PROTEIN OXIDATION DURING FROZEN STORAGE OF BEEF PATTIES WITH ADDED DOG ROSE (*Rosa canina L.)* EXTRACT

Mariana Utrera[1], David Morcuende[1] and Mario Estévez[1*]

[1]Department of Animal Production and Food Science (Food Technology Section), Faculty of Veterinary, University of Extremadura, Cáceres, Spain

Abstract – The effects of frozen storage and the addition of Rosa canina L. (RC) extract on protein oxidation, moisture losses and hardness of beef patties was investigated. Protein oxidation occurred during the frozen storage of beef patties as the amount of specific protein oxidation markers, amino adipic semialdehyde (AAS) and aminoadipic acid (AAA), increased over time. The susceptibility to undergo further protein oxidation during processing was enhanced by frozen storage. The addition of the RC extract inhibited the formation of aminoadipic acid (AAA). The antioxidant effect may respond to the protecting effect of phenolic compounds, mainly procyanidins. However, the addition of RC incremented water release and hardness of beef patties, possibly due to the formation of bounds between phenolics and proteins.

Key Words – Hardness, Phenolic compounds, Lysine oxidation

I. INTRODUCTION

Frozen storage is one of the most valuable preservation methods for meat since allows distribution in a larger extent, and is used to retard undesirable biochemical reactions and stop microbial spoilage in meat [1]. However, mechanical damage on cell structure and cryoconcentration of solutes in the unfrozen phase are induced during freezing due the formation and growth of ice crystals [2]. Therefore, some deteriorative changes on proteins have been mostly ascribed to denaturation during frozen storage causing changes in meat tenderness [2]. However, more recently proteins have been identified as possible targets for oxidative reactions [3], and a loss of meat quality has been ascribed to protein oxidation. These chemical reactions may have an impact on the water holding capacity, redness and tenderness of meat [3]. Lysine is one of the most susceptible amino acids to oxidation [3]. The oxidative deamination of lysine leads to the formation of α-aminoadipic semialdehyde (AAS), which can undergo further oxidation into α-aminoadipic acid (AAA) [3]. Nowadays, the antioxidant activity of natural materials rich in phenolic compounds is being extensively investigated as synthetic antioxidants have been linked to potential health hazards. However, contradictory effects of phenolic compounds on the oxidative stability of meat proteins have been reported [3]. Extracts of rose hips (Rosa canina L., RC), have shown high antioxidant activities in vitro and the addition to porcine burger patties has resulted in delaying oxidation [4]. Nevertheless, the overall effects of phenolic compounds are driven, among a diverse variety of factors, by the oxidative conditions generated during processing of meat [3]. Hence, the use of RC in different meat products needs to be further investigated.

The objective of this study is to investigate the effectiveness of RC extracts as inhibitors of protein oxidation when added to beef patties subjected to frozen storage.

II. MATERIALS AND METHODS

A. Materials

All chemicals were supplied from Merck (Merk, Darmstadt, Germany) and Sigma Chemicals (Sigma–Aldrich, Steinheim, Germany). Water used was purified by passage through a Milli-Q system (Millipore Corp., Bedford, MA, USA). Beef *quadriceps femoris* muscles belonged to industrial genotypes slaughtered and purchased in a local slaughterhouse in Cáceres (Spain). Meat was freed from visible fat, immediately chopped into pieces (2 cm^3), kept at 4°C during 8 h and used as such for patties manufacture.

B. Methods

Manufacture of porcine patties Depending on the addition of the phenolic-rich extract, two types of patties were prepared, control and treated. The basic recipe was as follows (g/kg raw batter): 732 g meat, 244 g distilled water and 24 g sodium chloride. In treated patties 50 g of the distilled water were replaced by 50 g of RC extract. All ingredients were minced in a cutter until a homogeneous raw batter was obtained (9 min). Burger patties were formed using a conventional burgermaker (~80 g/patty), to give average dimensions of 10 cm diameter and 1 cm thickness. In total, 8 burger patties per type were prepared in two independent manufacturing processes. Patties were stored in individual oxygen permeable polyethylene bags and dispensed in trays. Frozen patties (F) were subsequently stored for 20 weeks at -18°C in the dark. After the frozen storage, patties were thawed at 4°C during 10 h. Four patties were subjected to analysis (frozen raw patties, FR), while the other four were placed on trays and cooked at 170 °C for 18 min in a forced-air oven. After cooking, the remaining four samples were allowed to cool down at room temperature and analyses were performed (frozen cooked patties, FC). Meanwhile, fresh patties were immediately analysed (unfrozen raw patties, UFR) or subject to cooking (unfrozen cooked patties, UFC) as described before.

HPLC-FLD analysis of AAS Samples (5 mg protein) were derivatized with 50 mM p-aminobenzoic acid (ABA) and subsequently hydrolyzed with 6N HCl according to the procedure described by Utrera, et al [5]. Hydrosylates were dried in vacuo, reconstituted with 200 µL milli-Q water and filtered through PVDF syringe filter. Samples were injected in a HPLC as described by Utrera et al [5]. Peaks areas corresponding to AAS-ABA were plotted against an ABA standard curve. Results are expressed as mmol of carbonyl compound per mg of protein.

HPLC-FLD analysis of AAA Samples (5 mg protein) were hydrolyzed with 3 N HCl and subsequently derivatized with 0.2 mM 9-fluorenylmethyl chloroformate (FMoc) according to the procedure described by Utrera et al. [5]. Samples were injected in a HPLC as described by Utrera et al [5]. Peaks areas corresponding to AAA-FMoc were plotted against a standard curve. Results are expressed as mmol of AAA per mg of protein.

Moisture loss determination Moisture losses of patties were calculated as percentage of water weight lost after each process stage (thawing, and cooking loss).

C. Statistical analysis

Data were analyzed by Analyses of variance (ANOVA) using a one-way model and Tukey tests at 95% confidence level by SPSS (v. 15).

III. RESULTS AND DISCUSSION

The amount of AAS significantly increased during frozen storage up to 3 fold-times in beef patties (Table 1). The present results confirm that frozen storage enhanced the oxidation of lysine as a result of the release of catalytic iron by the disruption of cell membranes and the probable concentration of pro-oxidant solutes around proteins. In this oxidative environment, proteins are exposed to and readily accessed to radicals, and react primarily with more susceptible amino acids [3]. In addition, cooking of both UF- and F-patties caused increments in the amount of AAS. However, the increment was higher in F-patties than in the unfrozen counterparts. Results denoted that the high temperatures reached during cooking also promoted the oxidative deterioration of lysine by membrane disruption and inactivation of antioxidant enzymes [6].

Table 1. AAS (mmol/mg protein) in beef patties

	Control		Treated	
	UF	F	UF	F
Raw	$101.1^{b,y}$ ±21.7	$287.9^{a,y}$ ±55.1	$93.5^{b,y}$ ±17.9	$224.8^{a,y}$ ±37.4
Cooked	$173.4^{c,x}$ ±39.6	$930.1^{a,x}$ ±103.9	$151.8^{c,x}$ ±25.6	$668.3^{b,x}$ ±121.8

Data are expressed as means ± standard deviation. Means with different superscript (a-b) within a row and (x-y) within a column are significantly different ($p<0.05$)

No significant effect was exerted by the addition of RC in the formation of AAS in UF-patties (Table 1). In contrast, an antioxidant effect was exerted in F-patties after cooking, which could be attributed to the ability of RC

phenolics to act as radical scavengers [7]. Procyanidins and catechins have been reported as the major components of RC extracts [4]. The antioxidant potential of these compounds is attributed to its chemical structure, mainly due the high number of hydroxyl groups [7]. In addition, procyanindins are known to be effective against a variety of radical species, principally superoxide anion, due the extensive conjunction between 3-OH and B-ring catechol groups which, together with abundant β_{4-8} linkages, endow a polymer with significant radical scavenging properties [7]. The contradictory effects of RC phenolics against lysine oxidation could be ascribed to the location of lysine residues in the protein since the susceptibility of amino acid side-chains in proteins to oxidation depends on the exposure to the aqueous medium and the nearby amino acids [3].The degree of polymerization of procyanidins may hinder the interaction between protein radicals and phenolics and in consequence, lysine radicals were stabilized in a minor extent in the treated UF-patties. On the other hand, the antioxidant effect observed in F-patties could be promoted by modifications on the electronic arrangement of proteins consequence of the oxidative loss of amino groups. These new electronic arrangement can modify water-protein and protein-protein interactions and caused a new spatial conformation of proteins [3], which, in turn, could lead to a major exposure of lysine to radicals.

The total amount of AAA only increased significantly in patties subjected to both frozen storage and cooking (Table 2), highlighting that the further oxidation of AAS into AAA requires strong oxidation conditions (the presence of oxygen and an oxidising agent, H_2O_2) [3].

Table 2. AAA (mmol/mg protein) in beef patties

	Control		Treated	
	UF	F	UF	F
Raw	$9.3^{ab,x}$ ±1.4	$10.8^{a,y}$ ±1.0	$8.1^{b,y}$ ±1.3	7.9^{b} ±0.8
Cooked	$11.9^{b,x}$ ±2.6	$17.6^{a,x}$ ±3.9	$16.2^{a,x}$ ±2.9	7.9^{c} ±1.6

Data are expressed as means ± standard deviation. Means with different superscript (a-b) within a row and (x-y) within a column are significantly different ($p<0.05$)

As expected, the technological process applied to patties probably caused damage in the cell structure of meat which resulted in water release [6]. Water release was induced by cooking in both, control and treated patties (Table 3). Major losses were found in F-patties as the physicochemical damage caused by ice crystals in myofibrils and the sarcolema hinders the total reabsorption of the water that migrated from sarcoplasm to the intercellular space because of osmotic changes during freezing, into myofibrils during thawing [8]. In addition, the alteration of the overall electrical arrangement of proteins resulted from the oxidative damage of proteins could lead to loss of water-protein interaction and the successive water release [3].

Table 3. Moisture losses (%) in beef patties

	Control		Treated	
	UF	F	UF	F
Purge	-	1.4^{b} ±0.2	-	1.6^{a} ±0.4
Cooking loss	30.4^{c} ±4.5	35.1^{b} ±2.2	35.9^{b} ±1.7	50.2^{a} ±2.3

Data are expressed as means ± standard deviation. Means with different superscript (a-b) within a column are significantly different ($p<0.05$)

Also, higher moisture losses were found in beef patties with added RC extract, mainly in raw F-patties and in cooked UF- and F-patties (Table 3). This could be ascribed to the probable loss of water-protein interactions that keep water absorbed into myofibrils, which are replaced by phenolic-protein bounds.

IV. CONCLUSION

Frozen storage induced the oxidation of proteins. Protein oxidation is involved in the loss of quality of beef patties. Addition of RC extracts to beef patties could have antioxidant and pro-oxidant effect towards the formation of protein oxidation products, since protein oxidation it is implicated in many different kinds of detrimental reactions. Data suggest that RC extracts have an antioxidant effect towards AAA formation, however it had the potential to contribute to AAS formation and the consequently increments in water release and hardness.

ACKNOWLEDGEMENTS

Mario Estévez thanks the Spanish Ministry of Science and Innovation for the contract through the "Ramón y Cajal (RYC-2009-03901)" program and the support through the project "*Protein oxidation in frozen meat and dry-cured products: mechanisms, consequences and development of antioxidant strategies*; AGL2010-15134". Mario Estévez thanks the European Community for the economical support from the Marie Curie Reintegration (ERG) Fellowship (PERG-GA-2009-248959 - Pox-MEAT). Mariana Utrera thanks the University of Extremadura (Uex) for the pre-doctoral grant (Human Resources Recruitment Program "C Action").

REFERENCES

1. Hui, Y. H. (2004). Handbook of frozen foods CRC Press.
2. Genot, C. (2003). Congelación y calidad de la carne. Editorial Acribia.
3. Estévez, M. (2011). Protein carbonyls in meat systems: a review. Meat Science 89: 259–279.
4. Ganhão, R., Estévez , M., Kylli, P., Heinonen, M. & Morcuende, D. (2010). Characterization of selected wild mediterranean fruits and comparative efficacy as inhibitors of oxidative reactions in emulsified raw pork burger patties. Journal of Agricultural and Food Chemistry 58: 8854–8861.
5. Utrera, M. & Estévez, M. (2012). Oxidation of Myofibrillar Proteins and Impaired Functionality: Underlying Mechanisms of the Carbonylation Pathway. Journal of Agricultural and Food Chemistry 60: 8002−8011.
6. Min, B.& Ahn, D. U. (2005) Mechanism of lipid peroxidation in meat and meat products: A review . Food Science and Biotechnology 14: 152-163.
7. Heim, K., Tagliaferro, A. R. & Bobilya. (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. The Journal of Nutritional Biochemistry 13: 572–584.
8. Calvelo, A. (1981). Recent studies on meat freezing. In Developments in meat science. Lawrie, R. Elsevier (pp 125-159).