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Effect of Meat Cooking on Physicochemical State and in Vitro Digestibility of Myofibrillar Proteins

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The effect of meat cooking was measured on myofibrillar proteins from bovine M. *Rectus abdominis*. The heating treatment involved two temperatures (100 °C during 5, 15, 30, and 45 min and 270 °C during 1 min). Protein oxidation induced by cooking was evaluated by the level of carbonyl and free thiol groups. Structural modifications of proteins were assessed by the measurement of their surface hydrophobicity and by their aggregation state. With the aim of evaluating the impact of heat treatment on the digestive process, myofibrillar proteins were then exposed to proteases of the digestive tract (pepsin, trypsin, and α-chymotrypsin) in conditions of pH and temperature that simulate stomach and duodenal digestion. Meat cooking affected myofibrillar protein susceptibility to proteases, with increased or decreased rates, depending on the nature of the protease and the time/temperature parameters. Results showed a direct and quantitative relationship between protein carbonylation ($p < 0.01$) and aggregation ($p < 0.05$) induced by cooking and proteolytic susceptibility to pepsin. However, no such correlations have been observed with trypsin and α-chymotrypsin.

KEYWORDS: Cooking; meat; protein oxidation; hydrophobicity; aggregation; digestibility

INTRODUCTION

In modern society meat is almost always cooked prior consumption. Cooking is used to inactivate pathogenic microorganisms and to develop sensorial qualities. By generating free radicals and decreasing antioxidant protection, cooking favors lipid and protein oxidation as well as color degradation. Effect of cooking on lipid oxidation (*1–4*) and its consequences on meat flavor and odor (*5–7*) have been well studied. Color modification induced by elevated temperature has also been largely investigated (*8–10*). Conversely, the effect of cooking on protein oxidation is not so documented, and its consequences on protein digestibility are still unknown.

Oxidation of proteins leads to various amino acid modifications (*11–14*). Active oxygen species attack the side chain of basic amino acids (lysine, histidine, arginine) and can convert them into carbonyl derivatives. These carbonyl groups can then react with free amino groups to form amide bonds. Oxidative processes on proteins can also decrease thiol groups by forming disulfide bridges. Finally, aromatic amino acids can also be oxidized. For example, oxidation of tyrosine leads to the formation of dityrosine bridges. When they are formed between different proteins, these bridges initiate polymerization and formation of aggregates. Such amino acid oxidation and protein aggregation have been described in meat (*15–17*). In meat, interaction of proteins with aldehydic products of lipid peroxidation can also lead to aggregation by forming Schiff bases (*18, 19*). Oxidation and thermal denaturation of proteins can also induce structural changes leading to an increase of protein surface hydrophobicity, and this phenomenon has been recently reported in myofibrillar proteins (*17, 20*). By NMR (*21, 22*) and differential scanning calorimetry (*21*) it has been demonstrated that thermal denaturation of meat protein affects water mobility with important implications in water-holding capacity and cooking losses.

The interaction between protein oxidation and proteolysis has for years been extensively studied in biomedical sciences with contrasting effects reported. For example, increase of surface hydrophobicity has been described to enhance protein degradation by proteases (*23–25*), whereas intermolecular cross-links and formation of aggregates in highly oxidative conditions can reduce protein susceptibility to enzymatic proteolysis (*25, 26*).

Conversely, in meat, little is known about the effect of oxidation on protein digestibility, but the efficiency of this process is essential to ensure nutrient bioavailability. Indeed, proteins serve as an important source of energy and essential amino acids for humans, especially for some segments of the population such as the elderly and seriously ill persons for whom essential amino acid requirements are higher to limit the process of degenerative loss of skeletal muscle mass and strength called sarcopenia (*27, 28*). Moreover, nonhydrolyzed or partially hydrolyzed proteins are extensively fermented by colonic flora

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into phenol and *p*-cresol, which are potentially mutagenic products, increasing in that way the risk of colon cancer (*29, 30*). In a model system, limited studies have been performed to link chemical oxidation and digestibility of meat proteins (*17, 31, 32*); however, results have been inconsistent and contradictory. Increased or decreased digestibility has been observed depending on the level of oxidation and the presence or not of reducing agents. Moreover, levels of oxidation observed in these model systems are often very far from those measured in meat. Recently, we have established the link between myofibrillar protein oxidation and digestibility during meat aging (*33*); however, data on meat cooking are still lacking.

The present study was designed to explore the effect of meat cooking on physicochemical changes and in vitro digestibility of myofibrillar proteins from bovine muscle. Myofibrillar protein digestibility is presented and discussed in relationship to protein oxidative modifications (amino acid oxidations, hydrophobicity, and aggregation state of proteins).

MATERIALS AND METHODS

Animals and Samples. The experiment was carried out with bovine M. *Rectus abdominis*. Four animals (Charolais heifers) were killed in the experimental slaugtherhouse of the INRA Theix Research Centre. Twenty-four hours after animal death, when ultimate pH was reached (pH_{24h} measured on four animals = 5.63 ± 0.04), small samples of muscle were cut in parallelepipeds (0.5 × 0.5 × 2 cm) in parallel with muscle fibers and cooked.

Chemicals. 2,4-Dinitrophenylhydrazine (DNPH), 2,2′-dithiobis(5-nitropyridine) (DTNP), Bromophenol Blue (BPB), Nile Red, pepsin (from porcine gastric mucosa), trypsin, and α-chymotrypsin (from porcine pancreas) were purchased from Sigma.

Meat Cooking. The experiment involved two treatments that simulate meat cooking. First, four samples were placed in sealed polypropylene test tubes (inner diameter = 10 mm and thickness = 1 mm) and heated at 100 °C in a digital temperature-controlled dry bath (Block-heater, Stuart-Scientific) during 0, 5, 15, 30, and 45 min. This treatment reflected meat cooking in an oven for which a similar temperature can be reached at core. Four samples were placed in sealed aluminum tubes (inner diameter = 6 mm and thickness = 1 mm) and heated at 270 °C during 1 min in an oil bath. This treatment reflected more frying, by which meat is exposed to a high temperature during a brief time. The center temperature of the samples was measured with a digital thermometer fitted with a thin temperature probe. After cooking treatments, samples were cooled at room temperature for 15 min to reach 18–20 °C and then frozen at −80 °C until use.

Isolation of Myofibrils. Myofibrils were prepared according to the method of Ouali and Talmant (*34*) with some modifications as outlined by Martinaud et al. (*15*). The purity of myofibrillar proteins was assessed by SDS-PAGE as previously described (*17*). Electrophoretic profiles show that myofibril preparations were free of sarcoplasmic protein contamination. Especially, there was no trace of albumin (68 kDa), creatine kinase (42 kDa), and myoglobin (18 kDa), proteins mostly present in the sarcoplasmic fraction (results not shown).

Determination of Carbonyl Content. To determine level of carbonyl group formation, proteins were treated with DNPH using the method of Oliver et al. (*35*) with slight modifications (*15*). The results are expressed as nanomoles of DNPH fixed per milligram of protein. Each determination was performed in duplicate.

Determination of Free Thiol Group Content. Thiol oxidation was measured according to a modification of Ellman's method using DTNP (*36*). The results are expressed as nanomoles of free thiol per milligram of protein. Each determination was performed in duplicate.

Determination of Protein Surface Hydrophobicity. Hydrophobicity of myofibrillar proteins was determined using the hydrophobic chromophore BPB according to the method of Chelh et al. (*20*) with slight modifications. This method has been developed in our laboratory especially for the determination of surface hydrophobicity of nonsolubilized proteins. Myofibrillar proteins were suspended at the concentration of 2 mg/mL in 20 mM phosphate buffer at pH 6. To 1 mL of myofibril suspension was added and mixed well 40 μL of 1 mg/mL BPB (in distilled water). A control, without myofibrils, was done by the addition of 40 μL of 1 mg/mL BPB (in distilled water) to 1 mL of 20 mM phosphate buffer at pH 6. Samples and control were kept under agitation, at room temperature, during 10 min and then centrifuged 15 min at 2000*g*. The absorbance of supernatant, corresponding to free BPB, was measured at 595 nm against a blank of phosphate buffer. The amount of BPB bound, obtained by difference between total and free BPB, was used in this study as an index of hydrophobicity. Each determination was performed in duplicate.

In parallel, we have also developed a microscopic technique of the measurement of protein hydrophobicity. Thin transverse sections (10 μm) of meat samples, frozen in isopentane cooled by liquid nitrogen, were sliced with a microtome (Microm HM 560) and mounted on microscopic glass slides. Samples were exposed for 1 h to BPB (0.1 mg/mL) in 20 mM phosphate buffer at pH 6 plus 0.1 M NaCl. After removal of the BPB solution, samples were washed gently four times by PBS at pH 6.75 to remove unbound probe. Samples were then mounted with crystal mount. Microscopic observations were performed using an Olympus BX61 microscope. The observations were all performed in the same magnification (×200). Images were acquired using an Olympus DP 71 digital camera and the Cell F Softward. To estimate the level of protein hydrophobicity, we developed a semi-quantitative method based on color measurement of microscopy photographs. Images were converted into color photography using Adobe Photoshop Album Starter (edition 3.0) and printed with an optimal quality on a matt photographic paper. Color coordinates of images were then determined in the CIE $L^*a^*b^*$ (1976) system with a Uvikon 933 (Kontron) spectrophotometer, and (b^*) was used in this study as a tracer of the blue coloration. Microscopic observations have been done in duplicate, but on only one animal. Therefore, no statistical analysis of these results has been performed, and effects are mentioned for information only.

Determination of Protein Aggregation. Protein aggregation was evaluated by using the specificity of Nile Red to fluoresce after binding to aggregates (*37*). Protein aggregates were detected by a front face fluorescence technique. Before measurement, myofibrillar protein concentration was adjusted to 1 mg in 1 mL of 20 mM phosphate buffer at pH 6, and 10 μL of a stock solution of Nile Red (0.32 mg in 1 mL of ethanol) was added. The mixture was mixed well, and then 200 μL was placed into microplate cuvettes of 6 mm diameter. The fluorescence was measured with a standard spectrofluorometer Perkin-Elmer LS 50B fitted with a front surface accessory (Perkin-Elmer Plate Reader) for the direct measurement of a solid sample. Analyses were performed at optimum excitation and emission wavelengths (λ_{ex} = 560 nm and λ_{em} = 620 nm) with excitation and emission slit settings at 10 nm. For each sample the low autofluorescence of myofibrillar proteins was subtracted. Fluorescence intensities were expressed in arbitrary units (au). Each determination was performed in duplicate.

Protein aggregates were also detected by fluorescence microscopy according to the method developed on immunoglobulin aggregates by Demeule et al. (*37*) with modifications suited to the muscle tissue. Sample sections were performed as described in the preceding section and exposed for 3 h to 0.04 μM Nile Red in 20 mM phosphate buffer at pH 6 plus 0.1 M NaCl. After removal of the Nile Red solution, samples were washed gently four times by PBS at pH 6.75 to remove unbound probe. Samples were then mounted with crystal mount. Fluorescence microscopy was performed using an Olympus BX61 transmission fluorescence microscope with a CY3 filter. The observations and image acquisitions were all performed in the same magnification (×200) and the same lighting conditions (exposure time = 210 ms). As with BPB coloration, fluorescence microscopy observations have been done in duplicate, but on only one animal.

In Vitro Digestibility. Protein digestibility was assessed as previously described by Santé-Lhoutellier et al. (*17*) with slight modifications. Myofibrillar proteins were suspended in 33 mM glycine buffer at pH 1.8 (gastric pH), and final concentration was adjusted at 0.8 mg/mL. Proteins were first digested by gastric pepsin (5 units/mg of myofibrillar proteins) for 1 h at 37 °C. Digestion was terminated by the addition at various times (0, 10, 20, 30, 40, 60 min) of 15% (final concentration)

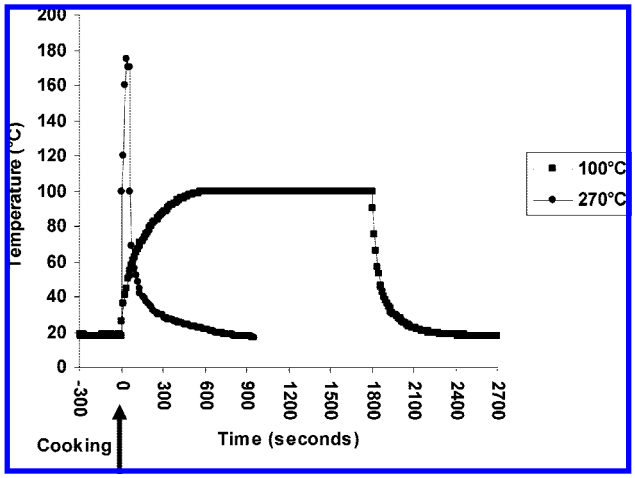

Figure 1. Temperature profiles in the center of meat cooked for 30 min at 100 °C and for 1 min at 270 °C and cooled for 15 min at room temperature. Values are means of four independent determinations.

trichloroacetic acid (TCA). After centrifugation for 10 min at 4000*g*, the content of hydrolyzed peptides in the soluble fraction was estimated by absorbance at 280 nm and the rate of proteolysis was expressed in optical density units by hour (ΔOD/h). The nonsoluble fractions of the 30 min pepsin hydrolysate was washed twice in 33 mM glycine buffer at pH 8 (duodenal pH), and final concentration was adjusted at 0.8 mg/mL in this same buffer. Proteins were then hydrolyzed for 30 min at 37 °C by a mixture of trypsin and α-chymotrypsin (6.6 and 0.33 units/mg of myofibrillar proteins). Digestion was terminated by addition at various times (0, 5, 10, 20, 30 min) of 15% (final concentration) TCA, and the rate of proteolysis was determined as previously described. Each determination of digestibility by pepsin and pancreatic proteases was performed in duplicate.

Statistical Analysis. All values are reported as the mean ± SEM of four independent determinations. The effect of cooking time was tested by an analysis of variance (ANOVA) under the SAS system. The mixed procedure with time repetition was used. The relationships between the different parameters were assessed by calculation of Pearson correlation coefficients.

RESULTS AND DISCUSSION

Effect of Cooking Time on Meat Temperature. The effects reported in this study were the results of different cooking times at 100 and 270 °C plus 15 min of cooling at room temperature. The time–temperature profiles recorded during this process in the center of meat are displayed in **Figure 1**.

100 °C Cooking. After 5 min of cooking, corresponding to the first biochemical measurement, the meat temperature was only 88 °C. The maximum temperature (between 99 and 100 °C) was reached after 9 min of cooking and then remained stable. Cooling at 18 °C induced a rapid decrease of meat temperature, and after 15 min, the final temperature (18–20 °C) was reached.

270 °C Cooking. A rapid increase of meat temperature was observed, and the maximum temperature (170 °C) was reached after only 40 s. This maximum was measured in the center of the sample. Surface temperature (not determined here) was probably higher. A rapid temperature decrease was also observed at the end of this cooking.

Effect of Cooking on the Carbonyl Group Formation. **Figure 2** shows that, during cooking, myofibrillar proteins are particularly prone to oxidation. At 5 min of the 100 °C cooking we can already observe a 2-fold increase in carbonyl groups. Then, carbonyls continually increased with time to reach a maximum after 45 min. ANOVA revealed an effect of the cooking time on this parameter, which was close to the

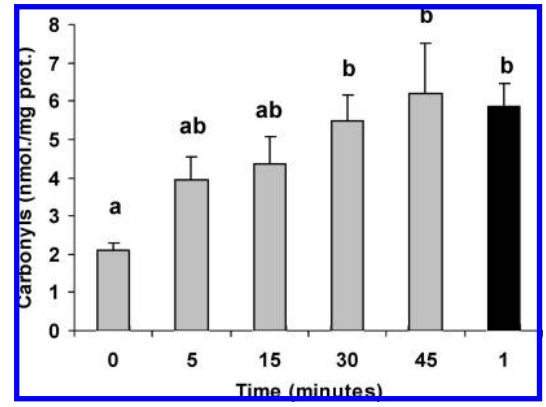

Figure 2. Effect of cooking (gray bars, 100 °C; black bar, 270 °C) on the carbonyl content of myofibrillar proteins. Values are means ± SEM of four independent determinations. For each time of measurement, values not bearing common letters differ significantly ($p < 0.05$).

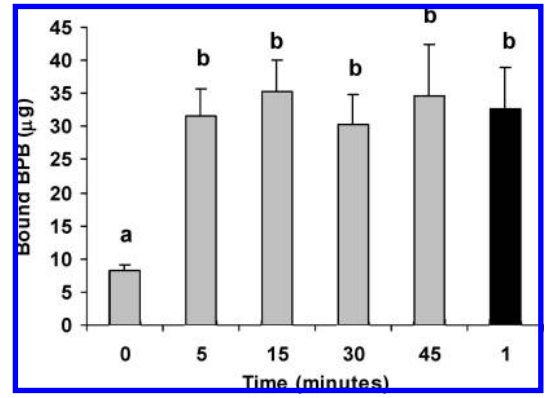

Figure 3. Effect of cooking (gray bars, 100 °C; black bar, 270 °C) on myofibrillar protein surface hydrophobicity measured by absorbance at 595 nm of the BPB probe. Values are means ± SEM of four independent determinations. For each time of measurement, values not bearing common letters differ significantly ($p < 0.05$).

significance ($p = 0.055$). Differences between cooked meat and raw meat were significant only after 30 min ($p < 0.001$). The quick-cooking at high temperature (1 min/270 °C) had also a significant effect on carbonyl group formation ($p < 0.001$), and the value obtained was very close to that observed after 45 min of the 100 °C cooking. By comparison, during bovine meat refrigerated storage, the same carbonyl level was only reached after 10 days (*15*). This rapid progress of protein oxidation during cooking can be attributed to the loss of antioxidant protection of muscle (*38, 39*). Moreover, during cooking, iron can be released from heme and non-heme proteins (*40, 41*) in its catalytic form, which can promote the formation of free radicals.

Effect of Cooking on Free Thiol Group Level. The susceptibility of myofibrillar proteins to oxidation during cooking was also monitored by the estimation of the free thiol groups (results not shown). The decrease of free thiol groups measured during cooking at 100 and 270 °C was low (respectively, 35 and 16%) and not significant ($p > 0.05$). This decrease might correspond to the oxidation of accessible free thiol groups from cysteine residues located at the protein surface, the inside cysteine residues being protected against free radical attack even during a long heating time. Even if the magnitude of thiol oxidation was low, because of its external localization, this phenomenon might have an important effect on protein–protein interaction and aggregate formation.

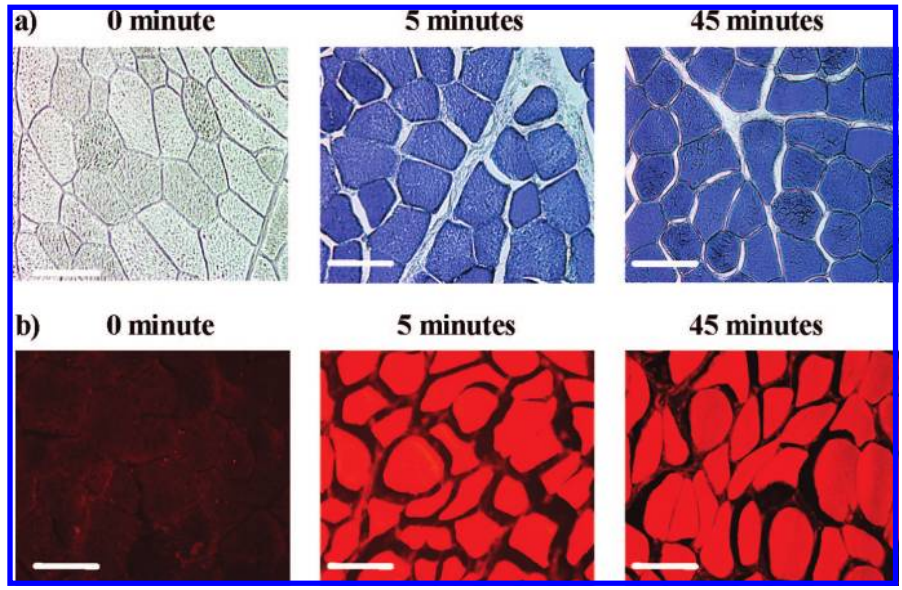

Figure 4. (**a**) Effect of 100 °C cooking on protein hydrophobicity measured by microscopy with Bromophenol Blue staining. (**b**) Effect of 100 °C cooking on protein aggregation measured by Nile Red fluorescence microscopy (white scale bar = 100 μm).

Effect of Cooking on Protein Surface Hydrophobicity. In view of its capacity to monitor subtle changes in the chemical and physical states of protein, hydrophobicity can be a suitable parameter to estimate protein denaturation. ANOVA revealed a cooking time effect on this parameter, which was very near the significance ($p = 0.059$). An important (approximately 4 times) and significant ($p < 0.05$) increase of protein hydrophobicity was already observed at 5 min of the 100 °C cooking, and no significant changes were subsequently observed (**Figure 3**). This increase of surface hydrophobicity was due to conformational changes, that is, the unfolding of myofibrillar proteins, and exposure of nonpolar amino acids to the surface of proteins. The fact that protein hydrophobicity reachs its maximum, whereas the carbonyl groups always increase, indicates that, during cooking, oxidation is probably not the only cause of structural change. The rupture of hydrogen bonds has been largely described to be the major cause of protein unfolding during heat treatment. The 270 °C cooking had also a significant effect on protein hydrophobicity ($p < 0.05$), and the value obtained was similar to those observed at 100 °C. Such an effect has been described during thermal denaturation of actomyosin of threadfin bream (*42*). These authors, using the ANS fluorescent probe, described a 2.5-fold increase in protein hydrophobicity between 30 and 70 °C and attributed this change in hydrophobicity mainly to myosin. During the heating treatment of purified myofibrils from pork muscle, we have also observed an important increase of hydrophocity, which was positively correlated to heating time and temperature (*20*).

To localize this change in protein structure at the cellular level, microscopic observations of muscle tissue stained by the same BPB probe were performed, and photographs are displayed for raw meat and after 5 and 45 min of the 100 °C cooking (**Figure 4a**). An important increase of blue coloration was observed after cooking, which was almost the same after 5 or 45 min. This result confirms that changes in protein surface hydrophobicity appear very early during meat cooking. The blue coloration was uniform inside the cells. This result is in contradiction with a precedent study (*43*) showing a heterogeneity of protein oxidation in muscle cells with a higher level of carbonylation of membrane proteins. Moreover, we can observe

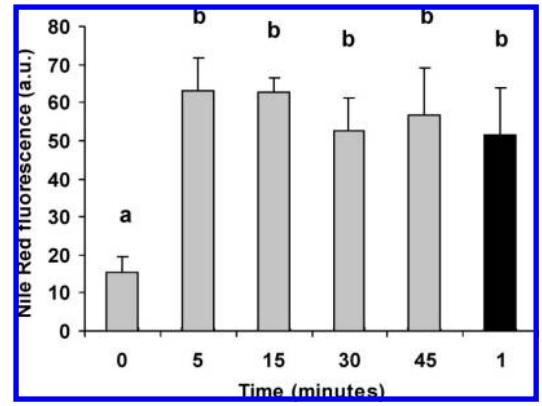

Figure 5. Effect of cooking (gray bars, 100 °C; black bar, 270 °C) on myofibrillar protein aggregation measured by Nile Red fluorescence spectroscopy. Values are means ± SEM of four independent determinations. For each time of measurement, values not bearing common letters differ significantly ($p < 0.05$).

an important blue coloration of the connective tissue demonstrating that collagen fibers also develop hydrophobic properties during their thermal denaturation.

Effect of Cooking on Protein Aggregation. Characterizing protein aggregation requires appropriate analytical methods that do not change or which induce minimal changes in the local environment of the protein. This paper describes an adaptation of the technique of Demeule et al. (*37*), which consists of analyzing protein aggregates by front face fluorescence spectroscopy after staining by the Nile Red phenoxazone dye. Nile Red has been described to be very sensitive for the detection and analysis of protein aggregates, for example, in immunoglobulin (*37, 44*) and in β-galactosidase aggregation (*45*), in calcitonin fibrillation (*37, 46*), and in amyloidic structures implicated in Parkinson's and Alzheimer's diseases (*47, 48*). In a comparative study, Demeule et al. (*37*) have demonstrated that Nile Red was able to bind protein aggregates as well as Thioflavine T and Congo red commonly used, in medical research and pathology laboratory, for the detection of amyloids.

ANOVA revealed a high significance effect ($p < 0.01$) of the 100 °C cooking time on protein aggregation measured by the fluorescence of Nile Red. An important (approximately 4

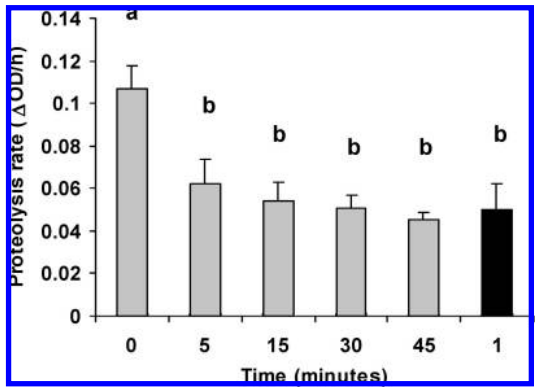

Figure 6. Effect of cooking (gray bars, 100 °C; black bar, 270 °C) on the proteolysis rate of myofibrillar proteins by gastric pepsin. Values are means ± SEM of four independent determinations. For each time of measurement, values not bearing common letters differ significantly ($p < 0.05$).

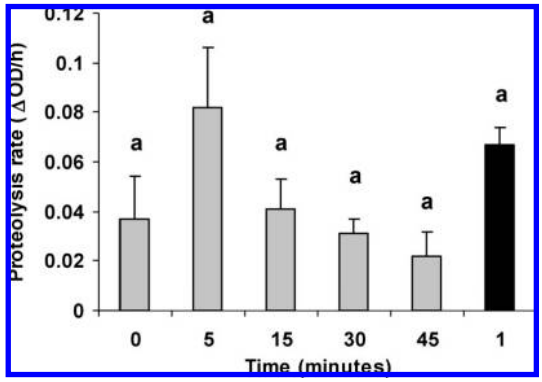

Figure 7. Effect of cooking (gray bars, 100 °C; black bar, 270 °C) on the proteolysis rate of myofibrillar proteins, previously treated with pepsin, by pancreatic trypsin + α-chymotrypsin. Values are means ± SEM of four independent determinations. For each time of measurement, values not bearing common letters differ significantly ($p < 0.05$).

times) and significant ($p < 0.01$) increase of fluorescence was already observed at 5 min (**Figure 5**). A slow decrease of fluorescence was then observed with increasing cooking time. This fluorescence decrease could be attributed to the possible absorption of the excitation light or to the reabsorption of emission fluorescence light by various chromophores as Schiff bases or Maillard and Amadori products, which can be formed by the interaction of myofibrillar proteins and lipids or sugars during cooking. Such chromophores have been described in myofibrillar proteins during a chemical oxidation (*18*) or meat storage (*19*). One minute of heating at 270 °C induced a lower, but still significant ($p < 0.05$), increase of fluorescence.

To localize protein aggregation in cells, this phenomenon has also been studied by fluorescence microscopy using the Nile Red probe (**Figure 4b**). Raw meat exhibited a very low red fluorescence, whereas the 100 °C cooked meat exhibited a high red fluorescence, which was almost the same after 5 or 45 min. These microscopy pictures show that fluorescence was homogeneous in inside the cell. A low fluorescence of the connective tissue was also observed.

These two approaches (BPB coloration and Nile Red fluorescence) show similar results, on isolated myofibrillar proteins as well as directly on muscle cells, indicating the probable implication of protein surface hydrophobicity in myofibrillar protein aggregation.

Effect of Cooking on Protein Digestibility. Figure 6 shows an important decrease of the pepsin activity on myofibrillar proteins after the 100 °C cooking. A 42% decrease, which was highly significant ($p < 0.001$), was already observed at 5 min. Then, pepsin activity decreased more slowly, and a maximum of 58% decrease was measured after 45 min. ANOVA confirmed this time effect on pepsin activity ($p < 0.01$). The fast cooking at 270 °C induced the same decrease in pepsin activity as a 30 min cooking at 100 °C. In a recent study, we have observed a comparable decrease of pepsin activity on chemically oxidized myofibrillar proteins (*17*). On the contrary, we have demonstrated that meat refrigerated storage had no significant effect on myofibrillar protein digestibility by pepsin (*33*). These conflicting results clearly show that only a process giving a high level of protein oxidation affects protein digestibility by pepsin, which is the first step in digestion (**Figure 7**).

The activity of pancreatic proteases (trypsin + α-chymotrypsin) on myofibrillar proteins, previously treated with pepsin, was also influenced by cooking time at 100 °C. With this mix of proteases we can observe a biphasic response. A 2-fold increase of activity was observed between 0 and 5 min followed by a rapid decrease, which reached a maximum, when compared with raw meat, of 40% after 45 min. No significant effect ($p > 0.05$) of cooking time was noted by ANOVA. These results are in agreement with those of Liu and Xiong (*32*) showing a biphasic response when proteolysis by trypsin and chymotrypsin was measured on oxidized myosin. Contrary to results observed on pepsin, the 1 min heating at 270 °C induced an important (but not significant) increase in protease activity, and the value obtained was of the same order as activity measured after 5 min of heating at 100 °C.

During the digestive process, gastric and pancreatic proteases act in a sequential manner; consequently, the effects measured on protein digestibility might be additive. If we sum the effects measured on both proteases, we can observe that a quick cooking (100 °C/5min or 270 °C/1 min) has virtually no effect on protein digestibility when compared with the potential digestibility of raw meat. In this case the lower degradation by pepsin is compensated by the increased proteolysis observed with pancreatic proteases. On the contrary, a dramatic decrease of

Table 1. Correlation Matrix between Parameters of Oxidative Modification (Carbonyl Groups, Free Thiol Groups, Hydrophobicity, and Aggregation) and Protease Activities[a]

	carbonyls	free SH	hydrophobicity	aggregation	pepsin activity	trypsin + α-chymotrypsin activity
carbonyls	1					
free SH	−0.043 NS	1				
hydrophobicity	0.049 NS	−0.400 NS	1			
aggregation	0.137 NS	−0.628 ***	0.844 ***	1		
pepsin activity	−0.517 **	0.293 NS	−0.308 NS	−0.408 *	1	
trypsin + α-chymotrypsin activity	0.164 NS	−0.046 NS	0.036 NS	0.157 NS	0.226 NS	1

[a] Twenty-four observations are included in the analysis. Significance is noted as follows: $p > 0.05$, NS; $p < 0.05$, *; $p < 0.01$, **; and $p < 0.001$, ***.

digestibility can be observed for the longest cooking times. For example, after 45 min of cooking at 100 °C, the cumulative decrease of digestibility was from 75%.

Correlations between Oxidation and Digestibility Measurements. A correlation study was performed to establish links between oxidative modifications (carbonyl, free thiol, hydrophobicity, and aggregation) and proteolysis rates (**Table 1**). A negative and highly significant correlation was measured between pepsin activity and carbonyl group formation. The change of an amino group by a carbonyl group can modify the redox properties of proteins and so can disturb the recognition of proteins by proteases. We also note a negative and significant correlation between pepsin activity and aggregate formation, confirming the impact of this phenomenon on the recognition of proteins by proteases. Surprisingly, no such correlation was observed between pepsin activity and protein surface hydrophobicity, whereas, during a chemical oxidation, Santé-Lhoutellier et al. (*17*) have described a significant effect of hydrophobicity on this activity. Due to the biphasic response of myofibrillar proteins toward proteolysis by the pancreatic enzymes, no significant correlations were noted between oxidative parameters and trypsin + α-chymotrypsin activity. Finally, **Table 1** confirms the implication of the disulfide bridges and the protein surface hydrophobicity in the aggregation process, whereas carbonyl groups did not affect the formation of protein aggregates. This result was surprising because carbonyl groups have been described to react with nonoxidized amino groups of protein to give an amide bond, which can be implicated in aggregate formation (*16, 17*).

In an attempt to minimize nutritional loss in meat products, the possibility of using reliable parameters of protein state that can monitor the severity of thermal processing must be considered. The effect of thermal processing on myofibrillar protein digestibility by pepsin can be monitored by the level of carbonyl groups or by the aggregation state of proteins, whereas no oxidative parameters, measured in this study, reflect the effect of heat on the activity of pancreatic proteases. Polymerization with other biomolecules (carbohydrates or lipid oxidation products) has not been studied here, but this would be an obvious area to continue within to gain further understanding of protein digestibility in relation to oxidative modifications.

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