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Lipid and Protein Oxidation in Charqui Meat and *Jerked Beef*

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ABSTRACT

In this study, the changes in the lipid (Lox) and protein oxidation (Pox) were measured quantitatively by TBARS and carbonyl methods, respectively, throughout the salting and drying steps of charqui meat (CH) and jerked beef (JB) preparation and their storage up to 60 days. The experiment was carried out on CH samples treated with brine (20.0%) and JB with same brine solution added with sodium nitrite (0.02%). After 60 days of storage, the carbonyl substances in CH were 2.77nmol mg^{-1} while in the JB samples, there was 61.0% oxidation inhibition. The TBARS determination revealed a Lox inhibition by approximately 5-fold in the latter samples. These results indicated that in the metmyoglobin molecule, the nitrite kept the Fe in the Fe^{2+} state in JB samples whereas in CH, the Fe was oxidized to Fe^{3+}, which catalyzed the oxidation reactions more efficiently, leading to the higher development of Lox and Pox.

Key words: protein oxidation, charqui, jerked beef

INTRODUCTION

Lipid (Lox) and Protein oxidations (Pox) are the resultant of an imbalance of pro-oxidant/antioxidant contents. Therefore, it is necessary to reach the oxidation homeostasis conditions in the food system in order to avoid them. By its composition and because of the harsh conditions to produce Charqui, this popular salted meat product seems to be excellent model to examine further these oxidative reactions. According to the Brazilian legislation, Charqui meat (CH) should contain 40-50% moisture and 10-20% salt (Brasil 1962) and 0.75 is the final value of its water activity, ranking it as an intermediate moisture meat product (Torres et al. 1994; Shimokomaki et al. 1998). On the other hand, a derivative cured meat product, commercially known as *Jerked beef* (JB) has officially been defined by the Brazilian legislation as having maximum 55% moisture, 50 ppm sodium nitrite, 18% salt a final a_w value of 0.78 and should be vacuum packed (Brasil 2000). Thus, technologically JB is an improvement from CH (Shimokomaki et al. 2003). Both products are the results of the so-called hurdle technology application in their processing (Leistner 1987; Torres et al. 1994). As recently described, salt, sodium nitrite, dehydration and packaging are hurdles sequentially applied to inhibit the

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microorganism's development (Shimokomaki et al. 1998). There is a selection for the halophylic bacteria in the particular fermentative microorganisms as demonstrated by Pinto et al. (2002). The conditions of CH preparation have been discussed elsewhere (Shimokomaki et al. 1998). The addition of NaCl in the meat preparation under these conditions changes the meat functional properties, in particular alter the iron state from ferrous (Fe^{2+}) of oxymyoglobin to ferric (Fe^{3+}) of metmyoglobin through the Fenton reactions, thus becoming higher meat pro-oxidant (Youssef et al. 2003). Under these conditions, NaCl is capable of enhancing the production of ROS catalyzing, in cascade reactions, the meat lipids and protein oxidation measured by the TBARS and carbonyl substances, respectively (Youssef et al. 2003; Montero et al. 2005).

There are several reports on the application of antioxidants in order to minimize these undesirable reactions in the food systems (Park et al. 2006; Ooizumi and Xiong 2006; 2008; Lund et al. 2007). Cured salts are essential ingredients for meat processing and nitrite plays a strong antioxidant role as originally reported by Pearson et al. (1977). Nitrite also acts as lipids antioxidant in the mechanical deboning of chicken meat (Trindade et al. 2008) and also in CH and JB (Youssef et al. 2003; 2011). Thus, the objective of this work was to determine quantitatively the amount of lipids and proteins oxidations through the TBARS and carbonyl determinations, respectively. The inhibition of these oxidations by sodium nitrite was evaluated throughout the charqui meat and jerked beef processing and storage up to 60 days.

MATERIALS AND METHODS

Meat samples

Zebu (Bos indicus) meat samples were taken from *Vastus lateralis m.* and kindly donated by a commercial charqui (n=6) and jerked beef (n=6) industry located in the Londrina city, Parana state.

Charqui meat and Jerked beef processing

The CH processing steps followed the techniques described elsewhere (Shimokomaki et al. 1998). Essentially, brine of 20% NaCl (w/v) was automatically injected in *Vastus lateralis* m. followed by dry salting on a concrete floor for five days. Subsequently, after washing to remove the excess of meat surface salt, samples were submitted to drying directly in the sun and wind, hung upon stainless steel rails under a maximum temperature of 40-45°C in the summer. The dried and salted products were finally vacuum packed. The JB followed similar processing, except that sodium nitrite (0.02%) was added to the brine for the injection salting step.

Sample preparation for analysis

Samples of 16g in duplicate were dialyzed against the distilled water in order to remove the salt residue and freeze dried for further analysis.

Metmyoglobin determination (MMb)

Five grams of ground desalted and freeze dried CH and JB were solubilized in 25 mL of phosphate buffer (pH 6.8) according to Krzywicki (1982). This solution was treated in a homogenizer (Turrax) at 13,500 rpm for 10s and left for 1h. The mixture was centrifuged at 5,000g and 4°C for 30min. The supernatant was filtered in a Whatman #1 paper. The absorbance of the filtrate was read in a spectrophotometer at 525, 572 and 700nm and the MMb concentration was calculated using equation 1:

$$MMb=\{1.395-[(A_{572}-A_{700})/(A_{525}-A_{700})]\}x100 \quad \text{(equation 1)}$$

A_{525}= absorbance at 525nm of the supernatant
A_{572} = absorbance at 572nm of the supernatant
A_{700}= absorbance at 700nm of the supernatant.

Metmyoglobin was evaluated in triplicate and expressed in percent (%).

Lipid oxidation determination

Lipid oxidation was evaluated in triplicate following Tarladgis et al. (1964) method as described in Soares et al. (2004). The results were expressed in mg TBARS kg^{-1} of the sample.

Protein oxidation determination

Protein oxidation in triplicate was determined by the derivatization with 2,4-dinitrophenylhydrazine as described in Fagan et al. (1999). Briefly, 3.0g of freeze dried samples in triplicate were solubilized in 25.0mL of buffer (pH 7.4), containing $Na_4P_2O_7$ (2.0mM), Tris-maleate (10mM), KCl (100mM), $MgCl_2$ (2.0mM) and EGTA (2.0mM). The mixture was homogenized in Turrax twice at 8,000g in an ice bath and centrifuged at 800g and 4°C for 20min. The supernatant was filtered through the cheesecloth and was further centrifuged at 38,720g and 4°C for 1h. After filtration through the

cheesecloth, an aliquot of 1.0mL was treated with 5.0mL the HCl/acetone (3:1) and centrifuge twice for 20min at 4°C. The pellet was treated with 5.0ml of 10% TCA. After centrifugation at 800g and 4°C for 20min, finally, the pellet was dissolved in 1.0mL buffer as described with 1.0ml of DNPH solution. Blanks were prepared by adding 1.0mL buffer and 1.0mL 2.0M HCl to a separate aliquot of albumin solution, which was left under agitation in darkness at 4°C for 60min. Trichloroacetic acid was added to a final concentration of 7%, and the samples were centrifuged at 11,000g for 20 min and the pellet was treated three times with ethanol/ethyl acetate (1:1) in 10mM HCl and centrifuged at 800g and 4°C for 20min. The pellet was solubilized with guanidine (6.0M) in phosphate buffer (pH 2.3) and after centrifugation, the carbonyl concentration was read at 380nm and plotted in an albumin solution standard curve. The carbonyl content was determined spectrophotometrically at 380nm, using a molar absorbance coefficient of $22{,}000^{M-1}cm^{-1}$.

Statistical Analysis

All results were analyzed using the program STATISTICA for Windows version 5.0 (StatSoft, 1995). The effect of antioxidant application was evaluated through the ANOVA and the Tukey test (5%) was used to assess the significance of difference.

RESULTS

Metmyoglobin content (MMb)

Table 1 shows the MMb amount found throughout the processing and storage of the CH and JB. The amount of MMb in the CH was approximately 3-fold in relation to JB samples, irrespective of storage time ($p<0.05$).

Table 1 - Metmyoglobin level (%) found in charqui meat and in jerked beef throughout processing and storage up to 60 days.

Days	Charqui meat	Jerked beef
1 (brining)	20.27^{a} ±1.43	5.41^{b} ±0.32
8 (after drying)	68.50^{c} ±2.11	20.46^{d} ±1.76
30 (storage)	72.05^{e} ±2.32	25.56^{f} ±1.1
60 (storage)	71.86^{g} ±2.05	25.84^{h} ±1.4

Triplicate mean with different letters on the same line differs significantly ($p<0.05$) by the Tukey test.

Lipid oxidation

Table 2 shows the TBARS values found in the CH and in JB samples. At first day of processing, the TBARS results did not show any difference ($p<0.05$). However, from the 8th days of storage, the TBARS values increased gradually, reaching its maximum at the 60th day of the storage.

Table 2 - Levels of TBARS (mg kg^{-1}) found in charqui meat and jerked beef throughout processing and storage up to 60 days.

Days	Charqui meat	Jerked beef
1	0.057^{a} ± 0.002	0.023^{a} ± 0.002
8	0.11^{b} ± 0.009	0.04^{c} ± 0.003
30	0.11^{d} ± 0.008	0.045^{e} ± 0.002
60	0.25^{f} ± 0.011	0.05^{g} ± 0.003

Triplicate mean with different letters on the same line differs significantly ($p<0.05$) by the Tukey test.

Protein oxidation

Table 3 showed that the carbonyl substances found in both the samples were not different on the 1st and 8th days of processing. However, there was a gradual increase during the storage, reaching a maximum at 0 days of the storage. Moreover, from the 30th day, the levels of carbonyl substances were significantly higher in the CH ($p<0.05$) compared to JB.

Table 3 - Levels of carbonyl substances (nmol mg^{-1}) of charqui meat and jerked beef stored up to 60 days.

Days	Charqui meat	Jerked beef
1	$0.32^{a} \pm 0.04$	$0.28^{a} \pm 0.03$
8	$0.70^{b} \pm 0.05$	$0.54^{b} \pm 0.06$
30	$1.07^{c} \pm 0.08$	$0.76^{d} \pm 0.07$
60	$2.77^{e} \pm 0.12$	$1.08^{f} \pm 0.08$

Triplicate mean with different letters on the same line differs significantly ($p<0.05$) by the Tukey test.

DISCUSSION

These results corroborated clearly that under the harsh conditions of the CH and JB processing, the oxidative reactions occurred not only for the Lox as reported previously (Torres et al. 1989; Youssef et al. 2003) but also for the Pox.

However, there was difference in intensity pattern during the storage for these reactions as Lox appeared to occur more intensely after eight days of processing (Table 2) as previously reported (Youssef et al. 2003) increasing approx. 5-fold at the 60^{th} day of storage. Pox started occurring after the 30^{th} day after initiation of salting (Table 3), reaching maximum value at the 60^{th} day of storage. This study showed that the pigment myoglobin suffered changes under the high salt concentration. The MMb amount was approximately 3-fold in the CH, showing the deleterious role of NaCl over myoglobin from the start of salting up to 60 days of storage. Nitrite protected this oxidation from occurring in the JB (Table 1). It was clear that from the start to approximately eight days of processing, the ROS components were being synthesized and becoming available for the further oxidation first of lipid and finally of protein. Previously studies have shown that free radicals were formed and hexanal was detected at first days under the salting process (Torres et al. 1988). Therefore, the possibility of Lox development could not be ruled out as further free radicals would be formed enhancing ultimately the Pox reactions. Several authors have reported the existence of direct relationship between the disruption of heme molecule during the heat treatment and the free iron would increase its concentration having as consequence gradual oxidative degradation in the cooked meat (Miller et al. 1994). Estévez et al. (2004; 2005) reported a significant correlation between the concomitant increase of free iron and the TBARS content. The results reported herein also showed that there was a direct relationship between the concentration level of the MMb and TBARS, suggesting that Fe^{3+} formation was induced by the oxidative environment promoted by the salting conditions catalyzing ROS synthesis as previously observed (Youssef et al. 2003).

Therefore, the higher availability of the ROS increased the oxidative reactions for both the Lox (Table 2) and Pox (Table 3). The inhibition of both the Lox and Pox in JB was most probably due to the presence of sodium nitrite packed under the vacuum. The present results corroborated the previous observation for the Lox in the JB samples and for the first time for the Pox. At least two possibilities existed to explain the role of nitrite as an oxidation inhibitor in the cured samples. Firstly, it chelated the free Fe^{3+} formed because of the high salt concentration, and thus, its pro oxidant was inhibited. Secondly, it reacted with the meat pigments, thus forming nitrosylmyoglobin-Fe^{2+} and enhancing the oxidation, while CH–free nitrite samples would form MMb-Fe^{3+}, favoring these oxidation reactions. The harsh conditions of the CH processing under the high concentration of salt and by drying under the sun at 40-45^{o}C for several days would denature the myofibrillar proteins (Shimokomaki et al. 1998) and collagen (Biscontini et al. 1996), thus, the gradative protein denaturation was positively correlated with the Lox as reported in dark chicken meat (Jing Li and King 1996).

CONCLUSIONS

Both, the lipids and proteins oxidations occurred during the salting, drying and storage of the charqui meat and jerked beef. However, there was a time difference in their installation: lipids oxidation being the first to occur and later protein oxidation possibly by the gradual formation of the ROS from the lipid oxidation. The presence of sodium nitrite was capable of partially inhibiting these undesirable reactions throughout the processing and storage of the jerked beef.

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