PROTEIN AND MYOGLOBIN OXIDATIVE STABILITY DURING 120 DAYS OF FROZEN STORAGE OF BURGERS ADDED WITH OREGANO EXTRACT

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Abstract – The aim of this study was to evaluate the influence of the addition of different amounts of oregano (*Origanum vulgare*) natural extract in relation to oxidative parameters of lamb burgers during 120 days of frozen storage. A total of five batches was prepared: without antioxidants (control), with sodium erythorbate (ER) and three with different concentrations of natural extract [ORE-1, ORE-2 and ORE-3, calculated according to three different colorimetric methods: Folin-Ciocalteau, DPPH· radical inhibition and FRAP, respectively]. Burgers were submitted to measurements of color stability (as oxy/metmyoglobin ratio, reflectance at 630/580 nm) and the protein oxidation was evaluated by the loss of thiol groups. Regarding color, results showed that metmyoglobinwas increased (P<0.001) during storage time for all the batches, presenting at 120 days lower values in the samples containing ER (P<0.001).However, lamb burgers with higher contents of oregano extract (ORE-2 and ORE-3) presented (P<0.05) higher thiol values. In conclusion, the addition of oregano extract, in concentrations calculated by DPPH· and FRAP methods, can represent a good alternative to frozen meat products, being effective against protein oxidation, despite negative effects in color stability along 120 days storage at -18±1ºC.

Key Words – *Origanum vulgare*, metmyoglobin, thiol.

I. INTRODUCTION

Numerous free radicals generated are associated with the oxidative degradation of proteins, and the myoglobin is one of the proteins most susceptible to oxidation in meat and meat products. Oxymyoglobin [MbFe(II)-O_2] is the heme-protein responsible for the red color, being the formation of metmyoglobin [MbFe(III)] resultant of their oxidation, promoting the discoloration of meats [1].

Herbal and plant extracts have been reported as effective in the oxidative protection of meat and their derivatives due to their high content of phenolic compounds with capacity to scavenge reactive radicals [2].

Nowadays, the meat industry is increasingly searching for natural alternatives, with similar effects to synthetic antioxidants, to minimize oxidative process of meat and meat products and, in addiction, aiming the production of healthier food [3].

Thus, the objective of this study was to evaluate the effects of oregano extract as natural antioxidant, added to lamb burgers in different concentrations, calculated from the results of antioxidant equivalence to sodium erythorbate, as well as to verify protein and myoglobin stability of the meat products during 120 days of frozen storage (-18±1ºC).

II. MATERIALS AND METHODS

A. Obtaining of natural extract

The extract of oregano was obtained in triplicate using a solvent mixture of acetone, ultrapure water and acetic acid glacial, respectively 70, 28

and 2% at a ratio of the 1:50 (g/mL). After grinding, agitation, centrifugation, filtration, to each 20 mL of extract concentrated and lyophilized, was performed the ressuspension of the samples in 5 mL ultrapure water [4].

B. Evaluation of antioxidant capacity to definition of natural extract concentrations

The extract, as well as the sodium erythorbate, were evaluated for phenolic compounds by Folin-Ciocalteau, and antioxidant activity by $DPPH^{\cdot}$ (2,2-diphenyl-1 picrylhydrazyl) and FRAP (Ferric ion Reducing Antioxidant Power), as described by Wootton-Beard *et al.* [5], with modifications. From these results, three volumes of natural extract to be added in the burgers were calculated in order to show equivalent antioxidant power as 500 ppm sodium erythorbate.

C. Processing of the burgers

Burger formulations consisted of meat (84%), and fat trimmings (14%) of lamb, salt (2%) and antioxidant (natural or synthetic). The meat and fat were thawed at 4 ºC for 12 hours and minced separately using disc of 4 mm. Five batches were manufactured in three different times: control without antioxidants (CO), with sodium erythorbate (ER) and with three amounts of natural extract (ORE-1, ORE-2 and ORE-3).

The burgers were formed using a manual molder (112 mm diameter x 2 cm height) and individually separated with polyethylene films, weighing 95-100 grams. Burgers were frozen in ultra freezer and packed in polypropylene bags, being immediately stored at -18 ºC. A total of 30 burgers were analyzed (5 batches x 3 different times of manufacture x 2 samples of each batch) in each sampling point.

D. Indirect estimate of metmyoglobin

The burgers were subjected to objective color analysis through evaluation system L*, a* and b* parameters of system CIE-L*a*b*, using a portable colorimeter (MiniScan XE, HunterLab, USA), with illuminant D65, observation angle of 10º and open cell of 30 mm [6]. Six readings at different points of the surface of each burger were measured after atmospheric exposure.

The objective color measures allowed to indirect estimate metmyoglobin formation,in relation to oxymioglobin, from the ratio between reflectance values of 630 and 580 nm, obtained from spectral curves according to Pearce *et al.*[7].

E. Quantification of thiol groups

Protein oxidation was determined based on the procedure of Liu *et al.* [8], with modifications. The protein content total was quantified at 540 nm by using biuret reagent as standard. In duplicate, 0.5 mL of the samples (1 mg/mL) was dissolved in 2.0 mL urea-SDS solution (8.0M urea, 3% SDS, 0.1M sodium phosphate, pH 7.4) and incubated with 0.5 mL 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) reagent (10 mM in 0.1M phosphate buffer, pH 7.4) at room temperature for 15 min. Thiol groups, based in the absorbance of DTNB at 412 nm, were calculated using a molar absorptivity of 13.600 $M^{-1}.cm^{-1}$. The results were expressed nmol of thiol/mg of protein.

F. Statistical analysis

The results were analyzed using analysis of variance (ANOVA) from IBM SPSS Statistics 17.0 (IBM Corporation, Somers, NY, USA). A Duncan's test was performed to compare the mean values at a significance level of $P<0.05$.

III. RESULTS AND DISCUSSION

According to the results of antioxidant capacity, the concentrations of natural extracts was defined as 1.33, 1.78 and 2.40 mL/100 g, respectively based on Folin-Ciocalteau (ORE-1 burger), $DPPH^{\cdot}$ (ORE-2 burger) and FRAP (ORE-3 burger) colorimetric methods.

With respect to indirect estimate of metmyoglobin formation during storage, one observed decreasing values that indicate a significant increase on formation of superficial metymioglobin in relation to oxymioglobin ($P<0.001$) in all batches (Fig. 1). This behavior is similar to the observed by

Georgantelis *et al.* [9] in studies of frozen beef burgers.

The wavelength of 580 nm indicates the peak of absorption to oxymioglobin and 630 nm to metmyoglobin. This relation is an indicative of superficial oxidation of myoglobin, and the lower is this ratio (approaching to 1, the higher is the formation of metmyoglobin, with consequent discoloration [7].

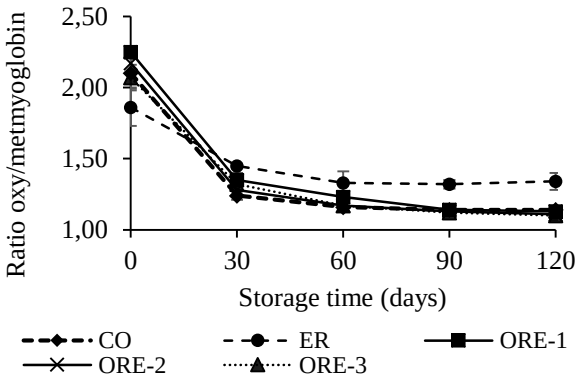

Figure 1. Formation of metmyoglobin during frozen storage.

During the storage time, there was a reduction of the values ($P<0.001$) and at 120 days, the samples containing sodium erythorbate presented a higher value (1.34±0.06) ($P<0.001$), less close to "1", in comparison to other samples, due to less formation of metmyoglobin.

However, the CO batch presented higher metmyoglobin formation ($P<0.001$), did not differing from the batches containing natural antioxidants ($P>0.05$), being this behavior probably attributed to the interference of extract color and chlorophyll oxidation, as verified by Akarpat *et al.* [10] in frozen burgers with water extract of nettle, stored by 120 days.

A lot of studies proved that the discoloration can be accelerated by lipid oxidation. But, beyond this behavior, the protein oxidation also is considered one of major causes of quality deterioration in meat and meat products [11, 12, 13, 14].

So, the oxidation of heme pigments can occur in consequence to protein oxidation, being the myoglobin a hemoprotein. The oxidative degradation results in denaturation of the globin and cleavage of the hematin pigment, and consequently in the release of heme iron, which promotes discoloration [15].

Despite Pearson's linear correlation coefficient confirming that the content of free thiols was positively related with the values of metmyoglobin (r= 0.387, $P<0.01$). With respect to loss of thiols, there was no differences ($P>0.05$) among all batches until 60 days, being observed maintenance of protein stability ($P>0.05$) in the ORE-2 e ORE-3 batches along the storage period evaluated. In addition, at 120 days these same batches presented higher values of free thiol groups ($P<0.01$), respectively of 49.13±1.97 and 47.98±4.07 nmol/mg of protein, proving occurrence of a lower oxidative stress of these samples (Fig. 2).

These results are similar to other authors, who found inhibitory effects of protein oxidation with the addition of natural antioxidants [16, 17].

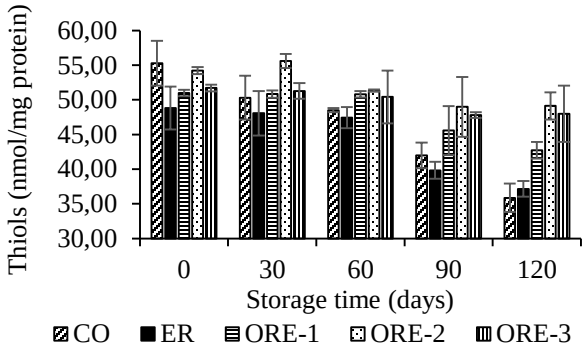

Figure 2. Quantification of thiol groups during frozen storage.

Thus, the ER batch showed less deterioration of color ($P<0.001$) at final of storage, because is a water-soluble compound which protected the pigment, while the extract inhibited more against oxidation. The protein radicals formed can induce the lipid oxidation, and consequently, the pigments may be also affected by pro-oxidant action [18].

IV. CONCLUSION

One can conclude that the natural antioxidant oregano extract, added to lamb burgers at

concentrations based on the results of DPPH$^{\cdot}$ and FRAP methods, is a good alternative to use in frozen meat products replacing synthetic sodium erythorbate, showing significant inhibition of protein oxidation, despite possible interferences in color stability.

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