Research Note

Peanut skin extract reduces lipid oxidation in cooked chicken patties

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ABSTRACT The objectives of this study were to evaluate the antioxidant capacity of peanut skin extract and its effect on the color and lipid oxidation of cooked chicken patties over 15 d of refrigerated storage. The extract was obtained using 80% ethanol and evaluated in terms of total phenolic content, reducing power based on the ferric reducing ability of plasma (FRAP) reagent, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. The patties were made with ground thigh fillets, chicken skin, and 2% salt. They were homogenized and divided into the following two groups: a control treatment without antioxidants and a peanut skin treatment with 70 mg gallic acid equivalent (GAE)/kg per patty. Analyses of the fatty acid profiles, instrumental colors (L^*, a^*, and b^*) and thiobarbituric acid reactive substances (TBARS) were performed on d 1, 8, and 15 of storage at 1 ± 1°C. The peanut skin extract resulted in a phenolic content of 32.6 ± 0.7 mg GAE/g dry skin, an antioxidant activity (FRAP) of 26.5 ± 0.8 6 μmol Trolox equivalent/g dry skin, and an efficient concentration (EC_{50}) of 46.5 μg/mL. The total unsaturated fatty acid was approximately 73%, and 39% of this fatty acid content was monounsaturated. The peanut skin extract slowed the decrease in the a^* values ($P < 0.05$) but reduced the L^* and b^* values compared to the control samples during storage ($P < 0.05$). Lipid oxidation was minimized by the peanut skin extract ($P < 0.05$), which resulted in a maximum value of 0.97 malondialdehyde (MDA)/kg compared to values that were close 19 mg MDA/kg patties in the control sample at the end of storage period. Thus, it can be concluded that although peanut skin extract causes little color change, it can be applied as a natural antioxidant to cooked chicken patties because it efficiently inhibits lipid oxidation in this product during refrigerated storage.

Key words: antioxidant, storage, phenolic compound, flavonoid, fatty acid

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INTRODUCTION

Lipid oxidation is a major cause of quality loss in meat products that results in changes in flavor, color and shelf life. To slow the effects of lipid oxidation, synthetic antioxidants are used, which has caused concern regarding the safety and toxicity of these compounds. Phenolic compounds are naturally found in plants and have been presented as an alternative that can partially or completely replace synthetic antioxidants in foods (Naveena et al., 2008; Liu et al., 2010).

Peanut skin (*Arachis hypogaea L.*) is discarded or used in animal feed because it has no relevant commercial value. However, antioxidant compounds such as phenolic acids, flavan-3ols, stilbenes, and their polymers are present peanut skin and exhibit anti-inflammatory and antimelanogenic activities (Hill, 2002; Ma et al., 2014; Tatsuno et al., 2012).

The antioxidant potentials of peanut skin extracts have been tested on ground beef, and these extracts have been shown to potentially retard lipid and pigment oxidation and to exhibit effects similar to those of synthetic antioxidants such as butylated hydroxyanisole (**BHA**) and butylated hydroxytoluol (**BHT**) and at lower concentrations (200 ppm or higher). In addition to its low effective concentration, the peanut skin extract does not lose its antioxidant capacity in the presence of other ingredients that characterize cured and emulsified products, which permits its application in other meat products (Yu et al., 2010; O'Keefe and Wang, 2006).

Due to the interest in antioxidants from natural and residual sources and the lack of information about peanut skin extract in ground chicken meat, this study

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aimed to evaluate the antioxidant effects of an ethanolic extract of peanut skin in cooked chicken patties during refrigerated storage.

MATERIALS AND METHODS

Extraction of Antioxidant Compounds from Peanut Skin

Peanut skin (variety Runner IAC886) was generously donated by Coplana – Industrial Cooperativa of peanuts. Prior to extraction, the kernel particles were removed and discarded, and the skins were kept under frozen storage. Extraction was performed according to the method of Infante et al. (2013) with modifications. Peanut skin (30 g) and 300 mL 80% ethanol were left in a water bath at 60°C for 50 min. Subsequently, the mixture was sonicated for 15 min at room temperature. The extract was centrifuged (5430R, Eppendorf, Hamburg, Germany) at 4,226 g for 15 min at 25°C and filtered through Whatman filter (Whatman Int., Maidstone, England) No. 3 paper. The extract was concentrated until it reached 20% of its initial volume (60 mL) at 55°C and −600 kPa. The extract was prepared daily for analyses and treatments.

Total Phenolic Content

The phenolic content of the peanut skin extract was determined according to the method of Georgé et al. (2005) with modification. Three hundred microliters of extract that was properly diluted in a test tube was mixed with 1,500 μL Folin-Ciocalteau reagent (Sigma-Aldrich Chemical Co., Bellefonte, PA) and left to rest for 2 min. Next, 1,200 μL sodium carbonate 7.5% solution was added to test tube, which was then left in a water bath at 50°C for 15 min. The reaction was cooled with tap water and read at 760 nm. The results were calculated based on 3 measures and are expressed as milligram gallic acid equivalent (**GAE**) (Vetec Química Fina Ltda, Rio de Janeiro, Brazil) per gram of dry peanut skin.

1,1-Diphenyl-2-picrylhydrazyl Radical Scavenging Capacity

The radical scavenging capacity was assessed according to the methods of Brand-Williams et al. (1995) with modifications. First, 3,150 μL 72 μM 1,1-Diphenyl-2-picrylhydrazyl (**DPPH**; Sigma-Aldrich Chemical Co., Bellefonte, PA) methanolic solution was mixed with extract volumes ranging from 0 to 150 μL for final volumes of 3,500 μL in test tubes. The reactions were left in the dark for 30 min and read at 515 nm. The relative free radical scavenging activity was calculated based on the absorbances of the control ($A_{515,Control}$) and the sample ($A_{515.Sample}$) using the following equation: scavenging activity (%) = $100 - (A_{515.Sample}/A_{515.Control}) \times 100$. The extract concentrations and their respective scavenging activities were plotted to express the results of 3 measures and are expressed as the efficient concentration ($\mathbf{EC_{50}}$), which is the amount of sample required to consume 50% of the initial DPPH concentration.

Table 1. Chicken patty formulations.

Ingredients	Control (g/100 g)	Peanut skin (g/100 g)
Chicken thigh	75.00	75.00
Chicken skin	20.00	20.00
Salt	2.00	2.00
Water	3.00	–
Peanut skin extract	–	3.00

Reducing Power

The antioxidant activity was assessed with the ferric reducing ability of plasma (**FRAP**) assay according to the method of Benzie and Strain (1996) with modifications. The FRAP reagent was prepared with 300 mM (pH 3.6) sodium acetate (Sigma-Aldrich Chemical Co., Bellefonte, PA) buffer solution, 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (**TPTZ**; Sigma-Aldrich Chemical Co., Bellefonte, PA) solution (dissolved in 40 mM HCl), and 20 mM ferric chlorate (Sigma-Aldrich Chemical Co., Bellefonte, PA) solution at proportions of 10:1:1, respectively. Next, 3,400 μL FRAP reagent was mixed with 100 μL properly diluted extract, left in a water bath at 37°C for 30 min, cooled in tap water, and read at an absorbance at 593 nm. The results were calculated based on 3 measures and are expressed as μmol Trolox equivalents (Sigma-Aldrich Chemical Co., Bellefonte, PA) per gram of dry peanut skin.

Preparation of the Chicken Patty Samples

Refrigerated chicken thighs with skin were purchased from a local butcher and ground in a 3 mm disc at 5°C. The ground meat was divided into 2 treatments (Table 1): the control treatment included water instead of the antioxidant extract, and the peanut skin treatment included 70 mg GAE extract/kg burger. Immediately after mincing, all ingredients for each treatment were hand-mixed, and 100 g portions were shaped into patty forms. The samples were cooked in an oven at 150°C until the internal temperature reached 80°C. The cooked samples were left to cool to room temperature, individually packaged in low density polyethylene pouches, and stored at 1 ± 1°C for 15 d.

Proximate Composition of the Chicken Patties

The proximate compositions were assessed in triplicate, and the moisture (method 950.46), protein (method 992.15), and ash (method 920.153) contents were determined according to the Association of Official Analytical Chemists methodology (AOAC, 2005). A high temperature lipid extraction system (ANKON XT10 Extractor, Macedon, NY) was used to the estimate lipid contents.

Instrumental Color Evaluation

The color parameters of the chicken patties were measured using a portable colorimeter (MiniScan XE HunterLab, Reston, VA) with a CIE-L*a*b* evaluation system for L* (brightness), a* (from the red to green range), and b* (from the blue to yellow range) values. A D65 illuminant was used with an observation angle of 10° and a cell opening of 30 mm. The average of 6 measures was obtained from each sample after exposure for 30 min at room temperature.

Measurement of Lipid Oxidation

Lipid oxidation was determined according to the method of Vynche (1970) with modifications. The sample (5 g) was homogenized with 25 mL 7.5% trichloroacetic acid solution (Synth, São Paulo, Brazil) containing 0.1% EDTA and 0.1% propyl gallate (Synth, São Paulo, Brazil) in Ultra-Turrax (Tecnal TE-102, Piracicaba, São Paulo, Brazil) for 2 min and filtered through Whatman No. 1 filter paper. A volume of 5 mL supernatant was mixed with 5 mL 0.02 M thiobarbituric acid solution in a test tube and heated to 98°C for 40 min and then cooled in tap water. The absorbance was measured at 532 nm. The results were calculated based on 3 measures of each sample and are expressed as milligrams of malondialdehyde (**MDA**) per kilogram of meat.

Fatty Acid Profiles of the Chicken Patties

After extraction from the cooked chicken patties according to the methods of Bligh and Dyer (1959), the fat was submitted to fatty acid methyl ester (**FAME**) gas chromatography to determine the fatty acid composition according to the official American Oil Chemists' Society methods Ce 2-66 and Ce 1-62 (AOCS, 1998). Under the following experimental conditions, the gas chromatography (GC) analyses were performed on a gas chromatograph (Shimadzu 2010 AF, Tokyo, Japan) with an automatic injector (Shimadzu AOC 20i, Tokyo, Japan) and a flame ionization detector: a nonbonded poly(biscyanopropyl siloxane) phase 0.20 μm, 100 m × 0.25 mm i.d. capillary column (Supelco SP-2560, Bellefonte, PA, USA); a helium carrier gas at a rate of 1.51 mL/min; an injection temperature of 250°C; a column temperature of 160°C that was increased to 245°C (at a rate of 3°C/min) and held at 245°C for 15 min; a detection temperature of 280°C; and an injection volume of 1.0 μL. The FAMEs were compared with external standards (Supelco, Bellefonte, PA). Quantification was based on the ratios of the area of each fatty acid to the area of the methyl tridecanoate C13:0 (Sigma-Aldrich, Bellefonte, PA) internal standard using the response correction factors of the flame ionization detector and the conversion of methyl esters of fatty acids to fatty acids (Aued-Pimentel et al., 2005).

Statistical Analyses

A complete randomized 2 × 3 factorial design with 2 treatments and 3 storage times was used to evaluate the effects of peanut skin extract on the color and lipid oxidation of the chicken patties. Duplicate treatments were evaluated at 1, 8, and 15 d of storage. The entire experiment was replicated twice. The results were analyzed with ANOVA in MINITAB (Minitab Inc.) version 16.2.1. The averages were compared between the treatments and storage times with Tukey tests at the 5% significance level.

RESULTS AND DISCUSSION

Phenolic Content and Antioxidant Activity of Peanut Extract

The phenolic content in the peanut skin extract was 32.6 ± 0.7 mg GAE/g of skin. The phenolic content of peanut skin extracts varies widely. Yu et al. (2006) studied the effect of skin removal by different technologies and observed values that ranged from 8.8 mg GAE/g dry skin for peeling after blanching to 101.5 mg GAE/g dry skin for peeling after roasting. Francisco and Resurreccion (2009) evaluated runner, Virginia, and Spanish peanut varieties and observed significant differences in the phenolic contents of the extracts (95.56 to 280.42 mg GAE/g dry skin).

The radical scavenging capacity of peanut skin extract had an EC_{50} of 46.5 μg/mL, which indicates a low effective concentration (i.e., high free radical scavenging activity). Radical scavenging is the major antioxidant mechanism of polyphenols (Yu et al., 2006). The results of the present study are concordant with those of Wang et al. (2007), who obtained an EC_{50} value of 30.8 μg/mL.

The reducing power, as determined with the FRAP assay, was 26.56 ± 0.8 μmol Trolox equivalent/g dry skin. Although the FRAP assay is a relatively new and underutilized methodology for antioxidant evaluation, it allowed for the assessment of the reducing power of peanut skin extract at low pH because the reaction occurred in an acid buffer solution. This reaction condition provided an additional result because antioxidant evaluation requires more than one methodology. The FRAP assay requires simple equipment and materials and is inexpensive, fast, accurate, and repeatable; however, it cannot be used to quantify the antioxidant activities of lipophilic compounds (Pérez-Jiménez et al., 2008).

Despite the relatively low phenolic content found in the present study compared to those reported by other authors, the observed phenolic content was indicative of the presence of antioxidant compounds in the skins discarded from peanut kernel processing. According to Ma et al. (2014), diverse antioxidant compounds are found in peanut skin, including phenolic acids (hydroxybenzoic acids, hydroxycinnamic acids, and their esters),

flavonoids (epicatechin, catechin, and their polymers), other flavonoids (isoflavones, flavanols, and flavones) and stilbene (resveratrol and trans-piceatannol). Large quantities of diverse phenolic compounds with radical scavenging and chelating activities are present in peanut skins, which might indicate their applicability to meat products (Milani et al., 2010; Wang et al., 2007; Yu et al., 2010).

Proximate Composition, Storage Stability, and Fatty Acid Profile

The patty compositions did not significantly differ according to treatment ($P > 0.05$). The mean moisture, protein, lipid, and ash compositions were 65.03 ± 0.23, 18.62 ± 0.10, 12.79 ± 0.38, and 3.03 ± 0.12%, respectively (data not shown). These results are in agreement with those of Naveena et al. (2006) but lower than those of Talukder and Sharma (2010).

The addition of the peanut skin extract to the chicken patties caused a slight darkening that resulted in lower L^* values compared to the control samples over the storage time ($P < 0.05$; Table 2). Throughout the 15 d storage time, no variation in the L^* values of the peanut skin treatment were observed. Variations in L^* values seem to depend on phenolic concentrations. O'Keefe and Wang (2006) observed significant reductions in L^* values with increases in phenolic content at concentrations above 400 ppm in cooked ground beef, but the same trend was not found by Yu et al. (2010) for peanut skin extract concentrations up to 0.1% in the same meat product.

Loss in redness was observed in both treatments, but the addition of peanut skin extract caused a significant reduction of the loss of a^* value ($P < 0.05$). Yu et al. (2010) found that peanut skin concentrations of 0.04% or higher can slow the loss in redness. According to these authors, the preservation of redness or at least slowing the loss of redness is positively related to the visual perception of quality. The yellowness remained stable after 15 d of storage in both treatments ($P > 0.05$). However, the addition of peanut skin extract resulted in significantly lower values than those observed in the control samples ($P < 0.05$).

Table 2. Effects of peanut skin extract on the colors and TBARS indices of the chicken patties during refrigerated storage.

Attribute	Control	Peanut skin
L^*		
D 1	$61.73 \pm 1.71^{b,A}$	$57.18 \pm 0.74^{a,B}$
D 8	$65.18 \pm 0.53^{a,b,A}$	$57.88 \pm 3.29^{a,B}$
D 15	$68.66 \pm 0.20^{a,A}$	$57.15 \pm 3.81^{a,B}$
a^*		
D 1	$5.68 \pm 0.39^{a,A}$	$5.65 \pm 0.24^{a,A}$
D 8	$3.00 \pm 0.13^{b,B}$	$4.50 \pm 0.49^{b,A}$
D 15	$2.90 \pm 0.26^{b,B}$	$4.90 \pm 0.50^{b,A}$
b^*		
D 1	$20.51 \pm 0.28^{a,A}$	$17.39 \pm 0.25^{a,B}$
D 8	$20.69 \pm 1.17^{a,A}$	$16.58 \pm 0.60^{a,B}$
D 15	$20.93 \pm 0.35^{a,A}$	$16.88 \pm 0.46^{a,B}$
TBARS (mg MDA equivalent/kg)		
D 1	$4.23 \pm 0.14^{b,A}$	$0.85 \pm 0.06^{a,b,B}$
D 8	$19.03 \pm 0.19^{a,A}$	$0.97 \pm 0.02^{a,B}$
D 15	$18.35 \pm 1.63^{a,A}$	$0.76 \pm 0.01^{b,B}$

Means ± standard deviations based on triplicate samples.

[a,b]The storage periods within the same treatment (column) with different superscripts are significantly different ($P < 0.05$).

[A,B]The treatments within the same storage period (row) with different superscripts are significantly different ($P < 0.05$).

Lipid oxidation was significantly reduced by the addition of peanut skin extract throughout the storage period ($P < 0.05$). The control treatment samples exhibited a maximum oxidation level of 19.03 ± 0.19 mg malondialdehyde (MDA) equivalent/kg after 8 d. In the same period, the peanut skin treatment samples exhibited an oxidation level of 0.97 mg MDA equivalent/kg. Additionally, the peanut skin treatment samples exhibited thiobarbituric acid reactive substances (**TBARS**) values under the sensory threshold of 0.5 to 1 mg MDA equivalent/kg (Tarladgis et al., 1960). A similar antioxidant effect was observed by O'Keefe and Wang (2006) after 14 d in ground beef for peanut skin extract concentrations above 200 ppm; these concentrations were found to reduce lipid oxidation from more than 10 mg MDA equivalent/kg to between 4 and 6 mg MDA equivalent/kg. Low TBARS values are desirable, particularly in chicken meat, and prevent the deterioration of cooked meat (Avila-Ramos et al., 2013). With the same purpose of inhibiting lipid oxidation with a natural extract, Naveena et al. (2013) studied the effects of rosemary leaf extract on cooked chicken patties. Treatments with 130 ppm of carnosic acid from the rosemary extract resulted in 0.2 mg MDA equivalent/kg compared to 1.0 mg MDA equivalent/kg in the control treatment after 28 d of storage at 4°C.

The difference found in the TBARS values between the treatments in the beginning of the storage period was due to the patty cooking process. Heat is responsible for the disruption of muscle cell structure, the inactivation of antioxidant enzymes, and the release of iron from myoglobin, which catalyzes lipid oxidation. In contrast, the phenolic compounds from the peanut skin might, in addition to radical scavenging activity, exhibit metal chelating activity that results in the formation of stable complexes with heme and nonheme ions that inhibit lipid oxidation and the loss of color in meat products (Min et al., 2010; Yu et al., 2010).

Table 3 presents the fatty acid profiles of the chicken patties. The main fatty acids were palmitic acid followed by oleic and linoleic acids. Polyunsaturated fatty acids represented approximately 34% of the total fatty acid content, and the total unsaturated content represented 74% of the fatty acid content. Saturated fatty acids accounted for 26% the fat in the chicken patties, which represents a nutritional disadvantage due the hypercholesterolemic effect. Despite the high level of fat oxidation (Table 2), unsaturated fatty acid was present in an elevated proportion. The fatty acid profile results agree with those of Feddern et al. (2010) and Hautrive et al. (2012) in terms of saturated, monounsaturated, and polyunsaturated fatty acid compositions.

Table 3. Fatty acid composition (%) of the chicken patties.

Fatty acid	Common name	Control	Peanut skin
C14:0	Myristic acid	0.97	0.84
C14:1	Myristoleic acid	–	0.24
C16:0	Palmitic acid	20.96	20.23
C16:1	Palmitoleic acid	5.84	5.18
C18:0	Stearic acid	4.46	4.77
C18:1	Oleic acid	33.19	33.55
C18:2	Linoleic acid	31.59	31.82
C18:3	Linolenic acid	3.00	2.94
C23:0	Tricosanoic acid	–	0.44
Σ Saturates		26.39	25.96
Σ Monounsaturates		39.03	39.38
Σ Polyunsaturates		34.59	34.90

Means of duplicate samples (SD < 3%)

In conclusion, peanut skin extract possesses a substantial amount of phenolic compounds with antioxidant activities in vitro. Although it caused a slight darkening of the samples, the application of peanut skin extract to the cooked chicken patties at a level of 70 mg GAE/kg significantly inhibited lipid oxidation and preserved redness. The meat industry could use peanut skins as a natural source of phenolic compounds that act as antioxidants to inhibit lipid and myoglobin oxidation.

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