, TBARS values in P1 (0.2% walnut leaf powder) were similar to those in C1 (0.1% BHT) and significantly lower than in C0. TBARS values increased with the prolongation of the storage period. After 15 days of refrigerated storage, TBARS values in samples with 0.5% WLP increased from 0.06 to 0.15 mg MDA/kg, which indicated only small oxidative changes. The high content of phenolic compounds in walnut leaf powder, namely phenolic acids, flavonoids, and naphthoquinones, may be responsible for its powerful antioxidant activity in cooked pork patties. Phenolic compounds, i.e. phenolic acids and flavonoids, act by donating electrons and reacting with free radicals to convert them to more stable products and terminate free chain reactions (Das et al., 2012).

The delay of lipid oxidation by plant powders and extracts has been shown in previous studies testing lotus and barley leaf powders in pork meat (Choe et al., 2011), *Moringa oleifera* leaf extracts in goat meat patties (Das et al., 2012), *Agave angustifolia* extracts (López-Romero, Ayala-Zavala, Peña-Ramos, Hernández, & Gonzáles-Rios, 2018) and *Cudrania tricuspidata* leaves extracts in pork patties (Cuong & Chin, 2018). Sesamol and olive leaf extracts were effective as natural functional ingredients in retarding lipid oxidation in fresh or heat-processed pork sausages kept at 4°C for 21 days (Hayes, Stepanyan, Allen, O’Grady, & Kerry, 2011), while Pateiro et al. (2018) have shown that guarana seed extracts delayed lipid oxidation in pork patties during refrigerated storage. Also, Albertos et al. (2017) showed that olive leaf powder is a valuable source of natural antioxidants and can be used to protect minced fish muscle from lipid oxidation.

**pH**

The pH variation in patties during refrigerated storage is shown in Table 2. After 15 days, the pH was recorded with the lowest values in the control sample (C0), while the highest values were found in samples with walnut leaf powder added. After 15 days of refrigerated storage, the pH level slightly decreased in C0 and C1 while no significant changes were recorded in P1 and P2. These results are in good agreement with those presented by Park and Jin (2007) who also found that the pH of pork patties containing Bokbunja (*Rubus coreanus*) extract was stable during 12 days refrigerated storage. Similar observations on the reduction in pH have recently been reported during storage of refrigerated chicken patties incorporated with moringa (*Moringa oleifera*) leaf powder (Elhadi, Elgasim, & Ahmed, 2017).

**Total phenolic content**

Walnut leaf powder used in this study had a total phenolic content of 2898.28 mg GAE/100 g dw and an ABTS antioxidant activity of 16.28 mmol Trolox/100 g dw.

The total phenolic content in pork patties containing WLP was significantly (*P < .05*) higher compared to the control and BHT samples (Table 2). Das et al. (2012) observed an increase in the phenolic content of goat meat patties with added *Moringa oleifera* leaves extract while Devatkal, Narsaiah, and Borah (2011) reported that kinnon and pomegranate by-product extracts significantly increased the total phenolic content of raw chicken patties during refrigerated storage.

### Table 2. TBARS values, pH, total phenolic content and ABTS antioxidant activity of the pork patties during refrigerated storage for 15 days.

| Treatment | 0 | 5 | 10 | 15 |
|-----------|---|---|----|----|
| TBARS values (mg MDA/kg) | | | | |
| C0        | 2.81 ± 0.26<sup>A</sup> | 3.02 ± 0.34<sup>B</sup> | 3.28 ± 0.29<sup>B</sup> | 3.37 ± 0.35<sup>B</sup> |
| C1        | 0.31 ± 0.04<sup>B</sup> | 1.36 ± 0.23<sup>C</sup> | 1.74 ± 0.22<sup>C</sup> | 2.06 ± 0.19<sup>C</sup> |
| P1        | 0.32 ± 0.06<sup>B</sup> | 0.82 ± 0.11<sup>B</sup> | 1.23 ± 0.16<sup>C</sup> | 1.34 ± 0.09<sup>C</sup> |
| P2        | 0.06 ± 0.02<sup>B</sup> | 0.07 ± 0.02<sup>B</sup> | 0.13 ± 0.02<sup>B</sup> | 0.15 ± 0.03<sup>B</sup> |
| pH        | 6.00 ± 0.03<sup>B</sup> | 6.04 ± 0.03<sup>B</sup> | 5.93 ± 0.06<sup>B</sup> | 5.85 ± 0.05<sup>B</sup> |
| C1        | 6.11 ± 0.03<sup>C</sup> | 6.04 ± 0.02<sup>B</sup> | 5.93 ± 0.05<sup>B</sup> | 5.97 ± 0.05<sup>B</sup> |
| P1        | 6.03 ± 0.07<sup>B</sup> | 6.05 ± 0.05<sup>B</sup> | 5.98 ± 0.03<sup>B</sup> | 6.06 ± 0.07<sup>B</sup> |
| P2        | 6.09 ± 0.06<sup>B</sup> | 6.03 ± 0.02<sup>B</sup> | 5.97 ± 0.05<sup>B</sup> | 6.05 ± 0.04<sup>B</sup> |

Total phenolic content (mg GAE/100 g)

| Treatment | 0 | 5 | 10 | 15 |
|-----------|---|---|----|----|
| C0        | 7.35 ± 0.32<sup>Ca</sup> | 6.53 ± 0.40<sup>Bd</sup> | 5.68 ± 0.33<sup>Ca</sup> | 5.42 ± 0.18<sup>Ca</sup> |
| C1        | 6.85 ± 0.21<sup>Ca</sup> | 5.87 ± 0.35<sup>Ca</sup> | 5.17 ± 0.29<sup>Ca</sup> | 4.95 ± 0.25<sup>Ca</sup> |
| P1        | 9.00 ± 0.38<sup>Bd</sup> | 8.18 ± 0.26<sup>B</sup> | 8.00 ± 0.34<sup>B</sup> | 7.57 ± 0.44<sup>B</sup> |
| P2        | 12.27 ± 0.56<sup>Bd</sup> | 13.07 ± 0.66<sup>B</sup> | 13.22 ± 0.69<sup>B</sup> | 12.68 ± 0.57<sup>B</sup> |

ABTS antioxidant activity (mmol Trolox/100 g)

| Treatment | 0 | 5 | 10 | 15 |
|-----------|---|---|----|----|
| C0        | 0.49 ± 0.03<sup>A</sup> | 0.49 ± 0.02<sup>A</sup> | 0.49 ± 0.02<sup>A</sup> | 0.48 ± 0.02<sup>A</sup> |
| C1        | 0.69 ± 0.03<sup>B</sup> | 0.59 ± 0.03<sup>B</sup> | 0.56 ± 0.02<sup>B</sup> | 0.55 ± 0.02<sup>B</sup> |
| P1        | 0.62 ± 0.02<sup>B</sup> | 0.62 ± 0.03<sup>B</sup> | 0.61 ± 0.02<sup>B</sup> | 0.60 ± 0.02<sup>B</sup> |
| P2        | 0.72 ± 0.03<sup>B</sup> | 0.69 ± 0.02<sup>B</sup> | 0.75 ± 0.03<sup>B</sup> | 0.70 ± 0.03<sup>B</sup> |

* Data represent mean ± standard deviation of three replicates. Different lowercase letters indicate significant differences due to treatment (*P < 0.05*), while different uppercase letters are indicative of significant differences due to storage period (*P < 0.05*).
1 C0: control, meat without antioxidant; C1: meat + 0.1% BHT; P1: meat + 0.2% WLP; P2: meat + 0.5% WLP.

Los datos representan la media ± desviación estándar de tres repeticiones. Las letras minúsculas diferentes indican diferencias significativas debido al tratamiento (*P < 0.05*), mientras que las letras mayúsculas diferentes señalan diferencias significativas debido al periodo de almacenamiento (*P < 0.05*).
1 C0: control, carne sin antioxidante; C1: carne + 0.1% BHT; P1: carne + 0.2% WLP; P2: carne + 0.5% WLP.
storage. The total phenolic content significantly decreased during storage for 15 days in control and BHT added samples but didn’t show significant variations in samples with 0.5% walnut leaf powder.

**ABTS free radical scavenging activity**

Addition of WLP resulted in a significant increase in the antioxidant activity relative to the control (Table 2). Numerous previous studies reported similar results at the addition of antioxidant plant extracts or powders to meat and meat products (El-Gharably & Ashhour, 2011; Huang et al., 2011). The increase of WLP level from 0.2% to 0.5% determined a significant increase of the antioxidant activity. The addition of 0.5% WLP resulted in a higher increase of the antioxidant activity than the 0.1% BHT addition, but the differences between them were not significant.

The antioxidant activity of BHT-added samples significantly decreased (*P < 0.05*) during storage while that of the samples with walnut leaf powder was not significantly affected.

**Color**

Color parameters L* (lightness), a* (redness) and b* (yellowness), as well as the hue and saturation of samples and their variation during refrigerated storage for 15 days, are presented in Table 3. Initially, the mean values of lightness (L*) were significantly higher in control samples (C0 and C1) compared to P1 and P2, therefore the addition of the dark brown walnut leaf powder resulted in the darkening of patties. At this time there were no significant differences in the lightness of C0 and C1. The lightness decrease was also reported by other authors as a result of the addition of antioxidant plant powders to chicken meat patties (Devatkal et al., 2011; Naveena et al., 2008) or of vegetal antioxidant extracts to raw pork or ground chicken meat (Brannan, 2009; Qin et al., 2013).

The redness (a*) values were significantly lower in samples with WLP compared to control samples (C0 and C1), which is due to the green color of the leaf powder. Moreover, the a* values were significantly lower in P2 relative to P1 as a result of the higher walnut leaf powder content. Lower a* values compared to the control in samples prepared with the addition of powders from lotus or barley leaves were also reported by Choe et al. (2011), and they were attributed to the green color of the leaf powder. The decrease of the parameter a* was attributed by other authors to the reduction in the red color of the meat as a result of the addition of plant extracts containing greenish-brownish color pigments as antioxidants in patty samples (Erl, Kaya, & Simşek, 2018). Other authors explained the reduction of parameter a* by the interdependence between lipid oxidation and meat color oxidation (Lynch & Faustman, 2000). After cooking, there were no significant differences in yellowness (b*) and chroma (C) between samples. However, other authors reported decreases in yellowness at the addition of antioxidant extracts from grape seeds and skins to ground chicken meat (Brannan, 2009; Selani et al., 2011).

During refrigerated storage, there was a slight increase in the lightness (L*) of the control samples (C0 and C1), while in the samples with WLP addition L* values decreased, even more so in P2 samples (0.5% WLP). Previous studies have shown that the discoloration of meat is determined by oxidative processes and reducing enzyme systems (Faustman & Cassens, 1990). As a result, it can be assumed that in the control, the more intense oxidative processes caused the observed discoloration of the samples. Also, the a* values (red component) recorded constant decreases over the storage period in all samples. Declines

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**Table 3.** Color parameters of the pork patties samples during refrigerated storage for 15 days.

| Color parameters | Treatment | 0 | 5 | 10 | 15 |
|------------------|-----------|---|---|----|----|
| L*               | C0        | 76.00 ± 1.39<sup>abc</sup> | 75.85 ± 1.18<sup>abc</sup> | 74.04 ± 0.39<sup>bc</sup> | 76.73 ± 2.00<sup>bc</sup> |
|                  | C1        | 75.04 ± 1.08<sup>abc</sup> | 75.67 ± 0.97<sup>abc</sup> | 73.26 ± 2.03<sup>bc</sup> | 75.18 ± 0.64<sup>bc</sup> |
|                  | P1        | 74.18 ± 1.40<sup>abc</sup> | 73.93 ± 1.33<sup>abc</sup> | 70.25 ± 0.98<sup>bc</sup> | 69.80 ± 2.44<sup>abc</sup> |
|                  | P2        | 72.23 ± 1.86<sup>ab</sup>  | 70.60 ± 1.17<sup>ab</sup>  | 65.27 ± 1.68<sup>ab</sup> | 65.51 ± 0.37<sup>ab</sup> |
| a*               | C0        | 5.32 ± 0.19<sup>ab</sup>  | 4.49 ± 0.15<sup>ab</sup>  | 4.53 ± 0.28<sup>ab</sup> | 3.97 ± 0.04<sup>ab</sup>  |
|                  | C1        | 6.82 ± 0.22<sup>ab</sup>  | 6.16 ± 0.09<sup>ab</sup>  | 5.82 ± 0.05<sup>ab</sup> | 5.40 ± 0.10<sup>ab</sup>  |
|                  | P1        | 5.26 ± 0.46<sup>ab</sup>  | 4.54 ± 0.44<sup>ab</sup>  | 4.75 ± 0.39<sup>ab</sup> | 4.16 ± 0.30<sup>ab</sup>  |
|                  | P2        | 4.25 ± 0.24<sup>ab</sup>  | 3.85 ± 0.37<sup>ab</sup>  | 3.88 ± 0.24<sup>ab</sup> | 3.59 ± 0.07<sup>ab</sup>  |
| b*               | C0        | 13.10 ± 0.35<sup>abc</sup>| 13.84 ± 0.14<sup>ab</sup>| 14.27 ± 0.41<sup>bc</sup>| 13.96 ± 0.28<sup>ab</sup>|  |
|                  | C1        | 12.87 ± 0.33<sup>abc</sup>| 13.42 ± 0.20<sup>ab</sup>| 13.37 ± 0.11<sup>ab</sup>| 13.21 ± 0.16<sup>ab</sup>|  |
|                  | P1        | 13.19 ± 0.31<sup>abc</sup>| 12.11 ± 0.30<sup>abc</sup>| 12.64 ± 0.62<sup>ab</sup>| 12.25 ± 0.57<sup>bc</sup>|  |
|                  | P2        | 13.27 ± 0.52<sup>abc</sup>| 12.21 ± 0.54<sup>abc</sup>| 12.24 ± 0.60<sup>ab</sup>| 12.06 ± 0.05<sup>ab</sup>|  |
| C                | C0        | 14.14 ± 0.39<sup>abc</sup>| 14.55 ± 0.13<sup>abc</sup>| 14.98 ± 0.47<sup>abc</sup>| 14.51 ± 0.28<sup>bc</sup> |  |
|                  | C1        | 14.57 ± 0.58<sup>abc</sup>| 14.76 ± 0.21<sup>ab</sup> | 14.60 ± 0.07<sup>ab</sup> | 14.27 ± 0.18<sup>ab</sup> |  |
|                  | P1        | 14.20 ± 0.46<sup>abc</sup>| 12.93 ± 0.42<sup>abc</sup>| 13.50 ± 0.71<sup>abc</sup> | 12.94 ± 0.62<sup>ab</sup> |  |
|                  | P2        | 13.93 ± 0.57<sup>abc</sup>| 12.81 ± 0.62<sup>abc</sup>| 12.84 ± 0.69<sup>ab</sup>  | 12.56 ± 0.03<sup>ab</sup> |  |
| h                | C0        | 67.90 ± 0.20<sup>ab</sup> | 72.03 ± 0.64<sup>ab</sup> | 72.33 ± 0.71<sup>bc</sup> | 74.14 ± 0.15<sup>c</sup> |  |
|                  | C1        | 62.07 ± 0.25<sup>ab</sup> | 65.35 ± 0.21<sup>ab</sup> | 66.53 ± 0.11<sup>bc</sup> | 67.75 ± 0.16<sup>ab</sup> |  |
|                  | P1        | 68.28 ± 1.27<sup>ab</sup> | 69.47 ± 1.45<sup>ab</sup> | 69.42 ± 0.77<sup>ab</sup> | 71.25 ± 0.72<sup>ab</sup> |  |
|                  | P2        | 72.25 ± 0.30<sup>ab</sup> | 72.56 ± 0.94<sup>ab</sup> | 72.44 ± 0.53<sup>ab</sup> | 73.87 ± 0.37<sup>ab</sup> |  |

* Data represent mean ± standard deviation of three replicates. Different lowercase letters indicate significant differences due to treatment (*P < 0.05*), while different uppercase letters are indicative of significant differences due to storage period (*P < 0.05*).

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* Los datos representan la media ± desviación estándar de tres repeticiones. Las letras minúsculas diferentes indican diferencias significativas debido al tratamiento (*P < 0.05*), mientras que las letras mayúsculas diferentes señalan diferencias significativas debido al periodo de almacenamiento (*P < 0.05*).

1 C0: control, carne sin antioxidante; C1: carne + 0.1% BHT; P1: carne + 0.2% WLP; P2: carne + 0.5% WLP.
Figure 1. Appearance, color, flavor and overall acceptability scores of pork patties during refrigerated storage for 15 days. Different lowercase letters indicate significant differences due to treatment ($P < .05$), while different uppercase letters are indicative of significant differences due to storage period ($P < 0.05$).

Figura 1. Puntuaciones de apariencia, color, sabor y aceptabilidad general de los medallones de cerdo durante los 15 días de almacenamiento refrigerado. Las letras minúsculas diferentes indican diferencias significativas debido al tratamiento ($P < .05$), mientras que las letras mayúsculas diferentes señalan diferencias significativas debido al periodo de almacenamiento ($P < 0.05$).
of the a* values during storage were previously reported in cooked pork patties or minced meat with and without added antioxidants (Choe et al., 2011; Park, Hur, Song, & Park, 2007; Qin et al., 2013). Decrease in a* value may also indicate the change in color from red to brown, which may be due to the formation of metmyoglobin in the salt-containing samples (Devatkal et al., 2011). The decrement rate of the parameter a* was lower in the samples with 0.5% WLP compared to the control, which may be an indicator of the antioxidant activity of walnut leaves and of its effective role as a food antioxidant.

The yellowness (b*) decreased during storage in the samples with walnut leaf powder but increased in the control, while the hue values increased during storage in all samples. Such increases in hue angle during refrigerated storage were previously reported by Devatkal et al. (2011) in raw chicken patties while Qin et al. (2013) also reported decreases in color parameter b* during the 12-day storage of pork meat with and without the addition of antioxidant plant extracts.

Sensory analysis

The results of the sensory evaluation of pork patties are shown in Figure 1. The addition of walnut leaf powder had positive effects on the appearance and flavor both after cooking and during refrigerated storage. The increased storage led to a decrease in overall acceptability of pork patties with or without the addition of walnut leaf powder. However, samples with WLP at 0.5% showed higher scores of overall acceptability and flavor compared to the control throughout the storage period, proving that the walnut powder had the potential to reduce lipid peroxidation and to prolong the shelf life of cooked pork patties.

The acceptability of meat products containing plant extracts or plant powders with high phytochemical content is of great importance in the development of functional meat products (Hayes et al., 2011). The addition of walnut leaf powder determined positive changes in appearance, flavor and overall acceptability even with respect to the BHT-added samples. The control samples without antioxidant showed the most significant deterioration of flavor during storage, indicating the production of unpleasant flavors due to the lipid oxidation process. In some previous studies, the addition of plant extracts, such as green tea or pistachio seed hull extracts, did not have a significant effect on the sensory attributes of meat and meat products (Al-Juhaimei et al., 2017; Siripatrawan & Noipha, 2012), suggesting that natural antioxidant extracts and powders were considered acceptable by the panelists.

Conclusions

Walnut leaf powder incorporated at 0.5% in cooked pork patties was more effective than 0.1% BHT in reducing the lipid oxidation – effect that may be associated with the high antioxidant activity of WLP and the presence of phenolic compounds in its composition. The addition of WLP resulted in increased moisture retention and enhanced quality parameters. WLP-containing samples were found to present higher scores for appearance, color, flavor, and overall acceptability as well as a higher color stability during storage compared to control. These results indicate that walnut leaf powder has the potential to be used as a natural antioxidant to minimize oxidative problems in ground pork products. Additional research will be required to evaluate the effect of walnut leaf powder on microbial spoilage and the shelf life of meat products.

Disclosure statement

No potential conflict of interest was reported by the authors.

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