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Oxidative Stability of Lamb Meat Patties From *Longissimus dorsi* Muscle Stored Under Refrigeration

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Abstract

The aim of this study was to evaluate the oxidative stability of fresh lamb patties from *Longissimus dorsi* (LD) muscle, obtained from animals slaughtered at 10 months-age, during 9 days under refrigerated storage. Six batches (n = 6) of lamb patties were prepared and analyzed for thiobarbituric acid-reactive substances (TBARS), and for carbonyl content throughout 9 days at 4 °C. TBARS levels were stable ($p > 0.05$) during first five days of storage and a decrease ($p < 0.05$) in lipid oxidation was observed on day 9. Carbonyl content increased ($p < 0.05$) up to day 5, remaining constant ($p > 0.05$) from day 5 to day 9. We concluded that fresh lamb patties from LD muscle of animals slaughtered with 10 months of age was relatively stable to oxidative process during refrigerated storage without additional treatment, which may represent a viable and interesting alternative for refrigerated meat market.

Keywords: Meat cut, Lipid and protein oxidation, TBARS, Carbonyl content, Storage quality

1. Introduction

The sheep meat production have been increasing in the last years (FAO, 2017), and lamb meat is preferred by consumers due to milder flavor and greater softness than sheep meat (Pinheiro et al., 2009). Moreover, lamb meat presents higher polyunsaturated fatty acid (PUFA) content compared to beef and pork meat, which are important for human health (Wood, 1999). Nevertheless, due to their nutritional composition, this meat is highly prone to oxidation (Bonoli et al., 2007; Cunha et al., 2018; Guedes-Oliveira et al., 2018).

Lipid and protein oxidation are key factors for changes of color, flavor and texture, which are related to freshness, wholesomeness and, therefore, consumer acceptance (Canto et al., 2016; Mariutti & Bragagnolo, 2017). Myoglobin oxidation leads to formation of metmyoglobin (MetMb) resulting in meat discoloration (Mancini & Hunt, 2005), while protein cross-linking formation or protein breakdown cause texture changes (Lorido et al., 2016; Rodrigues et al., 2017). On the other hand, lipid oxidation generates secondary compounds responsible by the development of off-flavors (low molecular weight compounds), and color and texture changes (high molecular weight compounds) (Mariutti & Bragagnolo, 2017). Additionally, some authors suggest an relationship between both oxidative mechanisms, where protein oxidation

release large amounts of free iron, which act as pro-oxidant accelerating the lipid oxidation, and secondary compounds from lipid oxidation may react with amino acids, peptides and proteins favoring the protein oxidation (Wongwichian et al., 2015; Babakhani et al., 2016).

Due to high perishability, lamb meat is mostly marketed in frozen-form (Fernandes et al., 2013), however, efforts have been made to consumers choose fresh foods instead of frozen and processed foods (Oliveira & Silva-Amparo, 2018). In addition, the decrease in fresh meat consumption can affect the meat production sector impairing the agribusiness activity. Nonetheless, muscle source and slaughtering age directly influence on oxidative stability (Canto et al., 2016; Malva et al., 2016). *Longissimus dorsi* (LD) muscle is predominantly constituted of glycolytic fibers, which have lower amount of myoglobin, lipid, and oxygen consumption rate as well as higher endogenous functional compounds (Jayasen et al., 2015) than oxidative muscle fibers, and therefore are less prone to oxidation (Wilson et al., 1976; Morcuende et al., 2003). On the other hand, slaughtering age affects the levels of lipid and myoglobin, saturated/unsaturated fatty acids ratio, and endogenous antioxidant system of meat (Wen et al., 2015; Malva et al., 2016). Therefore, LD muscle from lambs slaughtered at 10 months of age could be an alternative to encourage the fresh meat local market promoting social and economic benefits in lamb-meat producing countries. In this context, the aim of the present study was to investigate the oxidative stability of lamb LD muscle obtained from animals slaughtered at 10 months-age and stored at 4 °C during 9 days.

2. Material and Methods

2.1 Experimental Design and Lamb Patties Preparation

Six (n = 6) *Longissimus dorsi* (LD) muscle (24 hour post-mortem) of Texel sheep were obtained of different animals slaughtered under humanitarian conditions at an age of 10 months in a commercial processing facility (Americana, São Paulo). The animals were pasture-fed until 3 months age and, then were confined and fed with a concentrated mix diet (corn silage, soybean meal, and mix of vitamins and minerals). Lamb LD muscles were vacuum-packed, placed in an ice-box, and transported to the laboratory. LD muscle was milled, homogenized in mixer and shaped into patties (5.5 cm diameter, 1.5 cm thickness, 35 g weight) using polystyrene petri dishes (60 mm × 15 mm). The fresh lamb patties were individually packaged on polystyrene trays with soaker pads, over-wraped with polyvinyl chloride (PVC) film (0.014 mm thickness, Orleplast Ind. Com. de Plásticos Ltda, Orleans, Santa Catarina, Brazil), and stored at 4 °C during 9 days. Lamb patties from LD muscle were analyzed for proximate composition (day 0), and for lipid and protein oxidation (days, 0, 5, and 9). All analyses were carried out in duplicate.

2.2 Determination of Proximate Composition

Moisture, protein and ash contents were determined according to the AOAC methods (AOAC, 2012). The moisture was determined by weight difference after samples drying (100-102 °C) until constant weight (AOAC method 950.46B). The protein content was estimated through Kjeldahl method using 6.25 as conversion factor (AOAC method 955.04), while the ash was determined gravimetrically after sample incineration in a muffle furnace at 550 °C (AOAC method 920.153). The total lipid content was cold-extracted and quantified using procedures described by Bligh and Dyer (1959).

2.3 Lipid Oxidation Measurement

The thiobarbituric acid-reactive substances (TBARS) were determined according to the method of Sinnhuber and Yu (1958) with modifications (Buege & Aust, 1978). Sample (5 g) was homogenized with 22.5 mL of trichloroacetic acid (TCA) aqueous solution at 11% (w/v) in an Ultra Turrax 18 basic (IKA, Wilmington, NC, USA). A sample alíquot (1.5 mL) was centrifuged at 15,000 × *g* for 15 minutes at 4 °C, 1 mL of the supernantant was transferred to a tube, and then 1 mL of thiobarbituric acid (TBA) aqueous solution at 20 mM (w/v) was added. The mixture was vortexed for 5 seconds, incubated for 20 hours in dark condition, and absorbance values were measured at 532 nm through UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The results were expressed as absorbance values.

2.4 Protein Oxidation Measurement

The carbonyl content was quantified using procedures described by Oliver et al. (1987) adapted by Armenteros et al. (2009) and Mercier et al. (1998). Sample (3 g) was homogenized with 30 mL of KCl aqueous solution at 0.15 M (w/v; pH 7.4) in an Ultra Turrax 18 basic (IKA, Wilmington, NC, USA) during 90 seconds, and then centrifuged at 15,000 × *g* for 20 minutes at 4 °C. Two equivalent supernatants (0.1 mL each) were introduced in eppendorf tubes, 1 mL of TCA at 10% (w/v) was added, the mixture was vortexed, and then centrifuged at 5,000 × *g* for 5 minutes at 4 °C. The supernatants were gently discarded and the residues were added of 1 mL of HCl at 2 N (v/v) for determination of protein content or 1 mL of 2,4-dinitrophenylhydrazine (DNPH) at 10 mM (w/v) for determination of carbonyl content. The mixtures were incubated in dark condition during 1 hour and were vortexed every 15 minutes. Before the last homogenization in vortex, 1 mL of TCA at 10% (w/v) was added, following centrifugation at 11,000 × *g* for 10 minutes at 4 °C. The supernatants were slowly discarded, and the residues were washed thrice through 1 mL of ethanol/ethyl acetate solution (1:1, v/v). At each washing step, the supernatant was discarded using a pippete, the mixture was vortexed and centrifuged at 15,000 × *g* for 10 minutes at 4 °C. After that, the final protein precipitate was dissolved in 1 mL guanidine hydrochloride at 6 M (w/v) in sodium phosphate buffer at 20 mM (w/v) with pH 6.5, and centrifuged at 11,000 × *g* for 10 minutes at 4 °C to remove insoluble particles. The protein content was determined at 280 nm, while carbonyl content was read at 370 nm using an UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The results were expressed as nmol of carbonyls/mg of protein.

2.5 *Statistical Analysis*

This experiment was repeated six times (n = 6). The differences of means between days of storage (0, 5, and 9) were evaluated by one-way ANOVA followed by Tukey's test at 95% of confidence level ($p < 0.05$) using XLSTAT software (Addinsoft, Paris, France).

3. Results and Discussion

3.1 Proximate Composition

The results of moisture, protein, lipid, and ash were 66.47±1.00, 18.32±0.39, 11.53±0.14, and 0.95±0.02, respectively. Similar results of protein and ash were observed in lamb meat (Babiker et al., 1990; Fernandes et al., 2012; Malva et al., 2016). In contrast, variations on moisture and lipid contents were found in literature. Babiker et al. (1990), Fernandes et al.

(2012) and Malva et al. (2016) observed higher moisture and lower lipid contents in lamb meat as compared to our findings. Amongst nutritional parameters, lipid content is mostly variable depending mainly on the slaughtering age, diet and muscle source (Canto et al., 2016; Malva et al., 2016). The studies evaluating proximate composition of lamb *Longissimus dorsi* (LD) muscle are scarce. Nonetheless, the lipid content tends to increase as slaughtering age increases due to consumption of concentrated feed for a longer period until slaughter (Malva et al., 2016). In addition, moisture content is inversely related to lipid content (Hunt and Hedrick, 1977). Tejeda et al. (2008) and Malva et al. (2016) observed higher moisture and lower lipid contents in lamb meat as compared to our findings, however, these authors evaluated younger lambs (40–75 days of age), explaining the results of the present study.

3.2 Lipid Oxidation

The TBARS values of the lamb patties from LD muscle maintained stable ($p > 0.05$) up to day 5 and, after this period, TBARS values decreased ($p < 0.05$) until 9th day of refrigerated storage (Figure 1).

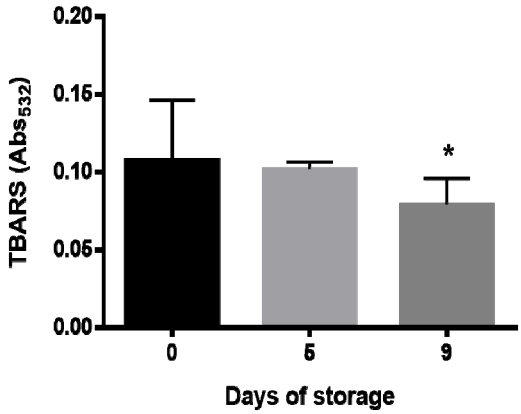

Figure 1. Absorbance values of thiobarbituric acid reactive substances (TBARS) at 532 nm of lamb patties from *Longissimus dorsi* (LD) muscle stored at 4 °C for 9 days

Error bars indicate means ± standard deviation (n = 6).

Means with asterisk (*) indicate significant difference ($p < 0.05$).

Although older lambs have higher intramuscular lipid content than younger lambs, meat from lambs slaughtered older has higher amount of saturated fatty acids (Malva et al., 2016), which are stable to lipid oxidation (Pegg & Shahidi, 2012). In addition, glycolytic muscles such as LD present lower lipid content, greater glycogen levels and endogenous functional compounds compared to oxidative muscles (Wilson et al., 1976; Lefaucheur, 2010; Peiretti et al., 2012). Jayasen et al. (2015) suggested that the anaerobic energy supply is most important for glycolytic muscles and, therefore, this type of muscle fiber require high levels of carnosine and anserine, which act as pH buffers in muscle in order to neutralize the pH shifts due to lactic acid accumulation. Moreover, anserine and carnosine convert free radicals in more stable products, due to ability to donate electrons, and cease the radical chain reactions (Peiretti et al., 2012), explaining our results.

The lipid oxidation may be affected by muscle source and composition of the lipid fraction, which is related to age at slaughter and diet (Oriani et al., 2005; Canto et al., 2016; Malva et al., 2016). However, there are lack of studies comparing the lipid oxidation in different lamb muscle sources as well as among different slaughtering ages and feed systems. Similarly, Luciano et al. (2012) observed a stability in TBARS values up to day 7 of refrigerated storage in lamb meat from animals slaughtered with 90 days of age and raised on pasture-feed system. Another study demonstrated that TBARS values maintained stable during 8 days of storage at 4 °C in lamb *Longissimus dorsi* muscle from animals submitted to pasture-feed system and slaughtered with 150 days of age (Luciano et al., 2013). Smeti et al. (2013) reported that TBARS values increased only on 6th day of refrigerated storage in *Longissimus dorsi* muscle of lambs slaughtered with 60 days of age and fed with pasture and concentrate.

The lowering in TBARS values on day 9 may be attributed to instability of the malondialdehyde during storage period, which can react with products from protein degradation (Giménez et al., 2002). The same phenomenon was observed in camel meat and pork meat stored under refrigeration (Maqsood et al., 2015; Li et al., 2017).

3.3 Protein Oxidation

The carbonyl content of the lamb patties from LD muscle are shown in Figure 2. The carbonyl content increased ($p < 0.05$) from day 0 to day 5, while maintained stable ($p > 0.05$) during the last days of refrigerated storage (days 5-9).

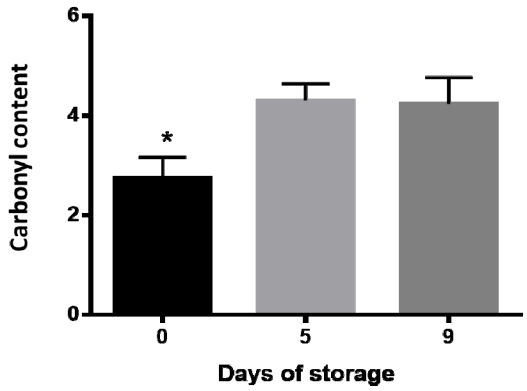

Figure 2. Carbonyl content (nmol of carbonyls/mg of protein) of lamb patties from *Longissimus dorsi* (LD) muscle stored at 4 °C for 9 days

Error bars indicate means ± standard deviation (n = 6).

Means with asterisk (*) indicate significant difference ($p < 0.05$).

LD is a glycolytic muscle and, therefore, has high glycogen levels favoring accumulation of lactic acid and increased post-mortem acidity (Lefaucheur, 2010). This fact accelerate the protein denaturation and improves the activity of proteolytic enzymes making proteins susceptible to oxidation (Cobos & Díaz, 2015). In addition, muscles from older animals tend to have a higher myoglobin content than younger animals (Lawrie, 1985), and the protein oxidation occur faster than the lipid oxidation (Santé-Lhoutellier et al., 2008), mainly when

exposed to oxygen environment, which accelerates the oxidation of proteins (Gómez & Lorenzo, 2012). Nonetheless, animals slaughtered older have usually a greater endogenous antioxidant capacity since it is determined by the energy metabolism and hypoxic response for prevent against free radicals and reactive oxygen species (Wen et al., 2015). On the other hand, LD muscle exhibit lower amount of myoglobin and oxygen consumption rate compared to oxidative muscles (Wilson et al., 1976; Morcuende et al., 2003). Therefore, our findings may be explained by balance between the protein susceptibility to oxidation from increased post-mortem acidity and endogenous antioxidant system more active in older animals. In agreement with our results, Ortuño et al. (2015) evaluated lamb meat from animals slaughtered at an age of 90 days, and observed an initial increase on carbonyl content from day 0 to day 7, while it remained constant from day 7 to day 11 of refrigerated storage. Santé-Lhoutellier et al. (2008) also found similar pattern on protein oxidation of lamb meat (M. longissimus dorsi) from animals slaughtered at 220 days. These authors reported that carbonyl content increased rapidly up to day 2 and, after this period, carbonyl levels tended to remain stable until the last day of refrigerated storage (day 7).

4. Conclusion

The results obtained in this study indicate no relationship between lipid and protein oxidation of LD muscle from lamb meat, which could be attributed to muscle-specific composition and biochemistry as well as slaughtering age. Fresh patties from LD muscle of lambs slaughtered at 10 months of age had a relative stability to oxidative process during 9 days at 4 °C without need of any additional treatment, representing an attractive alternative for improving the fresh meat local market of lamb-meat producing countries.

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