EFFECT OF CURING AGENTS (NITRITE/ASCORBATE) ON MYOFIBRILLAR PROTEINS SUBJECTED TO OXIDATIVE STRESS

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Abstract – Protein oxidation in meat systems involves severe chemical modifications, impaired functionality and loss of nutritional value. Nitrite and ascorbate have been used as preservers in meat for ages while their impact on protein oxidation is mostly unknown. The present study is devoted to elucidate the effect of curing agents (nitrite/ascorbate) on the stability of myofibrillar proteins (MP) oxidized *in vitro*. Nitrite (75 and 150 ppm) had a negligible effect on oxidizing proteins while ascorbate (250 and 500 ppm) had one of the most intense antioxidant actions against meat protein carbonylation described to date. On the other hand, nitrite induced the formation of nitrosation products (3-nitrotyrosine) that ascorbate was, yet again, able to inhibit. The present study reports innovative interactions between oxidative and nitrosative reactions in MP and explains the role of common additives on these relevant events.

Key Words – Protein carbonylation, Protein nitration, Nitrite, Ascorbate

I. INTRODUCTION

Preservation of meat with nitrite or nitrate has become important to the meat industry in controlling meat spoilage and in producing safe and palatable meat products at ambient temperature (1). However, the addition of this additive to food systems is controlled owing to the formation of the potentially toxic nitrosamines and other related health risks. Among the positive aspects, nitrite offers protection against growth of spore forming bacteria (i.e. *Clostridium botulinum* strains) and is involved in the formation of the color of cooked and dry-cured meat products (1). While nitrite is attributed a potent antioxidant activity on lipids, the effect of nitrite on protein oxidation has been scarcely studied (2). Nitric oxide is known to react with myoglobin and has a central role in the stability of cured colour. The impact of nitrite and related species on the most abundant meat proteins (myofibrillar proteins) is poorly understood. Recent dissertations highlight the potential implication of reactive forms of nitrite, reactive –nitrogen species (RNS) on the oxidative stress of food components. In medicine, peroxynitrite, a reactive form of nitrite, is known to induce chemical changes in proteins and those are related to health disorders (3). The impact of RNS on meat proteins is mostly unknown.

Ascorbate is commonly used with nitrite in cured meats. In addition to its recognized antioxidant and reducing activity, the ability of ascorbate to inhibit the formation of nitrosamines is highly appreciated. While ascorbate is recognized as an efficient quencher of radical species, the protection of this additive against the oxidative damage to meat proteins is a subject to be studied. Protein oxidation affects the conformation and functionality of myofibrillar proteins, particular sensory traits, involves a loss of nutritional value, and a potential toxicity risk (4, 5). Therefore, the potential implication of common additives such as curing agents, on these deleterious changes is of indisputable technological and scientific interest. To elucidate the impact of nitrite and ascorbate on myofibrillar proteins subjected to an in vitro oxidative stress was the objective of this study.

II. MATERIALS AND METHODS

Myofibrillar proteins (MP) were extracted from porcine longissimus dorsi muscle according to the method described by Utrera & Estévez (6). MP (4 mg/mL) in 0.6 NaCl and 8M 100 mM phosphate buffer, were oxidized using a hydroxyl radical generating system (25 μM Fe (III); 2.5 mM H_2O_2) at 37 ºC for 4 days and constant stirring. Depending on the addition of nitrite (0, 75 and 150 ppm) and ascorbate (0, 250 and 500 ppm), 9 different reaction units were prepared in triplicate (n=3). Upon complexion of the oxidation assay, samples were analyzed for the concentration of the protein carbonyl α-amino adipic semialdehyde (AAS) and the nitrosation degree (ND) which is

defined as the average number of 3-nitrotyrosine (3NT) residues divided by the total number of tyrosine residues in a protein molecule. The former was quantified by HPLC-FLD upon derivatization with p-amino benzoic acid (ABA) (6) while the latter was quantified by spectrophotometry according to the procedure recently described by Yang et al. (7). Analysis of variance (ANOVA), Tukey tests and correlation coefficients were run using SPSS. Unscrambler program enabled the design of the experiment and the calculation of response surfaces.

III. RESULTS AND DISCUSSION

As expected, the hydroxyl-generating system induced efficiently the formation of AAS (Table 1). The formation of a protein carbon-centered radical in a susceptible lysine residue from MP is followed by the formation of a carbonyl compound upon the catalytic action of a transition metal such as iron (5). The AAS is used as a reliable marker of protein oxidation in medicine and food science (4, 5). In meat systems, the carbonylation of MP has been linked to an impaired functionality and loss of nutritional value (5).

Table 1 Concentration of AAS (nmol/mg protein) in MP isolates with added nitrite and ascorbate.

	Nitrite (ppm)			
Ascorbate (ppm)	0	75	150	p
0	19.29x±0.67	17.86x±0.31	19.18x±1.07	0.105
250	1.56y±0.34	1.48y±0.48	1.26y±0.26	0.620
500	1.52a,y±0.25	0.71b,y±0.18	0.97b,y±0.06	0.004
p	<0.001	<0.001	<0.001	

p: significance level. Different letters within the same column (x-z) and the same row (a-c) denote significant differences between means. Lack of letters in raws or in columns denotes lack of significant differences between means.

The addition of nitrite, alone, had no significant effect on the formation of AAS (Table 1). The antioxidant mechanisms ascribed to this compound, and that may be effective against lipid oxidation, are not applicable to oxidizing proteins. Only when ascorbate was added up to 500 ppm, nitrite had a significant inhibitory effect against AAS formation. The protection of ascorbate was already significant and considerably remarkable at 250 ppm. Increasing its concentration to 500 ppm did not lead to a significant improvement. The lowest concentration of AAS was found in reaction mixtures with 500 ppm ascorbate and 75/150 ppm of nitrite. This finding is of great technological interest as nitrite and ascorbate are commonly combined at these concentrations in cured meat products to guarantee color stability and inhibit microbial growth. The present results confirm that this arrangement is also successful to control protein carbonylation. However, the antioxidant effect of ascorbate alone was so intense, that nitrite may be regarded to play a secondary role. Ascorbate is known to be an efficient scavenger of reactive oxygen species (ROS) such as the hydroxyl radical, the likely initiator of protein carbonylation in the present experiment. The resulting radical is considerable stable and do not form further reaction species (8). The protective effect of ascorbate towards MP is considerably higher than that described in previous papers for plant phenolics and other phytochemicals (9).

3NT was found in MP incubated in the absence of nitrite, which is consistent with the basal levels usually reported for proteins in living systems and derived from the physiological nitrosative stress (7).

Table 2 Nitrosation degree (ND) in MP isolates with added nitrite and ascorbate.

	Nitrite (ppm)			
Ascorbate (ppm)	0	75	150	p
0	0.04c±0.01	0.13b,x±0.02	0.31a,x±0.05	<0.001
250	0.02c±0.01	0.07b,y±0.02	0.15a,y±0.02	<0.001
500	0.02±0.01	0.03z±0.01	0.04z±0.01	0.054
p	0.055	0.001	<0.001	

p: significance level. Different letters within the same column (x-z) and the same row (a-c) denote significant differences between means. Lack of letters in raws or in columns denotes lack of significant differences between means.

The present study confirms that the ND increases in the presence of nitrite in a highly oxidative environment. In the present of hydrogen peroxide, nitrite forms peroxynitrite, also detected in the present samples (data not shown). Therefore, both iron and nitrite likely competed with hydrogen peroxide for the formation of ROS and RNS,

respectively. Iron, which was added at considerably high levels, was probably more effective at decomposing the hydrogen peroxide through the Fenton reaction to yield hydroxyl radicals. This would explain the remarkable higher levels of AAS, compared to previous studies (6). Whereas the formation of 3NT was promoted in the present conditions, the formation of the RNS and therefore, the intensity of the nitrosation reactions may have been higher at lower concentrations of transition metal. This extent requires further confirmation though. The addition of ascorbate inhibited efficiently the nitrosation of MP. A dose-dependent anti-nitrosation effect was observed at 75 and 150 ppm of nitrite. At 500 ppm of ascorbate, the ND was similar to the basal, which means that ascorbate inhibited completely the nitrosation damage to MP.

IV. CONCLUSION

The present paper describes for the first time the impact of curing agents on the formation of specific markers of oxidative stress on proteins. To date, ascorbate is found to be one of the most efficient inhibitors of protein carbonylation. In addition, it is also efficient at blocking the nitrosation of MP. These results may be considered to optimize food formulation and minimize the oxidative and nitrosative damage to meat proteins.

ACKNOWLEDGEMENTS

M.E. receives support from the Spanish Ministry of Economics and Competitiveness (SMEC) through the contract RYC-2009-03901 and the project AGL2010-15134 and from the Research Executive Agency from the European Community through the Marie Curie Reintegration Fellowship (PERG05-GA-2009-248959; Pox-MEAT). Adriana Villaverde is supported by a predoctoral FPI grant from the SMEC.

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