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Chitosan active films containing agro-industrial residue extracts for shelf life extension of chicken restructured product

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Abstract

This study aimed to develop chitosan films incorporating natural antioxidants from peanut skin (EPS) and pink pepper residue (EPP) extracts, as well as to evaluate their effects on lipid oxidation, pH, color, and microbial counts of a restructured chicken product. EPS had higher phenolic content and antioxidant activity compared to EPP. When both extracts were applied to chicken meat and the chitosan films, there were no differences for color, pH and total mesophilic counts compared to control at the end of the storage period. For lipid oxidation (peroxide value and thiobarbituric acid reactive substances), both extracts proved to be as effective as butylated hydroxytoluene to maintain the oxidative stability of the chicken product. The microbial counts of psychrotrophic microorganisms were significantly lower for treatments with active films. Chitosan active films with residue extracts may maintain the quality of chicken products due to their antioxidant and antimicrobial potential.

Keywords: Active packaging; Peanut skin; Pink pepper; Antimicrobial activity; Antioxidant activity.
1 Introduction

Currently, chicken meat has been highlighted by its great consumption (Sivarajan et al., 2017) and the main factors for this scenario are its nutritional quality and low-cost (Demirhan & Candoğan, 2017). However, this meat has some preservation issues, especially regarding lipid oxidation, which affects its quality, shelf life and sensory acceptability (Selani et al., 2011). As a consequence, sensory alterations, loss of nutritional quality, and the production of potentially toxic compounds are observed (Addis, 1986).

The industry of meat products uses synthetic antioxidants to prevent lipid oxidation. However, the use of these additives has raised concerns about their safety doses due to some possible health risks related to their consumption. This fact has resulted in greater caution in their use, thus increasing the need to replace them with other compounds (Fernandes, Trindade, Lorenzo, Munekata, & de Melo, 2016; Lorenzo, González-Rodríguez, Sánchez, Amado, & Franco, 2013; Nantitanon, Yotsawimonwat, & Okonogi, 2010; Pateiro, Lorenzo, Amado, & Franco, 2014). This has been reflected in the European community through the decrease in the use of synthetic antioxidants and the increase in the interest of the natural ones. Extracts of fruits, vegetables and their residues are potential sources of natural antioxidants, such as ascorbic acid, tocopherols, carotenoids and phenolic compounds.

The agro-industrial sector has grown in recent years, leading to an increase in residue generation. Most of them are discarded without proper treatment or used as fertilizers and animal feed, destinations that, a priori, do not generate economic gains, besides representing logistical and environmental bottlenecks at their disposal (Yi et al., 2009).

*Schinus terebinthifolius* Raddi is a plant belonging to the Anacardiaceae family, popularly known as pink pepper, aroeira, among others (Lorenzi & Matos, 2008). This pioneer species is native to Brazil and has no kinship with the family of traditional peppers (Degáspari, Waszczynskyj, & Prado, 2005). During product cleaning and selection, stems and leaves are removed and some non-standard fruits are rejected, constituting the residue of the production. The different varieties of pink pepper are characterized by their high contents of bioactive compounds, such as vitamin C, phenolic compounds, flavonoids and carotenoids (Alvarez-Parrilla, de la Rosa, Amarowicz, & Shahidi, 2011).
Peanut (Arachis hypogaea) is a legume of the Fabaceae family, native to South America. The largest number of species is found in Brazil, of which 46 of them are exclusively Brazilian (Freitas, Penaloza, Valls, 2003). The peanut residue is composed of its skin, which has a considerable amount of proanthocyanidins and procyanidins, both phenolic compounds that act as antioxidants (Oldoni et al., 2016).

In recent years, new packaging technologies have been developed to improve the preservation, quality and safety of foods. The active packaging is based on the concept of the incorporation of active components, which release or absorb substances for the purpose of extending shelf life while maintaining the quality and sensory characteristics of the food (Camo, Beltrán, & Roncalés, 2008; Lorenzo, Battle, & Gómez, 2014). Chitosan has attracted the attention of researchers due to their properties, mainly biodegradability, biocompatibility and the ability to form films (Devlieghere, Vermeulen, & Debevere, 2004). One of the characteristics of chitosan films is to act as an effective food preservative due to its antimicrobial activity (Portes, Gardrat, Castellan, & Coma, 2009; Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009). Although chitosan is a component of interest for the development of active packaging, it does not have a significant antioxidant activity. Due to this, the combination of chitosan active packaging with natural extracts with high antioxidant activity has been studied with positive results for meat preservation (Hassanzadeh et al., 2017; Krkić et al., 2013; Lekjing, 2016; Siripatrawan & Noipha, 2012).

Thus, this study aimed to develop and characterize active packaging obtained by the incorporation of antioxidant extracts of peanut skin and pink pepper residue in a chitosan film and to evaluate their effects on the physicochemical characteristics and oxidative stability of restructured chicken product.

2 Material e Methods

2.1 Residue collection and preparation of natural extracts

Peanut skin (cultivar Runner IAC 505), obtained by the blanching process, was supplied by CAP Agroindustrial (Dumont, SP, Brazil) and pink pepper residue (Schinus terebinthifolius Raddi), consisting of stems, leaves and rejected peppers, was provided by Agrorosa Ltda (São Mateus, ES, Brazil). Both materials were freeze-dried (Liotop L101, São Carlos, SP, Brazil),
ground in a knife mill (IKA A11, Basic, Staufen, Germany), sieved (40-mesh sieve, 420 μm) and stored at -18 °C.

Samples (1 g) were extracted with 10 mL of ethanol:water (80:20, v/v) for 25 min at 95 °C in a water bath (Quimis, Diadema, SP, Brazil) and for another 15 min in ultrasonic bath USC-1400A (Unique). The extracts were then centrifuged (Eppendorf 5810R) for 15 min at 5000 g and filtered qualitative filter paper. The supernatants, called extract of peanut skin (EPS) and extract of pink pepper residue (EPP) underwent chemical analyses and were incorporated into the chicken meat and the chitosan films.

2.1.1 Total phenolic content (TPC)

TPC was performed in triplicate, following the spectrophotometric method of Singleton, Orthofer, & Lamuela-Raventós (1999), using Folin-Ciocalteau reagent (Dinâmica, Diadema, SP, Brazil), gallic acid (Sigma Aldrich, St. Louis, MO, USA) as standard, and the results were expressed as mg gallic acid equivalent (GAE)/mL extract. For the technological application, the phenolic contents in mg GAE/mL extract were used to calculate the volume of EPP and EPS required to obtain 80 and 90 mg GAE, respectively. This volume was added for each kg of meat (for treatments with extracts added to the meat) or for the preparation of chitosan film needed to pack one kg of chicken product (for treatments with extracts added to the film).

2.1.2 Antioxidant activity (AA)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was performed according to Al-Duais, Müller, Böhm, & Jetschke (2009), with some modifications. The standard, control or samples (66 μL) were transferred to microplate wells with 134 μL of a 150 μM DDPH ethanolic solution (Sigma Aldrich, St. Louis, MO, USA). After 45 min, the absorbance was measured at 517 nm in a microplate reader (Molecular Devices, Sunnyvale, CA, USA). Trolox (Sigma Aldrich, St. Louis, MO, USA) was used as standard and the results were expressed as μmol Trolox/g.

The AA by the 2,2-azinobis-3-ethylbenzotiazoline-6-sulfonic acid (ABTS) method was assessed according to Al-Duais et al. (2009) with modifications. The ABTS radical was formed by the reaction between ABTS (7 mM) and potassium persulfate (140 mM), which were
incubated at 25 °C and protected from light for a period of 16 h. Once formed, the radical was diluted with ethanol to an absorbance of 0.700 ± 0.01 at 730 nm. The residue extracts (20 μL) were transferred to microplate wells with 220 μL of ABTS radical solution (Sigma Aldrich, St. Louis, MO, USA) and kept in the dark. After 6 min, the absorbance was read at 730 nm in a microplate reader. Ethanol was used as a blank and trolox as standard. The results were expressed as µmol Trolox/g.

The oxygen radical absorption capacity (ORAC) method was performed according to Melo et al. (2015), with modifications. The standard, control or samples (30 μL) were transferred to microplate wells with 60 μL of 508.25 nM fluorescein (Sigma Aldrich, St. Louis, MO, USA). Then, 110 μL of 76 mM 2,2’-azobis(2-methylpropionamide) dihydrochloride (AAPH) (Sigma Aldrich, St. Louis, MO, USA) was added. Both solutions were prepared with 75 mM potassium phosphate buffer (pH 7.4) that was used as blank. The reaction was incubated (37 °C) and the absorbance was measured every minute for 2 h at 485 nm (excitation) and 528 nm (emission), using a microplate reader. The results were expressed as µmol Trolox/g. All methods were performed in triplicate.

The superoxide anion (O·−) scavenging activity was determined according to Melo et al. (2015). For a final volume of 300 μL, 50μL of the EPS or EPP in different concentrations, 100 μL NADH (498 μM), 100 μL NBT (129 μM) and 50 μL PMS (16.2 μM) were added and dissolved in 19 mM potassium phosphate buffer (pH 7.4). A control was prepared by replacing the sample with buffer and a blank was prepared for each sample dilution replacing PMS and NADH with buffer. The samples were kept at 25 °C and readings were taken every minute during 10 minutes at 560 nm. The results were expressed as EC50, i.e., the mean quantity (mg/mL) of natural extract required to quench 50% of the superoxide radicals.

2.2 Preparation of chitosan active films

Chitosan films were prepared by dispersing 2 % (w/v) of chitosan (Primex – ChitoClear®, Siglufjordur, Iceland) in an aqueous acetic acid (1 %, v/v), which was homogenized in a magnetic stirring (IKA, RHB1, Wilmington, USA) at room temperature for 60 min. After the complete dissolution of chitosan, EPS and EPP were added to the film solution at the concentrations of 80 and 90 mg GAE/kg of meat (0.84% and 1.90 % v/v, mL
of extract per 100 mL of the chitosan solution), respectively. Glycerol (12.5 %, w/w of chitosan content), used as plasticizer, was added to the solution under magnetic stirring, at 180 rpm for 10 min. Then, 20 mL of the solution was poured into 9 cm round plastic petri dishes and dried in air-forced oven (FANEM, model 0023, Guarulhos, SP, Brazil) at 40 °C for 24 h.

The extract concentrations used in in this work were defined in a previous optimization study (Serrano-León, 2015), using response surface methodology and a central composite rotatable design. In this experiment, the extract concentration was one of the independent variables and ranged from 1 to 90 mg GAE/kg meat. The range of study was delimited to the maximum value of 90 mg GAE/kg meat to be close to the maximum limit of use of BHT in meats, according to the Brazilian legislation. The dependent variable was the value of thiobarbituric acid reactive substances (TBARS), which is an indicator of lipid oxidation. Based on the results, the optimal concentrations of EPS and EPP to maintain the oxidative stability of the chicken samples were 80 and 90 mg GAE/kg of meat, respectively.

2.3 Characterization of chitosan active films

2.3.1 Film thickness

Thickness (mm) was measured at five different locations of five films, using a micrometer (Mitutoyo, Tokyo, Japan).

2.3.2 Moisture content

The moisture of the films was determined in triplicate and the results were expressed as the percentage of weight loss after drying in an oven at 115 °C for 24 h (Mahmoud & Savello, 1993).

2.3.3 Water vapor permeability

Water vapor permeability was determined in five replicates, using the ASTM (American Society for Testing and Materials) E96-95 method (ASTM, 1995). Films were fixed on the top of test cells containing a desiccant (silica gel). These cells were placed in a chamber at 25 °C and 75 % RH (relative humidity) generated by a saline solution of sodium chloride. After 5
days of exposure, the permeation cells were weighed and the results used to calculate the water vapor permeability as water vapor transmission rate (WVTR).

2.3.4 **Total soluble matter (TSM)**

Three films were cut into 5-cm squares, dried in an oven (40 °C) and immersed in 100 mL of distilled water under stirring (shaker table Biomixer, TS-200a, Ribeirão Preto, SP, Brazil), during 24 h, at room temperature. The solution was filtered using a weighed filter paper, with the aim to retain the insolubilized film residues. Then the filter paper was dried (40 °C, 24 h) and weighed. The results were calculated by difference, in order to quantify the loss of solubilized matter (Sothornvit & Krochta, 2000).

2.3.5 **Mechanical properties**

Samples were evaluated by tensile test according to ASTM D882 method (ASTM, 1995). Films were cut into uniform rectangles of 2.54 cm x 10 cm long and then pre-conditioned at 75% RH and 25°C for 48 h. Tensile strength (TS), Young's modulus (E), and percentage elongation at break (ξ) were measured using a TexturePro CT V1.2 (Brookfield®, CT3 50K Texture Analyzer). The initial grip separation and crosshead speed were set at 50 mm and at 1 mm/min, respectively. There were 10 replicates per experiment.

2.4. **Preparation of chicken restructured product**

Boneless and skinless chicken thighs and drumsticks were obtained from a slaughterhouse (Rio Claro, SP, Brazil), ground (0.8 cm plate, Hobart 4B22-2, Troy, Ohio, USA) and divided into 6 treatments (Table 1), with about 2.5 kg each. Ground chicken meat (73.46% moisture, 16.87% protein, 7.82% fat, 1.05% ash) and the ingredients were homogenized in a cutter (Hobart 84142, Troy, Ohio, USA). In all treatments, 1.5% sodium chloride was added. The volume of EPP and EPS incorporated in the meat and film was previously determined and corresponded to the concentrations of 80 and 90 mg GAE/kg meat, respectively. For the treatment with synthetic antioxidant (SA), butylated hydroxytoluene (BHT) at the concentration of 100 mg BHT/kg of meat (limit of use in meat products according to the Brazilian Health Surveillance Agency) was previously dissolved in 5 mL of soybean oil (without synthetic antioxidant) and was added to the meat. In order to standardize this procedure, 5 mL of the
same soybean oil was added to the other treatments. After that, portions (50 g) were shaped in the form of burger. Treatments with active packaging were wrapped in chitosan films with residue extracts and all samples were aerobically packaged. All treatments were stored for 7 days in an incubator (ELETROlab, EL101/3, São Paulo, SP, Brazil) with controlled temperature (3 ± 1 °C) and lighting (1000 lux). Samples were analyzed after processing and after 4 and 7 days of refrigerated storage. This processing was replicated three times, on different days.

2.5 Analyses of the chicken product

2.5.1 pH and color

The pH was determined using a potentiometer (Oakton pH 300, 35618, Vernon Hills, IL, USA) with a glass penetration electrode (Digimed, Presidente Prudente, SP, Brazil). Color was determined using a colorimeter (Minolta CR-400, Konica Minolta, Osaka, Japan) with a measurement area of 8 mm in diameter, observation angle of 10° and illuminant C. The parameters were calibrated in a standard white porcelain with Y = 93.7, x = 0.3160 and y = 0.3323 and the color parameters were based in the CIELAB (Commission Internationale de l’Eclairage) color space: lightness (L*), redness (a* ± red-green), and yellowness (b* ± yellow-blue). Both analyses were performed in 5 samples of each treatment, with 3 readings per sample, after processing and after 4 and 7 days of refrigerated storage.

2.5.2 Peroxide value (PV)

Fat was extracted according to the method of Bligh and Dyer (1959), following the modifications proposed by Christie (2012) and Smedes and Thomasen (1996). PV was carried out in triplicate, according to American Oil Chemists’ Society AOCS - Cd 8-53 method (1990) and was calculated and expressed as milliequivalent peroxide per kg of sample.

2.5.3 Thiobarbituric acid reactive substances (TBARS)

TBARS determination was performed in triplicate according to Vyncke (1970) and Sørensen & Jørgensen (1996) with slight modifications described by Selani et al. (2011). 1,1,3,3-tetraethoxypropane was used as standard and the results expressed as mg of malonaldehyde (MDA)/kg meat.
2.5.4 Microbiological analysis

Microbiological analysis was performed using three samples per treatment, in duplicate. A 25 g portion of each treatment was homogenized with 225 mL of 0.1 % sterile peptone water (Difco, Detroit, MI, USA) and serial decimal dilutions were prepared. Mesophilic aerobic counts (MAC) were determined using 1 mL of suitable dilutions on pour-plates of plate count agar (Difco, Detroit, MI, USA) incubated at 35 °C for 2 days. For psychrotrophic aerobic counts (PAC), 0.1 mL of suitable dilutions was spread on to the surface of plate count agar and incubated at 7 °C for 10 days. Counts were expressed as the log\textsubscript{10} colony forming units per g of sample (log CFU/g).

2.6 Experimental design and statistical analysis

The study was a randomized block design, with three blocks (each block corresponding to an independent processing, performed on different days) with a 6 (treatments) x 3 (storage times) factorial arrangement. Analysis of variance (ANOVA) was carried out and the means comparison was performed by Tukey’s test (p<0.05). The Student’s t test was used to compare the mechanical properties of the films (p<0.05). All statistical analyses were performed using the R environment (R Core Team, 2015) with the ExpDes.pt package (Ferreira, Cavalcanti, & Nogueira, 2013).

3 Results and Discussion

3.1 Phenolic content and antioxidant activity

The results showed that the total phenolic content (TPC) of EPS was 2-fold higher (p<0.05) than that found in EPP (Table 2).

A similar TPC of ethanolic extracts of peanut skin was observed by Nepote, Grosso, and Guzman (2002), who reported 114.8 mg GAE/g. According to a previous study, the TPC found in peanut skin was much higher than that found in methanolic extracts of blanched and in-shell peanuts (de Camargo et al., 2012). This higher content in the skin may be explained by the fact that the outer layers of grains and seeds are more exposed to environmental aggressions and the phenolic compounds serve as plant defense against pests and pathogens.
According to Camargo et al. (2017), some of the phenolic compounds found in the peanut skin are protocatechuic acid, p-coumaric acid, ferulic acid, cis and trans-coutaric acid, ellagic acid, coumaroyl-rhamnose, dihydrodihydroxyprenylcoumaric acid, feruloyl pentoside, catechin, epicatechin, procyanidin dimer A and B, procyanidin trimer, procyanidin tetramer A1 and A2.

In relation to the phenolic content of EPP, the result is similar to that found by Bergamaschi (2016) (38.42 mg GAE/g), which also studied pink pepper residues (stems, leaves and rejected peppers). D’Sousa’Costa et al. (2015) evaluated different tissues of pink pepper and found TPC varying from 228.51 - 309.03 mg GAE/g for stem bark and 73.90 - 87.70 mg GAE/g for leaf extracts. The phenolic compounds identified in pink pepper residue extract that can be responsible for its antioxidant activity are catechin, p-coumaric acid, miricetin and epicatechin (Bergamaschi, 2016). Most of the published literature on pink pepper is related to the anti-inflammatory and antioxidant activities of its essential oil, with limited focus on the study of its residue.

Regarding the AA, from the four methods evaluated, EPS showed significantly higher activity in three of them (DPPH, ABTS and superoxide radical). The capacity of the EPS to scavenge the superoxide radical was similar to that of ascorbic acid (0.26 mg/mL) (Kong, Mat-Junit, Aminudin, Ismail, & Abdul-Aziz, 2012), but lower than that of Trolox (0.13 mg/mL) (Rodrigues, Mariutti, & Mercadante, 2013), a water-soluble vitamin E analog. For the ORAC method no significant difference was observed between the two residue extracts and this result may be related to the approach of each antioxidant assay. In general, antioxidant assays may be classified in methods using hydrogen atom transfer, single electron transfer and both mechanisms. DPPH and ABTS are methods related to both mechanisms (Prior, Wu, & Schaich, 2005), in addition to have an indirect approach, i.e., they measure the ability of an antioxidant to scavenge free radicals that are not associated with the real oxidative degradation (Roginsky and Lissi, 2005). On the other hand, the ORAC method measures the antioxidant inhibition of peroxyl radical induced oxidations (related to lipid peroxidation), reflecting classical radical chain breaking antioxidant activity by hydrogen atom transfer. In addition, factors such as the physical and chemical characteristics of the oxidants and antioxidants and the reaction media may explain the variation in the results from the use of different AA methods (Prior et al., 2005).
3.2 Characterization of chitosan active films

Chitosan films with residue extracts showed a smooth surface, a yellow transparent color, besides being flexible and homogeneous. They were physico-chemically (Table 3) and mechanically (Table 4) characterized.

The thickness of films containing peanut skin and pink pepper extracts was similar. Siripatrawan and Harte (2010) found the same thickness (0.06 mm) in chitosan films with green tea extract. The measurement of film thickness is important since it is the basis for calculating the water vapor transmission rate.

Chitosan films with natural extracts did not have significant difference for TSM. Film solubility is a relevant characteristic as it indicates the film behavior in an aqueous environment, such as water resistance, besides being an important factor to determine its biodegradability (Gnanasambadam, Hettiarachchy, & Coleman, 1997). Similar values (29.1 - 31.6 %) were reported by Martins, Cerqueira, and Vicente (2012) in chitosan films with of α-tocopherol, while Wang, Dong, Men, Tong, and Zhou (2013) found water solubility values ranging from 28 to 40% in chitosan films with tea polyphenols extract.

The moisture of both types of active films was similar. The water content of a filmogenic matrix influences the film barrier properties. An increase in water concentration leads to an increase in molecular motion, then modifying the diffusion and permeability properties of the films. This behavior has been previously reported by Mehyar and Han (2004). Similar film moisture values were found by Van Beest (2013) in chitosan films with essential oil of oregano (24%) and thyme (23.7%).

The water vapor permeability refers specifically to the permeation of water vapor at a set temperature through the film surface. The results for WVTR were similar for both films (Table 3). Film permeability depends on the chemical and morphological structure of the material and the ambient temperature. Chitosan films with extracts of pink pepper and peanut skin showed lower permeability than chitosan films with α-tocopherol, which presented values ranging from 6.0 to 7.3 x 10^{-4} g m^{-1} h^{-1} kPa^{-1} (Martins et al., 2012). Siripatrawan & Harte (2010) reported that variations in WVTR could be related to covalent interactions between the chitosan
matrix and the phenolic compounds that limit the availability of hydrogen to form hydrophilic bonds with water, then resulting in a decrease in the affinity of the film with water.

Regarding the mechanical properties, there were significant differences between films for TS and ξ. Peanut skin film tended to be more resistant and less flexible than pink pepper film, which showed the highest TS and the lowest ξ (Table 4). For the Young’s modulus, which indicates the stiffness of the material, no significant difference between the two films was verified.

Yoshida, Oliveira Junior, and Franco (2009) reported values of 21.15 MPa for TS and 17.10% for ξ in chitosan film (1% w/v). Comparing these results with those found in the present study, ξ values were very similar, while for TS they were lower. Bonilla, Talón, Atarés, Vargas, and Chiralt (2013) evaluated the mechanical properties of wheat starch-chitosan films with citric acid and obtained values of 34 MPa, 3%, and 0.141 GPa for TS, ξ and E, respectively. According to Ziani, Oses, Coma, and Maté (2008), variations in the results can be due to several factors such as: composition, obtaining source and degree of deacetylation of the chitosan, in addition to the presence of plasticizer, film preparation and storage.

3.3 Analyses of chicken product

3.3.1 pH

There was no significant effect of treatment, storage time and interaction between them for the pH and all treatments showed the same pH behavior during the storage time. The average pH of the samples was 6.40 ± 0.2

There are no reports of studies that have evaluated the application of pink pepper extract in meat or active films. For peanut skin, similar results were found in sheep patties with peanut skin extracts, since no significant difference in pH either among treatments or during the storage period was found (Munekata, Fernandes, de Melo, Trindade, and Lorenzo, 2016). The pH values obtained here are similar to those indicated by previous studies using natural extracts in chicken meat, such as grape residue extracts (Selani et al., 2011) and citrus extracts (Mexis, Chouliara, & Kontominas, 2012).
3.4 Instrumental color

No differences among treatments were observed for the three color parameters (L*: lightness, a*: redness, and b*: yellowness), while for the storage time just redness was significantly affected. There was no effect of interaction.

Lightness, redness and yellowness mean values of all treatments during the storage period were 68.00, 9.25, and 17.83, respectively. Despite the red color of EPS and yellow color of EPP, no significant changes in the characteristic chicken color were observed, probably due to the low concentrations used. Differently, Munekata et al. (2015) reported that chicken patties had their color affected by the addition of peanut skin extracts and O’Keefe and Wang (2006) found that concentrations higher than 400 ppm of peanut skin extract caused color changes in ground beef. It is known that color is one of the main attributes that determine food acceptance by consumers. In this sense, this study showed important results, which may be considered a positive aspect for the acceptance of the product.

Regarding redness, a significant drop in values during refrigerated storage was observed (10.09, 9.18 and 8.50 after processing and after 4 and 7 days of storage, respectively). This decrease in redness was probably related to the fact that the longer the meat is refrigerated stored, the greater the tendency for oxymyoglobin oxidizes to brownish metmyoglobin (Young & West, 2001). It may be also due to the occurrence of lipid oxidation, since free radicals may oxidize the iron atoms or denature the myoglobin molecules, resulting in meat discoloration (O’Grady, Monahan, & Brunton, 2001). Decrease in a* value was also observed during storage of chicken patties (Munekata et al., 2015), and sheep patties (Munekata et al., 2016), both with peanut skin extracts.

3.5.1 Peroxide value (PV)

For peroxide value, there was significant effect (p<0.05) of treatment, storage time and interaction between them (Figure 1A).

There was no effect of treatments on the PV of chicken products just after processing (2.45 mEq O₂/kg fat). This result was expected because the time between sample processing
and PV analysis was too short to show the possible effects of the antioxidants on the oxidative stability.

However, after 4 days of storage, the effectiveness of the treatments with antioxidants could already be observed, since CT showed the highest PV and all the other treatments had similar protection against lipid oxidation. At the end of 7 days, a similar trend was observed, with CT showing the highest production of hydroperoxides. The absence of antioxidants along with the presence of catalysts of the lipid oxidation (light, technological treatment, presence of salt and unsaturated lipids), resulted in the control showing a PV 49% higher than the values observed in the other treatments (Figure 1A). All treatments with natural antioxidants showed efficiency comparable to BHT and among these, DPP, DPS and FPP stood out regarding antioxidant protection of the chicken product.

PV was increased during storage in all treatments. This fact occurred at a higher speed in CT (4 days of refrigeration), due to factors previously mentioned. On the other hand, the other treatments, due to the presence of antioxidants, had the lipid oxidation delayed, with significant increase in PV only after 7 days of storage. According to Yu et al. (2010), in the presence of antioxidants, the formation of peroxides is slowed down through mechanisms such as metal chelating and free radical scavenging/quenching. The increase in PV during the storage was also observed by Yu et al. (2010) in ground beef with peanut skin extract. On the other hand, Larrauri et al. (2013) found that PV of salami samples without antioxidants decreased during the storage, suggesting hydroperoxide decomposition. For this reason, it is important to complement the PV with analyses that quantify more stable secondary compounds of the lipid oxidation, such as the thiobarbituric acid reactive substances.

3.5.2 Thiobarbituric acid reactive substances (TBARS)

For TBARS there was significant effect (p<0.05) of treatment, storage time and interaction between them (Figure 1B).

After processing, the TBARS values did not show significant differences among treatments. Once malonadehyde is a product of hydroperoxide decomposition, this result shows consistency with the PV, which also resulted in treatments without differentiation at day 0.
Similar results were found in sheep patties (Munekata et al., 2016) and ground beef (O’Keefe & Wang, 2006), both with incorporation of peanut skin extracts.

On the fourth day of storage, although no significant difference was detected among treatments with active films and CT, they also did not differ from treatments with direct antioxidant addition (SA, DPP and DPS), which were the ones with the lowest TBARS values. At the end of the storage period, DPP promoted the highest protection against lipid oxidation, decreasing TBARS values in 64% compared to control, followed by SA (51%) and DPS (50%). These results are in agreement with those of Munekata et al. (2015) and Yu et al. (2010), who reported decrease in TBARS values throughout the storage time of meat products.

Comparing the addition of the extracts directly into the meat with the addition of extracts into the film, DPP showed 23% higher efficiency than FPP to protect chicken product against lipid oxidation. For the treatments with peanut skin, DPS was 15% more effective than FPS. Although the incorporation of the extracts into the meat has shown greater efficiency in delaying lipid oxidation, FPP and FPS showed promising results, since they reduced TBARS values in 40.5% and 35.4% compared to control, respectively, with the advantages of limiting possible undesirable sensory attributes caused by the direct incorporation of the additives. To the best of our knowledge, there are no studies that have evaluated active packaging with incorporation of peanut skin or pink pepper extracts.

Regarding the efficiency of the natural antioxidants, although peanut skin extract has shown significant higher AA than pink pepper extract in DPPH, ABTS and superoxide radical methods, both extracts, either applied to the meat or to the film, provided similar protection against lipid oxidation of the chicken product. This behavior may be an indicative that the phenolic compounds of EPP have higher antioxidant potential in a meat matrix than those found in EPS. Similar result was observed by Packer et al. (2015), who verified that the most efficient natural extract for the maintenance of the oxidative stability of a chicken product was not necessarily the one with the highest AA when analyzed by antioxidant assays (DPPH and ABTS). According to these authors, this result may have occurred due to different mechanisms of antioxidant activity. In DPPH and ABTS methods, antioxidants are evaluated by electron and hydrogen atom transfer, while in foods, they are liable to undergo different/parallel mechanisms, such as electron transfer, hydrogen transfer and metal chelation.
Except for DPP, all the other treatments showed significant increase in TBARS during the storage period. This was expected due to intrinsic factors related to the processing of meat products (unsaturated lipids, salt, fine grinding, high temperature and incorporation of air), in addition to the light exposure of the samples (1000 lux). This light condition was specifically used to accelerate the lipid oxidation in order to evaluate the antioxidant effects of the natural extracts. Similarly to the PV results, CT had a more pronounced and faster development of the lipid oxidation compared to the other treatments.

3.6. Microbiological analysis

There was no significant effect of treatment and interaction between treatment and time, but for the storage time, a significant (p<0.05) increase in mesophilic aerobic counts (MAC) was observed.

Although there was no effect of the treatments on the MAC, FPP and FPS had lower microbial growth at 7 days of storage, with counts of 4.82 and 4.76 log CFU/g, respectively, against 6.63 CFU/g found in CT (Figure 2A). This decrease in microbial growth may have been given by the action of chitosan, since treatments without this component (AS, DPP and DPS) showed MAC counts very similar to control. The antimicrobial activity of chitosan may be justified by the presence of amino groups (positively charged), which interact with negatively charged bacterial cells membranes, leading to irreversible damage to the intracellular components of the microorganisms (Chen, Liau, & Tsai, 1998). Similarly, previous studies showed a decrease in MAC during storage of chicken meat with active films of chitosan and oregano oil (Petrou, Tsiraki, Giatrakou, & Sawaidis, 2012) and in pork sausages with direct addition of rosemary extract and chitosan (Georgantelis, Blekas, Katikou, Ambrosiadis, & Fletouri, 2007).

Regarding PAC, there was significant effect (p<0.05) of treatments, storage time and interaction (Figure 2B).

At the beginning of the experiment, and as expected, psychrotrophic aerobic counts (PAC) were similar for all treatments. However, after 4 and 7 days of refrigeration, some antimicrobial effect could already be observed, which was probably related to the presence of
chitosan, since FPS and FPP showed a significant decrease in microbial counts at the end of the storage period. These results demonstrate that the action of chitosan active packaging with natural antioxidants had an important effect on the growth of psychrotrophic microorganisms. This result is in agreement with that reported by Higueras et al. (2014), with chicken fillets packaged with chitosan/cyclodextrin + carvacrol films. Finally, this result agrees with the recently published study of Fernández-de Castro et al. (2016), who studied the antibacterial properties of chitosan films.

4 Conclusions

Extracts of peanut skin and pink pepper residue have considerable TPC and AA, mainly EPS. When natural extracts and BHT were added to the meat, DPP was the most effective in delaying lipid oxidation. Similar trend was observed when natural extracts were added to chitosan films, in which FPP stood out. This is an indicative that, although the antioxidant activity of EPP was lower than that of EPS in DPPH, ABTS and superoxide radical methods, EPP showed higher protection of the chicken meat against lipid oxidation.

Regarding the microbiological point of view, FPP and FPS were the most effective treatments since both showed inhibition of the growth of psychrotrophic microorganisms. Between the two treatments with pink pepper extract, FPP showed a greater action spectrum, because besides the antioxidant activity, it showed antimicrobial activity. None of the treatments altered the pH and color of the chicken product.

Chitosan active films with Brazilian agro-industrial residues extracts may maintain the quality of chicken products due to their antioxidant and antimicrobial potential.

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Selani, M. M., Contreras-Castillo, C. J., Shirahigue, L. D., Gallo, C. R., Plata-Oviedo, M., & Montes-Villanueva, N. D. (2011). Wine industry residues extracts as natural antioxidants in...


Table 1. Description of the treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>Control: without antioxidant</td>
</tr>
<tr>
<td>AS</td>
<td>Addition of synthetic antioxidant to the chicken product: 100 mg BHT/kg meat.</td>
</tr>
<tr>
<td>DPP</td>
<td>Direct addition of EPP to the chicken product: volume of extract equivalent to 90 mg GAE/kg meat.</td>
</tr>
<tr>
<td>DPS</td>
<td>Direct addition of EPS to the chicken product: volume of extract equivalent to 80 mg GAE/kg meat.</td>
</tr>
<tr>
<td>FPP</td>
<td>Addition of EPP to the chitosan active film: volume of extract equivalent to 90 mg GAE/kg meat.</td>
</tr>
<tr>
<td>FPS</td>
<td>Addition of EPS to the chitosan active film: volume of extract equivalent to 80 mg GAE/kg meat.</td>
</tr>
</tbody>
</table>
Table 2. Phenolic content and antioxidant activity (AA) of EPS and EPP

<table>
<thead>
<tr>
<th>Method</th>
<th>EPS (mg GAE/g)</th>
<th>EPP (mg GAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic content</td>
<td>107.69 ± 0.92ª</td>
<td>45.01 ± 0.54b</td>
</tr>
<tr>
<td>AA Methods with synthetic free radicals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPH- (µmol trolox/g)</td>
<td>776.46 ± 0.28ª</td>
<td>535.74 ± 0.32b</td>
</tr>
<tr>
<td>ABTS•+ (µmol trolox/g)</td>
<td>1,396.62 ± 1.25ª</td>
<td>931.00 ± 0.28ª</td>
</tr>
<tr>
<td>AA Methods with reactive oxygen species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORAC (µmol trolox/g)</td>
<td>152.15 ± 0.56ª</td>
<td>158.24 ± 0.92ª</td>
</tr>
<tr>
<td>Superoxide radical (O₂⁻)</td>
<td>0.27±0.06b</td>
<td>1.24±0.09ª</td>
</tr>
</tbody>
</table>

Mean ± standard deviation.

EPS: extract of peanut skin; EPP: extract of pink pepper residue.

Different letters in a row indicate significant difference (p<0.05) by the Tukey’s test.
Table 3. Thickness, total soluble matter (TSM), moisture and water vapor transmission rate (WVTR) of chitosan active films with residue extracts

<table>
<thead>
<tr>
<th>Film</th>
<th>Thickness (μm)</th>
<th>TSM (%)</th>
<th>Moisture (%)</th>
<th>WVTR ($10^{-8}$ g mm m$^{-2}$ h$^{-1}$ kPa$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPF</td>
<td>62.63 ± 2.89 $^a$</td>
<td>27.25 ± 0.27 $^a$</td>
<td>20.18 ± 0.44 $^a$</td>
<td>1.72 ± 0.07 $^a$</td>
</tr>
<tr>
<td>PSF</td>
<td>64.32 ± 3.01 $^a$</td>
<td>27.12 ± 0.41 $^a$</td>
<td>20.12 ± 0.82 $^a$</td>
<td>1.77 ± 0.08 $^a$</td>
</tr>
</tbody>
</table>

Mean ± standard deviation.

PPF: pink pepper residue film; PSF: peanut skin film;

Different letters in a column indicate significant difference (p<0.05) by the Student's t test.
Table 4. Mechanical parameters of chitosan active films with residue extracts

<table>
<thead>
<tr>
<th>Film</th>
<th>TS (MPa)</th>
<th>ξ (%)</th>
<th>E (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPF</td>
<td>16.40 ± 1.30</td>
<td>19.82 ± 1.70</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>PSF</td>
<td>18.84 ± 1.60</td>
<td>17.36 ± 0.60</td>
<td>0.12 ± 0.01</td>
</tr>
</tbody>
</table>

Mean ± standard deviation.

TS: Tensile strength; ξ: elongation at break; E: elastic modulus; PPF: pink pepper film; PFS: peanut skin film.

Different letters in a column indicate significant difference (p<0.05) by the Student’s t test.
Figure caption

**Figure 1.** PV (A) and TBARS (B) values of chicken products during storage. CT: control; AS: synthetic antioxidant; DPP: direct addition of pink pepper residue extract; DPS: direct addition of peanut skin extract; FPP: chitosan film with pink pepper residue extract; FPS: chitosan film with peanut skin extract.

Significant differences (p<0.05) between treatments within a day of storage are denoted by different lowercase letters. Different capital letters denote significant differences (p<0.05) between 7 days of storage.

**Figure 2.** MAC (A) and PAC (B) of chicken products during storage. CT: control; AS: synthetic antioxidant; DPP: direct addition of pink pepper residue extract; DPS: direct addition of peanut skin extract; FPP: chitosan film with pink pepper residue extract; FPS: chitosan film with peanut skin extract.

Significant differences (p<0.05) between treatments within a day of storage are denoted by different lowercase letters. Different capital letters denote significant differences (p<0.05) between 7 days of storage.

Figures 1 and 2 should appear in color only on line.
Figure should appear in black and white
Figure 2

Figure should appear in black and white.
Highlights

- Chitosan films with residue extracts were studied as active packaging in chicken product.
- Extract of peanut skin had higher DPPH and ABTS values than that of pink pepper residue.
- Both extracts, either added to the product or to the films, were as effective as BHT.
- Chitosan films with natural extracts showed lower psychrotrophic counts.
- Active films with residue extracts may maintain the quality of chicken products.