A MIMETIC MODEL TO STUDY THE EFFECT OF MEAT COMPOUNDS ON PROTEIN MODIFICATIONS DURING HEATING

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Abstract – Meat cooking induces oxidations and conformation changes of proteins which can affect meat products quality and decrease their nutritional value. The aim of this paper was to investigate the effect of fiber type and cellular compounds (oxidants and antioxidants) on meat protein modifications (oxidation and denaturation) during heat treatments. Experiments were performed on a mimetic model composed of an aqueous suspension of myofibrillar proteins in a medium (pH and ionic strength) which reflected the muscle environment. Protein modifications were evaluated after 120 min heating (45°C or 75°C) by measurements of carbonyl groups and protein surface hydrophobicity. Results showed that myofibrillar proteins from glycolytic fibers were more sensitive to oxidation and thermal denaturation than proteins from oxidative fibers. Organic peroxide in interaction with iron induced less oxidation than hydrogen peroxide. Antioxidant enzymes were less efficient than vitamin E and carnosine to protect proteins against oxidation.

Key Words – hydrophobicity, myofibrillar proteins, oxidation.

I. INTRODUCTION

Meat is an important source of energy and essential amino acids for humans. Nevertheless meat processes can induce physicochemical changes (oxidation/denaturation) in proteins with a negative impact on technological, sensorial and nutritional properties of meat products [1]. To better understand the mechanisms involved in these changes, our researches were conducted to determine the kinetic laws which govern the oxidation and thermal denaturation of proteins during meat cooking. We worked on model systems which mimicked the biochemical composition of meat, in order to avoid confusing effect due to the uncontrolled biological variability and to assess independently the specific incidence of various compounds and heat treatment. A previous study on such model systems had assessed the relative contributions of chemical and thermal effects on protein states: (1) a basic model composed of an aqueous suspension of myofibrillar proteins and (2) a complex model in which oxidants were added in physiological concentrations [2]. Heating temperatures (45°C, 60°C, 75°C, 90°C) were applied during 120 min. The results showed: (i) a synergic effect of oxidants and heating temperatures on protein oxidations and (ii) a fast denaturation of proteins which was not affected by oxidants. The present work aims at determining kinetics in a complexified model which reflect meat: the effects of fiber type and additional oxidants/antioxidants on protein physicochemical changes induced by heating temperature were investigated.

II. MATERIALS AND METHODS

The basic model was composed of myofibrillar proteins in suspension (5 mg/ml) in a 40 mM phosphate buffer at pH 6; the same pH and ionic strength than muscle. In order to study the effect of fiber type: the rabbit muscles *psoas major* and *conoïdeus* which are known to be pure muscles [3] were removed one day *post mortem*. It was checked, using SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis), that *psoas major* muscle is a 100% α-white fiber type and *conoïdeus* muscle is a 100% β-red fiber type. Myofibrillar proteins were extracted from either *psoas major* (MP*pm*) or *conoïdeus* (MP*c*) muscles according to the method of Pietrzak *et al*., 1997 [4] and delipidated by a solvent (butanol/di-isopropyl ether) which preserved the protein state.
In the case of the complex model, a mixture of ferrous iron (0.2 mM; a value close to iron content in pork meat) hydrogen peroxide (2 mM) and ascorbate (0.1 mM) was added, in physiological concentration, just before heating. This mixture, which represents the main oxidative process in meat during cooking, produces hydroxyl radicals by reaction 1 (p3). In some experiments hydrogen peroxide was substituted by a model of organic

peroxides: the *tert*-butyl hydroperoxide (*t*-BuOOH). The muscle antioxidant effect was investigated by addition alone or in combination of three groups of antioxidants in physiological concentration: antioxidant enzymes (superoxide dismutase: 240 U/ml and catalase: 640 U/ml), trolox C (a water soluble analogue of α-tocopherol: 11.6 nM) and a dipeptide (20 mM of carnosine).

In each case, heating was performed at 45°C or 75°C, during 120 min, in a dry bath.

The protein oxidation was evaluated by measurement of carbonyls groups with the use of dinitrophenylhydrazine [5]. The protein thermal denaturation was evaluated from protein surface hydrophobicity measured using a hydrophobic probe: bromophenol blue [5]. Since oxidants have a negligible effect on protein thermal denaturation the effect of peroxides and antioxidants were only evaluated on protein oxidation [2].

The statistical analysis was performed by an analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS system. Values not bearing common superscript letters (a, b, c) showed significant differences between treatments ($p < 0.05$).

III. RESULTS AND DISCUSSION

A°) Effects of fiber type and heating temperatures (HT) on protein oxidation and denaturation.

As already observed with MP*pm*, the results obtained with MP*c* using both models showed: that protein oxidation (which was mainly due to lysine, arginine, proline and threonine amino acids) cannot be produced under the thermal process alone and needs oxidants while protein hydrophobicity is dominated by the thermal process.

Figure 1 shows the effect of fiber type on protein oxidation. The initial level of carbonyl groups, due to protein oxidation during storage, was significantly greater ($p < 0.001$) in the oxidative *conoïdeus* muscle. This is logical since compared to the glycolytic *psoas major* muscle it contains higher levels of myoglobin and mitochondria which can promote higher free radical production. ANOVA revealed significant effects of fiber type and heating temperatures (HT). At 45°C no significant effect of fiber type was observed. At 75°C the level of carbonyl groups increased significantly in the MP*pm*. This higher level of protein oxidation may be explained by a more important expression of Heat Shock Proteins (HSP guarantee a response to oxidative stress condition) in oxidative muscles (*conoïdeus*) [6]. Pulford *et al* [7] showed that soluble HSP may associate with myofibrillar proteins.

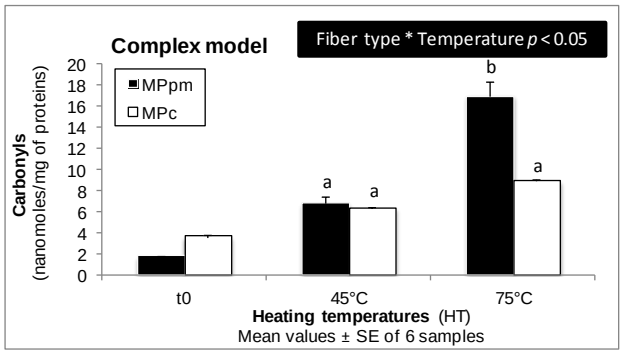

Fig. 1: Effects of fiber type on protein oxidation (120 min, at 45°C or 75°C, with oxidants).

Figure 2 depicts changes in protein structure. As a consequence of thermal denaturation an exposure of hydrophobic amino acids can occur to the protein surface. *Psoas major* had a higher initial level of hydrophobicity than *conoïdeus* muscle ($p < 0.001$). Protein surface hydrophobicity was affected by fiber type ($p < 0.001$) and HT ($p < 0.001$). The increase in surface hydrophobicity with HT was not the same in MP*pm* and MP*c* respectively: 25% and 60% at 45°C, 108% and 180% at 75°C. This could be explained by a higher level of hydrophobic amino acids in corresponding proteins (results not shown).

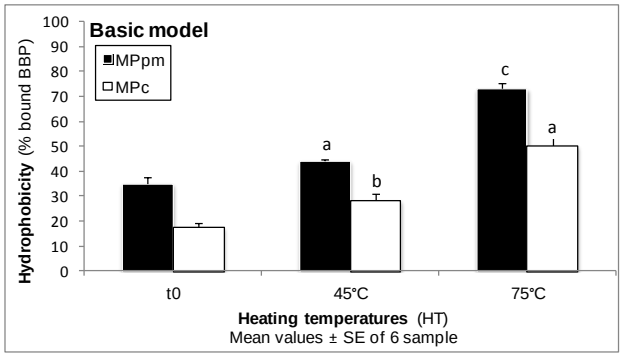

Fig. 2: Effects of fiber type on protein hydrophobicity (120 min, at 45°C or 75°C, without oxidants).

B°) Effects of two pro-oxidant peroxides (t-BuOOH and H_2O_2) on protein oxidation.

In meat products there is a combination of lipid peroxides (LOOL or/and LOOH) and hydrogen peroxides (H_2O_2), but their respective contribution on protein oxidation are unknown. During cooking myoglobin oxidation or/and glucose autoxidation can promote the production of superoxide radicals ($O_2^{\circ-}$) which can spontaneously dismute in H_2O_2. The lipid peroxides are produced during the propagation phase of lipid peroxidation. Using two peroxides (H_2O_2 or *t*-BuOOH) allowed to determine the relative contribution of each peroxides and the role of lipid peroxidation on protein oxidation. Peroxides induce protein oxidation by production of free radicals following the reactions (1) and (2):

(1) Fe(II) + ROOH → Fe(III) + HO^- + RO°
(2) Fe(III) + ROOH → Fe(II) + H^+ + ROO°
(R stands for either H or *t*-Bu or L)

Table 1: Effects of peroxides on protein oxidation (120 min, at 45°C or 75°C, with oxidants).

	45°C	***75°C***
H_2O_2	6.79 ± 0.70 *ac*	16.97 ± 1.30 *b*
***t*-BuOOH**	5.69 ± 0.09 *a*	9.55 ± 0.11 *c*

Mean values ± SE (n = 6).

ANOVA showed significant effects of peroxide type ($p < 0.001$), HT ($p < 0.001$) and their interaction ($p < 0.001$) on protein oxidation (Table 1). At 45°C no significant differences were observed between the two peroxides while at 75°C the level of carbonyls was significantly higher with H_2O_2. This difference can be explained by a higher rate constant for reaction (1) with H_2O_2; Rush *et al* [8] found that reaction of ferrous citrate with H_2O_2 was two fold higher than with *t*-BuOOH. Moreover, the radicals formed by *t*-BuOOH or H_2O_2 in reaction with iron attack quickly the substrate by abstraction of hydrogen atoms. The rate constants of these reactions are 10^6 $M.s^{-1}$ and 10^9 $M.s^{-1}$, respectively [9].

C°) Antioxidant effects on protein oxidation

Table 2 shows the antioxidant protection (AP) provided by different antioxidants towards protein oxidation. The AP is given by the following formula: AP = [(carbonyls without antioxidant - carbonyls with antioxidant) / carbonyls without antioxidants] x 100.

Table 2: Antioxidant protection against protein oxidation (120 min, at 45°C or 75°C, with oxidants and antioxidants).

	45°C	***75°C***
Antioxidant enzymes	53.30 % ± 5.06 *a*	22.57 % ± 6.74 *b*
Trolox C	48.82 % ± 4.82 *a*	41.76 % ± 4.14 *ab*
Carnosine	44.67 % ± 4.04 *ab*	30.98 % ± 5.78 *ab*

Mean values ± SE (n = 6).

A combination of antioxidant enzymes: superoxide dismutase (SOD) and catalase (CAT) was added. These two enzymes act in synergy: SOD catalyzes the conversion of $O_2^{\circ-}$ to H_2O_2 and CAT decomposes H_2O_2 into water and oxygen. In the model, the enzyme antioxidant protection decreased significantly during heating at 75°C. This can be attributed to thermal denaturation of CAT whose decreases over 85% in cooked meat from 60°C [10]. Then the model was complexified by addition of trolox C which is an analogue of α-tocopherol. As α-tocopherol, trolox C contains a phenolic group which can neutralize reactive oxygen species ($O_2^{\circ-}$, HO°) and lipid radicals. Temperature did not affect significantly the AP by trolox C (Table 2). The AP was also tested with carnosine (β-alanyl-L-histidine). The carnosine antioxidant effect may be ascribed to metal ion chelation and trapping of reactive oxygen species. As already observed with trolox C increasing temperature did not significantly decrease AP. Therefore the heat stability of trolox C and carnosine was higher than antioxidant enzymes. ANOVA revealed no significant interaction between HT and antioxidant. In order to investigate a synergic effect between antioxidants we also have tested in the same conditions a combination of antioxidant enzymes and trolox C. Whatever the HT, no synergic effect of the two antioxidants to increase AP was revealed ($p > 0.05$).

IV. CONCLUSION

The composition of our mimetic models can be well controlled and varied from very simple cases up to cases that reflect physico-chemical meat environment at best. This allows the identification of reactions which affect the protein modifications during heat treatments. This study demonstrates a fiber type effect on protein oxidation and thermal denaturation. Protein oxidation is affected by the composition of meat. Indeed radicals formed from organic peroxides are less reactive than hydroxyl radicals and the enzymatic antioxidant protection is less efficient during heat processes. The present study allows the alimentation of our data base with new results. The ultimate goal of our research is to predict through a mathematical tool the effects of heat treatments on the nutritional value of meat products. Thus to build up this tool our effort will be firstly focused on the modeling of protein oxidation and denaturation kinetics both in the mimetic models and in the meat. In a second time kinetic models will be combined to heat-mass transfer models to predict protein evolutions under realistic cooking conditions in large pieces of meat.

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