Impact of Myrciaria Dubia Peel and Seed Extracts on Oxidation Process and Colour Stability of Ground Lamb

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ABSTRACT
The present study investigated the influence of Myrciaria dubia peel and seed extracts (PE and SE) on colour and oxidation stability of ground lamb. Instrumental colour (L**, a* and b* values), pH, lipid oxidation (TBARS), and protein oxidation (carbonyls) were determined during 9 days of storage at 4 °C. Although the L* value was not affected by any enhancement type (BHT, PE, or SE), all samples exhibited a decrease on the a* and b* values (P < 0.05) during refrigerated storage. M. dubia extracts increased the lipid stability in ground lamb, however no positive effect on protein oxidation was observed. PE and SE samples had lower TBARS values compared with control and BHT counterparts. Due to its antioxidant effect on lipids, peel and seed extracts of M. dubia can be considered a viable strategy to improve the oxidative stability of ground lamb during storage.

1. Introduction
Meat quality deterioration involves the oxidation of both fatty acids and muscle pigments, processes which are synergistic and result in simultaneous loss of colour and odour/flavour quality (Viana, Canto, Costa-Lima, Salim, & Conte-Junior, 2016). In addition, proteins are also important substrates for oxidation reactions (Canto et al., 2016), the oxidation of myofibrillar proteins affect their functionality generally compromising the texture quality of processed meats (Cao, True, Chen, Youling, & Xiong, 2016).

Previous reports documented that the fat present in lamb longissimus thoracis et lumborum is composed of nearly 50% of unsaturated fatty acids where 36% are monounsaturated and 13% are polyunsaturated fatty acids (Hajji et al., 2016). Lipid oxidation is a well characterized three-step radical chain reaction comprised of initiation, propagation, and termination involving mainly fatty acid (R-), peroxy (ROO•), and hydroperoxide (H2O2) radicals. These reactions yield volatile compounds in meat related to off-flavours and rancid odour (Falowo, Fayemi, & Muchenje, 2014). In addition, these processes are self-propagating processes and can be associated with the off-flavours and off-odours produced during meat storage (Baron & Andersen, 2002; Faustman, Sun, Mancini, & Suman, 2010; Yin & Faustman, 1993). Proteins are also important substrates for oxidation reactions (Canto et al., 2016; Lorido, Ventanas, Akan, & Estévez, 2016) commonly associated with reactive oxygen species (ROS) leading to the formation for a variety of products including carbon-centred (P•), peroxy (POO•), alkyl peroxide (POOH), and alkoxy (PO•) radicals (Lund, Heinonen, Baron, & Estévez, 2011).

The food industry usually utilizes synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, tert-butylhydroquinone, and propyl gallate to mitigate the negative effects matrix oxidation of food. These synthetic
antioxidants have the advantage of low cost and high efficiency (Almeida et al., 2015), however there is a demand-driven growing interest by the consumer market for replacement of artificial additives with compounds from natural sources (Viana, Silva, & Trindade, 2014). Plant tissues including bark, leaf, root, fruit, and their parts are widely studied as potential sources of phytochemicals with potential use as substitutes of synthetic molecules (Armenteros, Morcuende, Ventanas, & Estévez, 2016; Ganhão, Morcuende, & Estévez, 2010; Jia, Kong, Liu, Diao, & Xia, 2012). Fruit by-products are interesting raw materials due to their economical aspect, furthermore several varieties exhibit well established crops and processing chains indicating a more reliable sourcing of raw materials (Guedes-Oliveira, Salgado, Costa-Lima, Guedes-Oliveira, & Conte-Junior, 2016). Previous authors reported that native fruits from rainforests exhibit high contents of polyphenols and organic acids with antioxidant potential (López et al., 2015; Rufino et al., 2010).

Myrciaria dubia (camu camu) is a berry from the Amazon Rainforest known for its high content of ascorbic acid (Zanatta & Mercadante, 2007) with potential to be utilized as an antioxidant ingredient (Fracassetti, Costa, Moulay, & Tomás-Barberán, 2013). In addition, the antioxidant activity of M. dubia is not solely attributed to ascorbic acid, but also to the high levels of phenolic compounds such as flavonols, anthocyanins, and ellagic acid (Gonçalves, Lajolo, & Genovese, 2010; Reynertson, Yang, Jiang, Basile, & Kennelly, 2008; Rufino et al., 2010; Zanatta & Mercadante, 2007). Peel and seed are by products from M. dubia processing, indicating a potential source of inexpensive raw material for the isolation of bioactive compounds to be utilized in food and pharmaceutical industries (Deng et al., 2012). Also, these by-products exhibit higher contents of antioxidant molecules than the pulp (Deng et al., 2012; Fracassetti et al., 2013).

Although there is an increasing wealth of information about natural alternatives to synthetic antioxidants utilized to improve the colour and chemical stability of meat products (Shah, Bosco, & Mir, 2014), there is a lack of information about the influence of M. dubia extracts on lamb products quality parameters. Ground meat is not allowed to contain synthetic antioxidants (Tarté, 2009), nonetheless the present study utilized butylated hydroxytoluene and ground lamb as a model with simpler chemical composition and manufacturing procedure than other processed meats. In this context, the aim of this study was to evaluate the efficacy of the extracts obtained from M. dubia peel and seed on the mitigation of lipid and protein oxidation, and colour stability of ground lamb during refrigerated storage.

2. Materials and methods

2.1 Plant material

Myrciaria dubia (HBK) McVaugh samples were provided by the Sindicato Rural de Iguape (24° 42′ 29″ S, 47° 33′ 19″ W) (Iguape, São Paulo, Brazil). The berries were manually peeled and the seeds separated. Seed and peel of M. dubia were individually dehydrated with forced-air for 24 h at 25 °C to constant weight. Dried seeds and peels were individually ground in a burr grinder (MSS-1B, Hario, Japan), sieved through a 0.125 mm mesh, and stored in amber-glass flasks at −20 °C before extraction.

2.2 Seeds and peel extracts of M. dubia

Seed and peel extracts were individually obtained by homogenizing the ground samples in 50% (v/v) ethanol in water solvent at a solute-to-solvent ratio of 1:10 (w/v). Extraction was done on a rotary shaker (TS – 2000 A, Biomixer, China) at 60 rpm for 120 min at 25 °C. The extracts were centrifuged at 13,000 × g (Sorvall ST16R, Thermo Scientific, São Paulo, Brazil) for 5 min at 4 °C, and the supernatants were kept at 4 °C in amber-glass flasks.

2.3 Total phenolic content (TPC)

Total phenolic content of the extracts was estimated by Folin-Ciocalteu method and expressed as mg gallic acid equivalents (GAE) per ml of the extract (Ainsworth & Gillespie, 2007). The standard curve ranging from 0.0 to 1.2 mg GAE was plotted with an r-square of 0.991. The extracts exhibited 1.8 ± 0.1 and 2.0 ± 0.2 mg of GAE/ml for the peel and seed starting materials, respectively. Furthermore, the extraction procedure of the peel yielded 1.2 g, while the seed provided 1.7 g per 100 g of starting material.

2.4 Application of seed and peel extracts in lamb meat

2.4.1 Lamb patties and storage conditions

Eighth untrimmed longissimus thoracis et lumbarum (LTL) muscle whole cuts were obtained 36 hours post-mortem from Texel animals harvested at 10 months of age; each muscle derived from a different animal. The cuts were individually ground and divided into four batters to be enhanced to 110% of the initial weight with either water (negative control; NC); 100 mg/kg BHT (positive control; PC); 100 mg/kg peel extract (PE); or 100 mg/kg seed extract (SE). Immediately after the enhancement the batches were homogenized for 5 minutes at low speed utilizing a bowl mixer equipped with a heavy dough hook attachment (SXB401, Arno, Brazil), and formed into patties (5.5 cm diameter × 1.5 cm thickness). Patties were placed on polystyrene trays with Soaker pads, over-wrapped with polyvinyl chloride (PVC) film (0.014 mm thickness, O2 transmittion rate 320 cm2 m−2 d−1, Orlep plast Ind. e Com de Plásticos Ltda, Orleans, Santa Catarina, Brazil), and stored for 9 days at 4 °C. The PVC film was not in direct contact with the sample surface.

2.4.2 Proximate composition and pH value

Moisture, lipids, protein and ash contents, and pH values were determined using official methods (AOAC, 2012). The pH value was determined from samples homogenized at 1:9 sample-to-water ratio utilizing a pHmeter (DM-22, Digimed, Campo Grande, Brazil).

2.4.3 Instrumental color

The instrumental colour was monitored using a spectrophotometer (CM-600D, Konica Minolta Sensing Inc., Osaka, Japan) equipped with illuminant D65, 8 mm aperture, and 10° standard observer (AMSA, 2012). Lightness (L* value), redness (a* value) and yellowness (b* value) parameters were recorded at three different locations on the patties surface. The colour difference between treatments was calculated considering the following equation:

$$
\Delta E_{ab}^* = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}
$$

The colour difference, colour parameter, and textural measurements were subjected to analysis of variance using Minitab 17 (Minitab Inc., State College, PA) at a 95% confidence level.
2.4.4 Lipid oxidation

TBARS was reported as mg MDA/kg meat (Yin, Faustman, Riesen, & Williams, 1993). Briefly, 5 g of sample were homogenized with 23 mL of 11% trichloroacetic acid (TCA) utilizing an Ultra Turrax T18 basic (IKA, Wilmington, NC, USA) for two cycles consisting of 1 min of homogenization followed by 1 min of ice-cold water bath. Aliquots of 1.5 mL of the homogenate were centrifuged at 15,000 × g for 15 min at 4 °C (Sorvall ST16R, Thermo Scientific, São Paulo, Brazil) to obtain a clear supernatant from which, 1.0 mL was reacted with an equal volume of 20 mM thiobarbituric acid solution. The test tubes were briefly vortexed and incubated for 18 h at 25 °C in the dark for colour development. Absorbance at 532 nm were recorded utilizing an UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Concentration of TBARS was calculated from standard curve (r² = 0.997) prepared with Malondialdehyde tetrabutylammonium salt (Sigma-Aldrich, Brazil).

2.4.5 Protein oxidation

Protein oxidation was estimated based on 2,4-dinitrophe-nylhydrazine (DNPH) derivatization method (Oliver, Ahn, Moerman, Goldstein, & Stadtman, 1987) with modifications (Armenteros, Heinonen, Ollilainen, Toldrá, & Estévez, 2009). Three grams of sample were homogenized utilizing Ultra Turrax T18 basic (IKA, Wilmington, NC, USA) in 30 mL of 0.75 M KCl solution (pH 7.4) for 90 s. Aliquots of 1.0 mL were centrifuged at 15,000 × g for 15 min at 4 °C (Sorvall ST16R, Thermo Scientific, São Paulo, Brazil), and then two 100 μL aliquots were individually vortexed with 1.0 mL of 10% TCA solution and centrifuged at 5,000 × g for 5 min at 4 °C. The pellets were either dissolved in 1.0 mL of 2 N HCl or with 1.0 mL of 20 mM DNPH in 2 N HCl solution for protein and carbonyls quantification, respectively. The homogenates were incubated for 1 hour at 25 °C with intermittent brief vortexing every 15 min following precipitation with 20% TCA and centrifugation at 11,000 × g for 15 min at 4 °C (Sorvall ST16R, Thermo Scientific, São Paulo, Brazil). The reagent for carbonyl estimation was prepared utilizing Ultra Turrax T18 basic (IKA, Wilmington, DE) 30 min of 0.75 M KCl solution (pH 7.4) to obtain a clear supernatant (Sigma-Aldrich, Brazil).

Eight batches (N = 8) of each treatment, from individual loins, were manufactured to evaluate the influence of enhancement (NC, PC, PE, and SE) and refrigerated storage (0, 5, and 9 days) on pH, instrumental colour, lipid oxidation and protein oxidation of ground lamb. Analysis of variance at 95% of confidence level (P < 0.05) was carried out to determine the significance of the independent factors (treatments and storage days) followed by Tukey’s test utilizing XLStat software (Addinsoft, Paris, France).

3. Results and discussion

3.1 Proximate composition and pH value

The ground lamb exhibited 66.47 ± 1.00 g/100g moisture, 18.32 ± 0.39 g/100g protein, 9.05 ± 0.02 g/100g ash, and 13.53 ± 0.14 g/100g lipid. The values of proximate composition of ground LD were similar to the ones reported in the literature for the lean LD (Fernandes, Freire, Carrer, & Trindade, 2013; Jaworska, Czaudema, Przybyski, & Rozbicka-Wieczorek, 2016). Nonetheless, in the present study the lipid content was greater than the values reported in the literature due to the formulation effect as the ground lamb contained the fat layer that was not trimmed from the LD in order to emulate a high fat content in a raw lamb meat product.

The pH for peel and seed extract presented values of 2.84 and 2.48, respectively. The pH value of ground lamb was not affected (P > 0.05) by the addition of either BHT, PE, or SE (Table 1). The pH values of lamb meat are in the range of 5.4

Table 1. Instrumental colour and pH values of ground lamb (N = 8) enhanced with Myrciaria dubia (camu camu) extracts and stored at 4 °C for 9 days.

| Parameter | NC | PC | PE | SE |
|-----------|----|----|----|----|
| L* (lightness) | 0 | 43.93 ± 1.90 | 43.19 ± 1.52 | 45.66 ± 2.56 | 43.99 ± 1.06 |
| 5 | 47.03 ± 0.71 | 46.53 ± 1.04 | 45.25 ± 0.53 | 42.67 ± 1.03 |
| 9 | 46.65 ± 1.32 | 45.43 ± 0.89 | 45.67 ± 1.83 | 43.30 ± 1.12 |
| a* (redness) | 0 | 15.88 ± 1.87 | 14.42 ± 1.47 | 11.13 ± 0.71 | 15.60 ± 1.87 |
| 5 | 7.31 ± 0.53 | 7.12 ± 0.92 | 6.93 ± 0.88 | 5.61 ± 0.86 |
| 9 | 7.15 ± 1.12 | 8.06 ± 0.65 | 6.51 ± 1.09 | 6.71 ± 0.93 |
| b* (yellowness) | 0 | 18.14 ± 1.81 | 17.19 ± 0.54 | 16.29 ± 1.53 | 13.33 ± 1.61 |
| 5 | 13.05 ± 0.90 | 13.09 ± 1.13 | 11.93 ± 1.29 | 9.72 ± 0.93 |
| 9 | 12.87 ± 1.12 | 12.27 ± 0.77 | 11.25 ± 1.17 | 10.19 ± 1.31 |
| pH | 0 | 5.50 ± 0.10 | 5.48 ± 0.03 | 5.33 ± 0.11 | 5.61 ± 0.18 |
| 5 | 6.15 ± 0.24 | 6.23 ± 0.28 | 6.06 ± 0.18 | 6.01 ± 0.34 |
| 9 | 6.16 ± 0.26 | 6.28 ± 0.26 | 6.16 ± 0.17 | 6.12 ± 0.14 |

NC: negative control; PC: positive control (100 mg/kg of BHT); PE: 100 mg/kg of peel extract; and SE: 100 mg/kg of seed extract. a–c: Means with different letters within a column indicate difference amongst days of storage (P < 0.05). x–z: Means with different letters within a row indicate difference amongst enhancement (P < 0.05).
3.2 Instrumental colour

The addition of either peel or seed extract did not affect (P > 0.05) the L* values at the beginning of the refrigerated storage (day 0; Table 1). However, at day 9 the patties enhanced with SE exhibited lower L* values (P < 0.05) than the other treatments. Differently than other treatments, SE patties exhibited the highest protein oxidation throughout the refrigerated storage, potentially affecting the L* values. Oxidation of meat pigments is responsible for the decrease of lightness values in meat (Villalobos-Delgado, González-Mondragón, Govea, Andrade, & Santiago-Castro, 2017). In addition, the decrease in lightness is potentially due to the characteristic dark colour of extracts (Selani et al., 2011). In the present study, PE and SE presented L* values of 26.90 and 28.70, respectively. M. dubia PE was dark-red in colour (a* 8.00; b* 2.82), while SE exhibited an orange colour (a* 2.58; b* 20.12). Beef patties enhanced with edible plants extracts (crown daisy, pumpkin, chamnamul, fatsia, leek, bok choy, acanthopanax, butterbur, soybean, and flower heads of broccoli) exhibited a decrease in L* values (Kim et al., 2013b).

Peel extract decreased lamb patties redness (a* values) at the beginning of the refrigerated storage (day 0; P < 0.05), whereas NC presented the highest value for redness 15.88; PE exhibited the lowest value at 11.13. Whilst at day 9, BHT-enhanced patties (PC) had the highest redness 8.06 (P < 0.05), negative control an intermediate value (7.05), and PE the lowest redness at 6.51. However, neither the synthetic antioxidant (BHT in PC) nor the M. dubia extracts (PE and SE) were able to attenuate the decrease on redness. Oxidation of myoglobin into metmyoglobin negatively affects the redness values (Kim, Cho, & Han, 2013a; Realini, Guárda, Díaz, García-Regueiro, & Amau, 2015) therefore, it was expected that by increasing the levels of reducing chemicals it would result in decreased pigment oxidation and improved colour stability. In addition, according to Zang et al. (2016) the carotenoids level of extracts may increase the red colour, however M. dubia fruits may not contain sufficient levels of carotenoids (Rufino et al., 2010) to affect the a* values in ground lamb.

Yellowness (b* values) was affected (P < 0.05) by the addition of seed extract, at day 0 NC showed the highest b* value (18.14) while SE the lowest (13.33). However, at days 5 and 9 the treatments with M. dubia extracts presented a more pronounced decrease (P < 0.05) in yellowness than NC and PC. At the end of storage (day 9), NC kept the highest yellowness (12.87) and SE the lowest (10.19). Previous authors reported that red grape pomace extract, decreased yellowness in pork burgers due to the phenolic compounds that act as potent free radical scavengers preventing lipid oxidation (Garrido et al., 2011). Radical species from lipid oxidation may also promote the oxidation of colour pigments and the formation of metmyoglobin. Metmyoglobin levels were among the most important factors contributing to meat redness and yellowness (Luciano et al., 2009).

The ΔE values in the Table 2, describe the colour differences between treatments. A ΔE of 1 or less is not perceptible by human eyes (Sharifzadeh, Clemmensen, Borggaard, Stoier, & Ersboll, 2014). All the treatments at days 0, 5 and 9 of storage presented perceptible differences of colour; whereas only NC and PC at day 5 presented ΔE value lower than 1. ΔE consists of the changes in lightness, redness and yellowness. The variation presented for PE and SE in those parameters (Table 1) may explain the different values of ΔE for patties with peel and seed extracts. This behaviour can be explained by the compounds found in each extract (Fracassetti et al., 2013).

### 3.3 Lipid oxidation

Peel and seed extracts affected (P < 0.05) the lipid oxidation values (Figure 1); in general, throughout the whole storage period lamb patties enhanced with PE and SE exhibited lower (P < 0.05) TBARS values than NC and PC counterparts. Similar to the present study, other authors reported a decrease on the lipid oxidation rate in ground beef enhanced with Plinia cauliflora peel and leafy green vegetable extracts (Almeida et al., 2015; Kim et al., 2013a). As expected, the refrigerated storage promoted an increase (P < 0.05) on lipid oxidation potentially due to enzymatic and non-enzymatic reactions (Grau, Guardiola, Boatala, & Codony, 2000; Selani et al., 2011).

The protective effect of the M. dubia extracts on lipid oxidation may be associated with the phenolic molecules present in this fruit. Previous reports documented that M. dubia fruit contains considerable amounts of anthocyanins (42.2 mg/100g), flavonoids (20.1 mg/100g), carotenoids (0.4 mg/100g), and ascorbic acid (1,882 mg/100g), which

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**Table 2.** Colour difference between ground lamb (N = 8) enhanced with Myrciaria dubia (camu camu) extracts and stored at 4 °C for 9 days.

| Parameter | Days | NC-PC | NC-PE | NC-SE | PC-PE | PC-SE | PE-SE |
|-----------|------|-------|-------|-------|-------|-------|-------|
| ΔE        | 0    | 1.89  | 5.38  | 4.82  | 4.23  | 4.11  | 5.61  |
|           | 5    | 0.52  | 2.14  | 5.74  | 1.75  | 5.36  | 3.64  |
|           | 9    | 1.64  | 2.00  | 4.31  | 1.87  | 3.26  | 2.60  |

NC: negative control; PC: positive control (100 mg/kg of BHT); PE: 100 mg/kg of peel extract; and SE: 100 mg/kg of seed extract.

NC: control negativo; PC: control positivo (100 mg/kg de BHT); PE: 100 mg/kg de extracto de cáscara; y SE: 100 mg/kg de extracto de semilla.
exhibit antioxidant activity and the ability to quench free radicals (Kaneshima et al., 2016; Marie et al., 2014; Rufino et al., 2010). Furthermore, anthocyanins, flavonoids, carotenoids and ascorbic acid may participate in the stabilization of lipid free radicals into less reactive forms, resulting in the protection against lipid oxidation (Jridi et al., 2018). Jia et al. (2012) attributed the inhibitory effect of black currant extract on lipid oxidation to its phenolic compounds and other biochemical compounds that contribute to the antioxidant activity. The present study illustrates the efficiency of the peel and seed extracts elaborated from M. dubia processing on the prevention of lipid oxidation in lamb patties.

### 3.4 Protein oxidation

Protein oxidation in ground lamb was evaluated by the formation of protein carbonyls (Figure 2). In this study,
only PC provided a protection against protein oxidation represented by lower (P < 0.05) carbonyl levels than the other treatments at the end of refrigerated storage (day 9). Furthermore, the addition of SE promoted an increase (P < 0.05) on the protein carbonyls content at day 0; and from day 5 until the end of the refrigerated storage, SE, PE, and NC exhibited similar (P > 0.05) carbonyl content values. It was previously reported that seed of *M. dubia* has higher contents of ellagitannins than pear and pulp (Fracassetti et al., 2013) and different content of phenolic compounds may exhibit pro-oxidant effects in a protein-liposome system (Viljanen, Kylli, Kivikari, & Heinonen, 2004).

The influence of fruit extracts on protein oxidation in meat products is a controversial topic as some authors documented that extracts from strawberry tree fruit, hawthorn, dog rose, elm-leaf black-berry (Ganhão et al., 2010), white grape (Jongberg, Torgren, Gunvig, Skibsted, & Lund, 2013), and dog rose (Armenteros, Morcuende, Ventanas, & Estévez, 2013) exerted antioxidant activity against the formation of protein carbonyls in meat, whereas other authors documented no protective effect against protein carboxylation when cured beef and pork hams were enhanced with apple pomace (Sun, Zhang, Zhou, Xu, & Peng, 2010). These conflicting results may be attributed to differences on the structure and levels of phytochemicals present in fruits (Estévez, Kylli, Puolanne, Kivikari, & Heinonen, 2008; Jia et al., 2012) and the extraction procedure (Jia et al., 2012; Jongberg et al., 2013). Moreover, plant phenolics are known as redox–active molecules that present antioxidant activities depending on their concentrations and the presence of other compounds (Sabeena Farvin, Grejsen, & Jacobsen, 2012; Zhang, Lin, Leng, Huang, & Zhou, 2013). The interaction between phenolic compounds and the formation of protein carbonyls are not well known, thus further investigations are necessary to improve the understanding about such mechanisms (Jia et al., 2012; Zhang et al., 2013).

The present study demonstrated that peel and seed extracts of *Myrciaria dubia* protected ground lamb against lipid peroxidation, however this effect was not observed on protein oxidative stability. These results suggest that the replacement of artificial antioxidants by *M. dubia* peel and seed extracts can be considered a viable solution in order to improve the lipid oxidative stability during the storage of lamb with minimal negative effect on the colour parameters. Further studies are necessary to improve the processing yield, and to identify and isolate the active phytochemicals from *M. dubia* by-products in order to optimize the application of extracts and promote the development of lamb products.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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