TENDERNESS AND OXIDATIVE STABILITY OF BEEF IN VACUUM AND MODIFIED ATMOSPHERE PACKAGING

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Abstract – During processing, storage and tenderization, meat is prone to biochemical changes due to proteolytic and oxidative processes. The demand for high-quality *in natura* products has contributed to the meat industry's development of new technologies, such as modified atmosphere packaging and tenderization, that provide uniform tenderness. Accordingly, the objective of this study was to evaluate the influence of vacuum and modified atmosphere packaging (MAP) on the protein and lipid oxidation and tenderness of fresh beef. *Longissimus thoracis* (LT) muscles from Nellore bulls (*Bos indicus*) were portioned into steaks 1.5 cm in thickness and distributed among several packaging treatments: vacuum; 75%O_2/25%CO_2 (75%O_2); 60%CO_2/0.2%CO/39.8%N_2 (0.2%CO) and 40%CO_2/0.4%CO/59.6%N_2 (0.4%CO) and held at 2 °C for 28 days of storage. The parameters studied were protein and lipid oxidation and the myofibrillar fragmentation index (MFI). A gradual tenderization of the meat was detected in the different packaging systems; however, tenderness was reduced by oxygen (O_2) levels that were high in comparison to MAP anaerobic conditions. The high O_2 accelerated protein oxidation process, which can affect the enzymes responsible for natural meat tenderization more than either a vacuum or atmospheres containing low concentrations of carbon monoxide (CO). This was corroborated by the analysis of MFI. The lipid oxidation increased when the storage period was prolonged to 28 days, regardless of modified atmosphere packaging.

Keywords – *Longissimus thoracis*, oxidation, proteolysis.

I. INTRODUCTION

Brazil, as a world leader in beef exports, is adapting to international market demands by investing new production patterns, processing and marketing to achieve the increasingly high levels of quality demanded by consumers. The beef production chain in Brazil is arranged and classified according to animal of origin: steers, bulls or cows. Meat from steers has the best quality attributes, and it is commonly vacuum packed and intended for foreign markets. Conversely, meat from bulls and cows is sold on the local market undifferentiated, at a lower price.

Among the technologies developed and applied by the food industry in other countries, MAP aims to maintain the quality and appearance of fresh meat during distribution and marketing through centralizing operations, labeling and product distribution and extending storage life. MAP is not a new concept in meat preservation, but it represents a potential marketing strategy for the national industry that supports the replacement of the traditionally distributed whole vacuum pieces.

Details are still limited in Brazil regarding the applicability of MAP and its effect on the quality parameters of beef from *Bos indicus* (Nellore) during tenderization. In this context, this paper presents original information on the protein and lipid oxidative stability and tenderness of steaks obtained from the *Longissimus thoracis* (LT) of bulls after refrigerated tenderization (2 °C) in different packaging systems for 28 days.

The objective of this study was to evaluate the lipid and protein oxidative stability and tenderness fresh meat from bulls in vacuum packaging and MAP.

II. MATERIALS AND METHODS

A. Animals and sampling

LT muscles from grass-fed bovine Nellore bulls (*Bos indicus*) between 30 and 36 months of age were used. The animals were slaughtered in a local slaughterhouse, and de-boning was performed 24 hours *postmortem*. Muscles were removed from the left side of half-carcasses (n=17), kept refrigerated at 2 °C and butchered 48 hours *postmortem* in an air-conditioned environment at 15 °C.

Steaks weighing 250 g that were 1.5 cm in thickness were placed in thermoformed laminate trays (polyester/polyethylene 23.6 x 16.4 x 4.5 cm) for MAP and shrink bags for vacuum packaging (Bemis Company®-Dixie Toga). Liquid-absorbing pads (Dri-loc, Cryovac®, Sealedair) with and absorbing capacity of 100 ml were placed in MAP trays. Sachets of self-activated O_2 were used (Post Soft Brazil®, LTDA.) in anaerobic trays with an absorption capacity of 300 cm^3. The cover trays closing the laminate film used an antifog system (moisture) and polyethylene sealant layer, polyamide outer layer and a barrier layer of ethylene vinyl alcohol (Bemis Company® - DixieToga); the thickness was 102 μm, the O_2 permeability was 2.5 $cm^3/m^2/24$ h, the temperature was 23 °C, the relative humidity (RH) was 0%, and the rate of water vapor permeability was 7 $g/m^2/24$ h, 38 °C and 90% RH. One hundred twenty experimental units with two LT slices each were packaged in vacuum and MAP containing the following: $75\%O_2/25\%CO_2$ ($75\%O_2$), $60\%CO_2/0.2\%CO/39.8\%N_2$ (0.2%CO) or $40\%CO_2/0.4\%CO/59.6\%N_2$ (0.4%CO). The storage periods were 0, 7, 14, 21 and 28 days.

B. Protein oxidation

5,5-dithiobis (2-nitrobenzoic acid) (DTNB) was used for the determination of free thiol groups in proteins [1]. The thiol content was calculated as nmol free thiol/mg protein. Triplicate measurements were performed for each meat sample, and the mean values were used for the statistical analysis.

C. Lipid oxidation

The extent of lipid oxidation was measured according to thiobarbituric acid reactive substance (TBARS) content according to the extraction method described by Vyncke [2] and modified by Sørensen & Jørgensen [3] with trichloroacetic acid (TCA) and 2-thiobarbituric acid (TBA). TBARS were measured in replicates and were expressed as mg malonaldehyde/kg meat.

D. Myofibrillar fragmentation index (MFI)

The MFI was determined for the LT samples using the method described by Culler *et al*. [4]. The absorbance was multiplied by 200 to generate a MFI value. Each sample was analyzed three times, and the mean values were used for the statistical analysis.

E. *Statistical analysis*

The statistical analysis was performed using the SAS 9.0 package, SAS Institute, Inc., Cary, NC. The data were analyzed using a randomized 4x5 (packaging x time) factorial design with three replicates per treatment. To investigate the differences between the treatments, variance analysis (ANOVA) was conducted. The differences between the means were analyzed using the Tukey test ($p < 0.05$).

III. RESULTS AND DISCUSSION

The protein oxidation process values represented by the free thiols in the steaks stored in vacuum and MAP at 2 °C for 28 days are shown Figure 1. For all of the packaging types, there was a tendency toward protein oxidation. The steaks in $75\%O_2$ had lower levels of free thiols at the end of the experimental period than those in the other types of packaging ($p < 0.05$). The high O_2 content in the atmosphere and prolonged storage encourage the acceleration of protein oxidation in meat and negatively influence its oxidative stability [5]. A recent study observed a decrease in beef steak tenderness when the beef was stored under high concentrations of O_2, and it determined that O_2 gas is the greatest facilitator of protein oxidation [6].

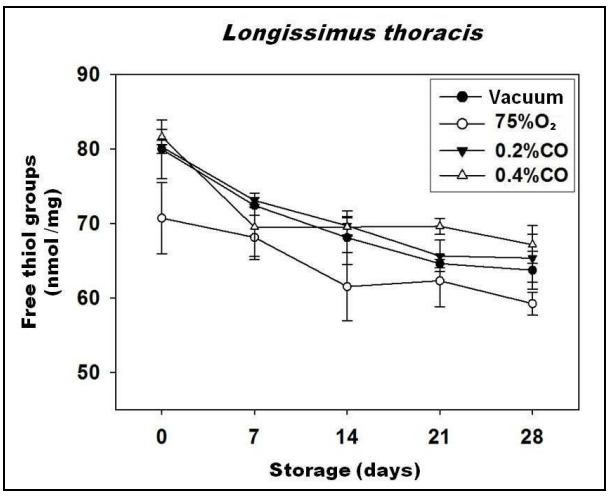

Figure 1 – Free thiol groups (nmol/mg) extracted with 5% SDS from LT bull steaks in vacuum and modified atmosphere packaging for up 28 days at 2 °C.

Steaks stored in anoxic packaging (vacuum, 0.2%CO and 0.4%CO) were less susceptible to protein oxidation, and a decelerate oxidation process could be observed. In this study, the protein oxidation for steaks in CO MAP less than that of vacuum-packed steaks, but the results were not significantly different.

The steaks stored under the highest CO concentration (0.4%CO) demonstrated lower protein oxidation at the end of the storage period. This phenomenon may be associated with CO binding to the muscle pigment myoglobin to produce carboximyoglobin, which is much more stable against oxidation than oxymyglobin due to the stronger binding of CO to the iron-porphyrin site on the myoglobin molecule [7]. Additionally, carboximyoglobin favors the formation of a desirable red color in meat [8] and wide acceptability by the consumer; commercially, this is a comparative advantage to traditional packaging under vacuum.

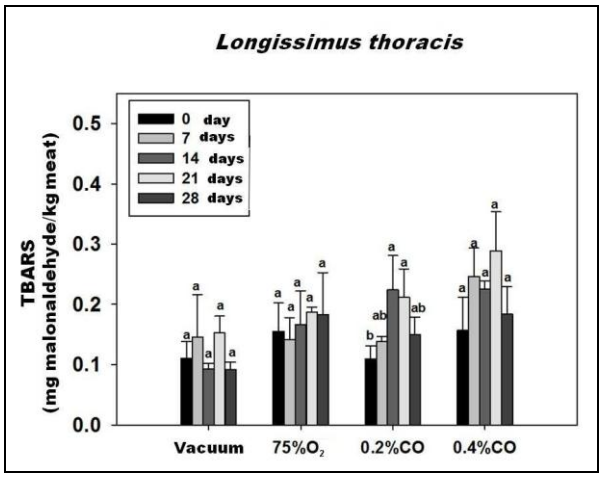

Figure 2 - TBARS (mg malonaldehyde/kg meat) from LT bull steaks in vacuum and modified atmosphere packaging for 28 days at 2 °C.

In contrast to protein oxidation, lipid oxidation showed unstable behavior throughout the experimental period (Figure 2). The TBARS values for the steaks in different packaging conditions infrequently increased up to 21 days of storage. However, there was a not significant reduction in the TBARS values for all of the package types on the 28th day of storage. This behavior may be associated with the combination of malonaldehyde with meat proteins to form stable compounds that lead to an underestimation of the final TBARS value [9].

At the end of the experimental period (28 days) higher TBARS values were observed in steaks in MAP (75%O_2; 0.2%CO and 0.4%CO) than those in vacuum packaging. Previous studies have suggested that high levels of O_2 and/or CO_2 in MAP, combined with prolonged periods of storage, promote the oxidation of lipids [10, 11].

The MFI of LT bull steaks in vacuum and MAP are shown in Table 1. Significant differences (p < 0.0001) were found in the MFI values in the initial days of the experiment (0 day), 48 hours *postmortem*.

Table 1. MFI from LT bull steaks in vacuum and modified atmosphere packaging for 28 days at 2 °C.

Time (days)	Packaging system			
	Vacuum	75%O_2	0.2%CO	0.4%CO
0	91.3 ± 3.9^{Aa}	65.3 ± 3.8^{Ab}	32.2 ± 2.8^{Cc}	40.7 ± 2.6^{Cc}
7	59.7 ± 1.3^{Cb}	33.4 ± 4.5^{Bc}	80.6 ± 3.0^{Ba}	53.6 ± 7.1^{Bb}
14	64.7 ± 1.0^{BCb}	63.5 ± 5.3^{Ab}	102.5 ± 3.0^{Aa}	64.9 ± 1.1^{ABb}
21	70.1 ± 2.7^{Bb}	58.5 ± 3.4^{Ab}	94.3 ± 7.7^{Aa}	71.6 ± 5.5^{Ab}
28	88.5 ± 2.2^{Aa}	57.6 ± 4.1^{Ac}	92.3 ± 3.0^{Aa}	71.9 ± 2.9^{Ab}

^{AB} Means in a column of subscribed letters differ significantly (p < 0.05). ^{ab} Means in lines with subscripted letters differ significantly (p < 0.05).

This phenomenon is associated with wide variability in animals; certain steaks suffered accelerated fragmentation within 48 hours *postmortem*, probably due to factors such as stress pre-slaughter or pH [12]. There were significant effects (p < 0.0001) for different the types of packaging throughout the experimental period. After 28 days of storage, the steaks in CO MAP showed a significant increase in MFI compared to the initial day due to the typical process of meat proteolysis [12]. However, the MFI in vacuum and 75%O_2 packaging showed no significant difference in MFI values at 28 days of storage when compared to the initial day.

The steaks stored in 75%O_2 had markedly smaller MFI values at the end of the experiment compared to the samples stored in oxygen-free (vacuum,

0.2%CO and 0.4%CO) packaging. The decreased MFI obtained from the high-oxygen atmospheres may be caused by protein cross-linking; the formation of cross-linked myosin-heavy chains has been shown to occur in pork samples after storage in 70%O_2/30%CO_2 [5], which corresponds to the 75%O_2/25%CO_2 condition in the present study. Another possible explanation may be that the degree of proteolysis was decreased due to the inactivation of μ-calpain, which has been shown to take place in beef [13].

Corroborating this study, Clausen *et al*. [14] studied steaks from *Longissimus dorsi* stored for 20 days at 4 °C in different atmospheres containing 50% and 80% O_2 and found that high O_2 levels had a negative influence on meat tenderness. The tenderness index was significantly correlated with the oxidative stability of proteins ($p < 0.01$, r = -0.2594) and lipids ($p < 0.05$, r = 0.1983) for the different atmospheres and storage periods (n=120).

IV. CONCLUSION

Based on the results from this study, we can conclude that the high O_2 levels in modified atmosphere packaging affects both tenderness and protein oxidation. This may reflect the cross-linking of proteins and/or reduced proteolysis. Lipid oxidation increased when the storage period was prolonged from 7 to 28 days, regardless of whether modified atmosphere packaging was used.

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