Comparison of Oxidative Processes on Myofibrillar Proteins from Beef during Maturation and by Different Model Oxidation Systems

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The aim of this work was to compare the oxidative processes occurring in myofibrillar proteins during meat maturation and after an *in vitro* exposure to different enzymic and nonenzymic oxidative systems. Myofibrils were prepared from bovine Longissimus lumborum and Diaphragma pedialis at day 1 and day 10 post-mortem. Myofibrillar protein oxidation was measured by the carbonyl content, with the 2,4-dinitrophenylhydrazine (DNPH) method, and by (thiol group) SH content with the 2,2′-dithiobis(5-nitropyridine) (DTNP) method. Polymerization and/or fragmentation of oxidized proteins were estimated by SDS-PAGE and Western blot analysis using a polyclonal antibody to myosin. Oxidation of myofibrillar proteins is dependent upon the different metal-catalyzed oxidation (MCO) systems. The increase in carbonyl content and also the decrease in SH content of myofibrillar proteins, after maturation of 10 days, were similar to those obtained after a 1 h incubation of myofibrillar proteins in the presence of several MCO systems. Electrophoretic studies showed that myosin was the protein the most sensitive to oxidation, and to a lesser extent, troponin T. Myosin-oxidative products were also detected by Western blot analysis.

Keywords: *Beef; myofibrillar proteins; protein oxidation; carbonyl content; SH-content; metal-ion catalyzed oxidation system; SDS−PAGE; Western blotting*

INTRODUCTION

Oxidative processes are known to be the major causes of meat quality deterioration such as flavor, color, and nutritional composition (Asghar et al., 1988). Oxidation of membrane phospholipids is the primary cause of off-flavors, in raw as well as in cooked meats, due to their high degree of unsaturation, and it is known that lipid oxidation is induced by oxy- and/or lipid-free-radical generation. The development of metmyoglobin (MetMb) responsible for color deterioration depends essentially on the rates of myoglobin autoxidation and oxygen consumption (Renerre, 1990). During myoglobin autoxidation, oxymyoglobin (MbO_2) separates into MetMb, H_2O_2, and $O_2^{\bullet -}$. In the presence of iron, $O_2^{\bullet -}$, which is transformed into hydroxyl radical $OH^{\bullet}$ by the Fenton reaction, can enhance lipid oxidation in meat (Harel and Kanner, 1985). Conversely, with model systems, many authors have shown that lipid oxidation is a promoter of myoglobin oxidation. In our laboratory, it was shown that membrane phospholipids, peroxidized by enzymic or nonenzymic systems, increased myoglobin autoxidation (Anton et al., 1993).

While lipid oxidation and discoloration processes, and their relationships, have been extensively studied (Renerre and Labadie, 1993; Gray et al., 1996), oxidation of proteins during meat storage (Mercier et al., 1995), has been less studied, and the mechanisms remain poorly understood. Stadtman (1990) showed that active oxygen species attack preferentially the side chain of amino acid residues and can lead to the conversion of some amino acid residues to carbonyl derivatives, to a loss of catalytic activity and to an increased susceptibility to proteolytic degradation. Stadtman (1990) showed that oxidation of the sulfhydryl groups may lead to the formation of either intra- or interprotein disulfide cross-linkages or to the formation of mixed-disulfide conjugates with glutathione, cysteine, or other low molecular weight mercaptans. Reznick et al. (1992) have shown an increase in protein oxidation in skeletal muscle after a short period of exercise. Xiong and Decker (1995) indicated that metal-catalyzed formation of the $OH^{\bullet}$ converted some amino acid residues to carbonyl derivatives.

Among the numerous questions, it is not known how closely the proteolysis during meat maturation is linked with oxidative processes. Contrary to Starke-Reed and Oliver (1989), who have studied the possible relationships between protein oxidation and proteolysis *in vivo*, Davies et al. (1987b), according to Stadtman (1990), indicated that the oxidized proteins had a higher susceptibility to proteolysis, an important change which could be linked to meat tenderness. Previously, we had shown that oxidation could affect myofibrillar proteins and changes in ultrastructure (Ouali et al., 1992) and cause the appearance of amorphous protein structures around the Z-lines. During metal-catalyzed myofibrillar proteins oxidation, Decker et al. (1993) observed that high molecular weight polymers were produced by disulfide linkages and were mainly derived from myosin and actin; fragmentation of proteins was also observed. Although the oxidative mechanisms may be implicated in the physical characteristics of meat, their role in texture and tenderness is still not known (Decker et al., 1993; Xiong and Decker, 1995). Blanchard and Mantle (1996) have indicated that free radical species may be implicated in the process of meat tenderization.

The aims of this work were to quantify the oxidation of myofibrillar proteins during meat maturation, compared to the *in vitro* action of different enzymic and nonenzymic oxidative systems on myofibrillar proteins, and to better understand the relationships between the two phenomena. Experiments were performed on bo-

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vine Longissimus lumborum, which is a fast-oxidative glycolytic muscle, and Diaphragma pedialis which is a slow-oxidative muscle (Renerre, 1984).

MATERIALS AND METHODS

Reagents. All reagents were purchased from Sigma Co. (Sigma-Aldrich, St Quentin Fallavier, France).

Isolation of Myofibrils. Myofibrils from bovine Longissimus lumborum (LL) and Diaphragma pedialis (DP) muscles were prepared at one (D1) and ten (D10) days post-mortem by a modification of the method of Ouali and Talmant (1990). Ten grams of muscle were homogenized with a Waring blender in 100 mL of a solution at pH 6.5 containing 150 mM NaCl, 25 mM KCl, 3 mM $MgCl_2$, 1 mM phenylmethanesulfonyl fluoride (PMSF) and 4 mM EDTA. This homogenate was ground with a Polytron (Bioblock, France) for 30 s, and collagen was eliminated by filtration on gauze. After 30 min of stirring in ice, the extract was centrifuged (Beckman, France) at 2000*g* for 15 min. The pellet was washed twice with 100 mL of a 50 mM KCl and 5 mM mercaptoethanol solution at pH 6.4 and once with 100 mL of 200 mM sodium phosphate buffer at pH 7.4. After washing, the pellet was resuspended in the same phosphate buffer, and the protein concentration (Gornall et al., 1949) was adjusted to 5 mg/mL.

Assay for Protein Oxidation. Oxidation of myofibrils were carried out using three hydroxyl radical ($OH^{\bullet}$) generating systems: (1) $FeSO_4$ (4 mM) and EDTA (8 mM) as used by Amici et al. (1989); (2) $FeCl_3$ (0.1 mM) and ascorbate (25 mM) as used by Rivett (1985), Amici et al. (1989), and Li et al. (1993), and (3) $FeSO_4$ (2.5 mM)/diethylenetriaminepentaacetic acid (DETAPAC) (2.5 mM)/H_2O_2 (2.5 mM) (Fenton reagent) as used by Curan et al. (1984) and Uchida et al. (1989). To produce superoxide anions ($O_2^{\bullet -}$) and H_2O_2, an enzymic system of a mixture of xanthine (2.5 mM) and xanthine oxidase (0.075 units/mL) (X/XO) was used as described by Davies and Goldberg (1987a). The system consisting of metmyoglobin (70 mM) and H_2O_2 (70 mM) was also used to generate tyrosine peroxyl radicals (Newman et al., 1991; Gatellier et al., 1995).

The myofibrils, prepared 24 h post-mortem in a 200 mM sodium phosphate buffer at pH 7.4 to accelerate oxidation, were incubated at 37 °C (Amici et al., 1989) for 1 h (T1), 2 h (T2), and 5 h (T5), with T0 time before oxidation. All experiments were replicated in triplicate.

Determination of Protein Carbonyl Content. Carbonyl groups were detected by reactivity with 2,4 dinitrophenylhydrazine (DNPH) to form protein hydrazones. The method of Oliver et al. (1987) was used with slight modifications. Two fractions of 500 μL myofibrillar protein solution were precipitated with 10% trichloroacetic acid (TCA; w/v; final concentration). After centrifugation (2000*g*/10 min) (Jouan, France), one pellet was treated with 2 N HCl and the other was treated with 0.2% DNPH (w/v) in 2 N HCl with agitation for 1 h at room temperature. The two fractions were then precipitated with 10% TCA (final concentration) and centrifuged. The pellets were washed twice with 1 mL of ethanol:ethyl acetate (1:1 v/v), and the solution was precipitated with 10% TCA (final concentration) and centrifuged. Proteins were then dissolved in 2 mL of 6 M guanidine with 20 mM sodium phosphate buffer at pH 6.5. Absorbance was measured at 365 nm (Kontron, France) for the DNPH-treated sample against an HCl control. Protein concentration was calculated at 280 nm in the HCl control using a standard bovine serum albumin (BSA) in guanidine. The amount of carbonyl was expressed as nmol of DNPH fixed/mg of protein using an absorption coefficient of 21 mM^{-1} cm^{-1} for protein hydrazones.

Determination of Protein Thiol Oxidation. Thiol oxidation was measured by a modification of Ellman's method using 2,2′-dithiobis(5-nitropyridine) DTNP (Winterbourn, 1990). Myofibrillar proteins were diluted in a 100 mM phosphate buffer at pH 8 containing 8 M urea. A 10 μL volume of 10 mM DTNP (stock solution in ethanol) was added to 1 mL of protein solution and, after an incubation period of 1 h at room temperature, the absorbance at 386 nm was measured against a blank of protein at the same concentration without DTNP. The absorbance of diluted DTNP was subtracted, and the thiol concentration was calculated using an absorption coefficient (386 nm) of 14 mM^{-1} cm^{-1}. The results are expressed as nmol of free thiol/mg protein.

Electrophoretic Analysis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) with 2-mercaptoethanol was used to monitor polymerization or fragmentation of the oxidized proteins. SDS-PAGE was performed in 12% acrylamide resolving gel (Laemmli, 1970). At day 1 post-mortem, 0.5 mL of sample was taken and diluted in 1 mL of solubilization buffer (50 mM Tris; 7.5%-SDS; 1−2% mercaptoethanol; 7.5% glycerol) at pH 6.8. Samples were heated at 100 °C for 5 min to denature the proteins. This procedure cuts disulfide bridges between and within the intact chains. Blue bromophenol was added (0.05%) to follow the migration front. After migration, the proteins were fixed with a solution composed of acetic acid (5%), ethanol (30%), and distilled water (65%). Detection of protein bands was performed by using Coomassie blue staining (0.12% solution). To better separate high molecular weight proteins, a second SDS-PAGE was performed in 7.5% acrylamide resolving gel (Laemmli, 1970).

Western Blot Analysis. Proteins were separated by SDS-PAGE according to the procedure of Laemmli (1970) and were transferred to a immobilidon-poly(vinylidene fluoride) (PVDF) membrane by means of a Bio-Rad Trans-Blot apparatus with a transfer buffer consisting of 25 mM Tris/192 mM glycine/20% (v/v) ethanol at pH 8.3 (Towbin et al., 1979). The membrane was washed with TBS (100 mM Tris-HCl; 150 mM NaCl; 0.05% Tween 20) at pH 8.0, and the membrane was blocked with the same buffer containing 3% fat-free dried milk.

The membrane was then incubated overnight with a polyclonal rabbit antimyosin antibody (Sigma) in TBS-milk solution (1/10 dilution) and washed three times with TBS solution. Then, the membrane was incubated for 2 h with a TBS solution containing a 1:10000 dilution of horseraddish peroxidase-conjugated goat antirabbit IgG (Sigma). The membrane was washed in TBS solution before detection of the peroxidase by immunoreactive material using 4-chloro-1-naphthol (3 mg/mL in 20% (v/v) methanol/80% (v/v) TBS) and H_2O_2 (0.01%, v/v). Comparisons were done between myofibrillar proteins oxidized after a maturation of 10 days and those after a 5 h incubation time at 37 °C with Fe^{2+}/EDTA and xanthine/xanthine oxidase.

Statistics. The unpaired Student *t*-test was used to determine the levels of statistical significance. All values are reported as the mean ±SD.

RESULTS AND DISCUSSION

Effect of Maturation on Carbonyl and Sulfhydryl Content. During meat maturation for 10 days, there was an increase in carbonyl content. At day 1, the carbonyl content of myofibrillar proteins was 3.1 ± 1.6 nmol/mg protein for LL muscle and 4.8 ± 1.5 nmol/mg protein for DP muscle, similar to those found by Murphy and Kehrer (1989) in chicken. Liu and Xiong (1996), in chicken, indicated that protein carbonyls of myofibril samples were about 1.5 nmol/mg protein. After 10 days storage, the carbonyl content of proteins increased to 5.1 ± 0.4 nmol/mg protein (+70%) for LL muscle and to 6.9 ± 0.9 nmol/mg protein (+44%) for DP muscle. These results were similar to those obtained by Liu and Xiong (1996) on chicken myofibril pellets.

The value of carbonyl content for DP muscle at day 1 was very high, perhaps due to artefacts during the myofibrils preparation itself, or the use of frozen stored meat (−80 °C for 3 months). Effectively, Reznick et al. (1992) showed that freeze/thaw cycle enhanced the carbonyl formation of proteins. Moreover, as cellular lipids are difficult to remove completely during protein isolation (Xiong and Decker, 1995), carbonyls found in myofibrillar proteins preparations could be partly derived from some residual lipids. Nucleic acids will also contribute to the carbonyl content of proteins in more

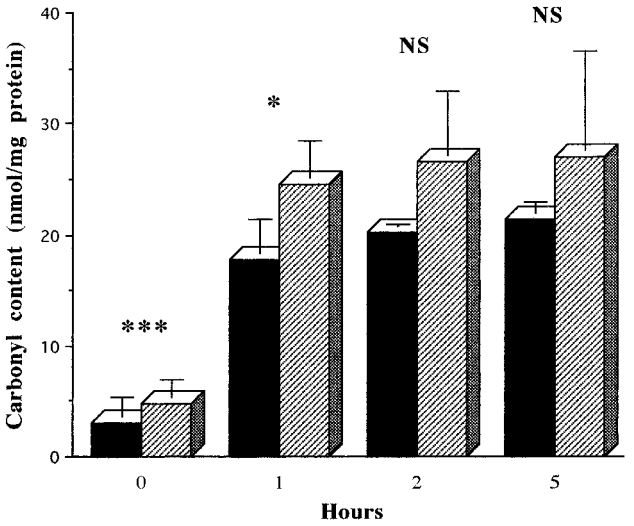

Figure 1. Carbonyl content in myofibrillar proteins of LL (solid) and DP (striped) muscles induced by ferrous iron (2.5 mM) and H_2O_2 (2.5 mM). Values are expressed as means of triplicate measurements. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

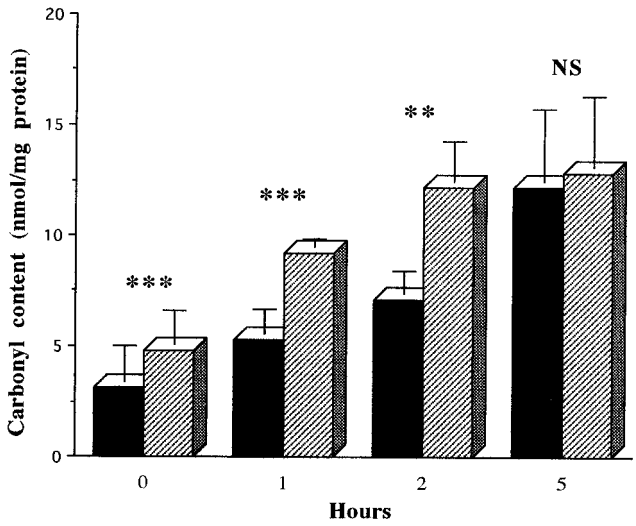

Figure 2. Carbonyl content in myofibrillar proteins of LL (solid) and DP (striped) muscles induced by ferric iron (0.1 mM) and ascorbate (25 mM). Values are expressed as means of triplicate measurements. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

crude extracts (Levine et al., 1990). In addition, as oxidized proteins are particularly susceptible to proteolytic degradation (Stadtman, 1990), the inclusion of only one protease inhibitor, PMSF in the homogenizing buffer, may not have been sufficient to prevent degradation.

Mackerel, stored as fillets during 8 days, showed a 2−5-fold increase in carbonyl content between day 0 and day 8 (Srinivasan and Hultin, 1995). Starke-Reed and Oliver (1989), cited by Stadtman (1992), have estimated that 2 nmol of carbonyl groups/mg of protein, an amount commonly found in young adults, represents damage to about 10% of the total cellular protein. However, these results underestimate the total oxidation, some of which does not lead to carbonyl derivatives (Stadtman 1992).

Because protein oxidation is also associated with a decrease in SH groups, the SH content was also analyzed. During 10 days storage, the SH content of myofibrillar proteins for LL muscle decreased 10% from 66.2 ± 8.4 to 59.6 ± 3.1 nmol/mg protein. For DP muscle, the values decreased 17% from 71.9 ± 12.2 to 60.2 ± 2.1 nmol/mg protein during the same period. With total proteins in beef muscles, there was a greater decrease in SH content for DP muscle than for LL muscle having 55 and 67 nmol/mg protein, respectively, after 10 days storage (Mercier, 1994).

Effect of Different Oxidative Systems on Carbonyl Content. As shown in Figures 1−3, metal-catalyzed oxidation processes resulted in an increase in carbonyl content. Regardless of the system, there was an increase in carbonyl content after a 5 h incubation. Among the different systems, the Fe^{2+}/H_2O_2 mixture was the most efficient system, giving a carbonyl content of 17.7 ± 3.1 for LL and 24.6 ± 3.3 nmol/mg protein for DP muscles after only 1 h (Figure 1) and a further increase in carbonyl content after a 5 h incubation. Regardless of the incubation time (Figure 1), there was a tendency to find more carbonyl groups in DP than in LL muscle. Uchida et al. (1992) found that iron/H_2O_2 caused the protein to degrade or polymerize, corresponding to modifications in amino acid composition. According to Stadtman (1990), Fe^{2+} binds to a metal-binding site of the protein, and the Fe^{2+}−protein complex reacts with H_2O_2 to yield an active oxygen species.

The Fe^{3+}/ascorbate system (Figure 2), after a 5 h incubation at 37 °C, produced a carbonyl content of 12.2 ± 3.2 for LL and 12.8 ± 3.2 nmol/mg protein for DP muscles. Decker et al. (1993) showed a carbonyl content of about 11 nmol/mg protein for turkey breast myofibrillar proteins. After a chemical incubation on sarcoplasmic proteins from turkeys, it was also observed that the results were dependent on the concentration of the different components: with 100 μM Fe^{3+}, an increase in ascorbate from 0.1 to 25 mM led to nearly a 3 fold increase in carbonyl content (Gatellier et al., 1996). As shown in Figure 2, after a 1 or 2 h incubation, the differences in carbonyl contents between the muscles were highly significant ($P < 0.01$) with the highest value noted with DP (SO) muscle. From 1 to 5 h incubation, the oxidation of myofibrillar proteins of LL muscle resulted in 3 fold increase in carbonyl content, results similar to those of Mercier (1994) obtained on sarcoplasmic proteins.

The Fe^{2+}/EDTA system, particularly for DP muscle, gave a lower evolution than that previously reported with Fe^{3+}/ascorbate. After a 5 h incubation at 37 °C, the carbonyl contents varied from 3.1 ± 1.6 nmol/mg protein to 10.7 ± 1.9 nmol/mg protein for LL muscle (Figure 3). Surprisingly and without explanation, from 1 to 5 h incubation, the carbonyl content in DP muscle was lower than in LL muscle.

The increase in carbonyl content with the MetMb/H_2O_2 system was low: after a 5 h incubation, carbonyl content was 8.9 ± 3.5 nmol/mg protein for LL and 8.7 ± 1.1 nmol/mg protein for DP muscles. As noted previously (Gatellier et al., 1995), the interaction of H_2O_2 with MetMb in model systems formed a myoglobin-derived radical (tyrosine peroxyl radical) which is a weaker radical than radicals previously found; with the ESR detection, no difference could be observed in the amount of 5,5-dimethyl-1-pyrroline 1-oxide (DMPO)−tyrosyl radical which was formed from LL and PM muscles (Gatellier et al., 1995).

The xanthine/xanthine oxidase system produced the lowest increase in carbonyl content from 1 to 5 h incubation, from 3.1 ± 1.6 to 4.5 ± 0.4 nmol/mg protein for LL muscle and from 4.8 ± 1.5 to 5.6 ± 1.9 nmol/mg protein for DP muscle. Nevertheless in our conditions,

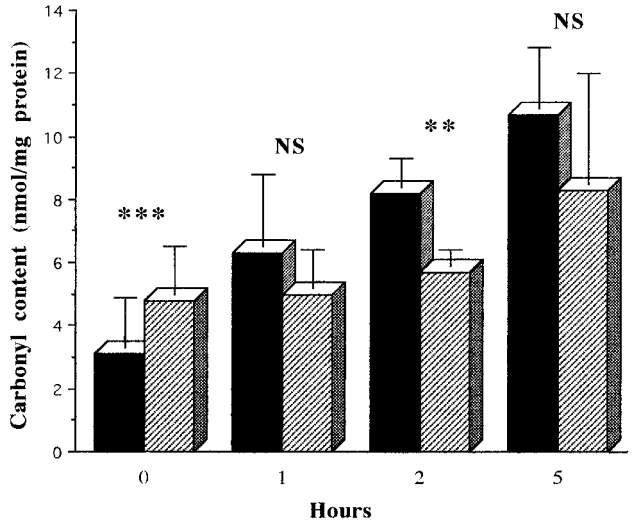

Figure 3. Carbonyl content in myofibrillar proteins of LL (solid) and DP (striped) muscles induced by ferrous iron (4 mM) and EDTA (8 mM). Values are expressed as means of triplicate measurements. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

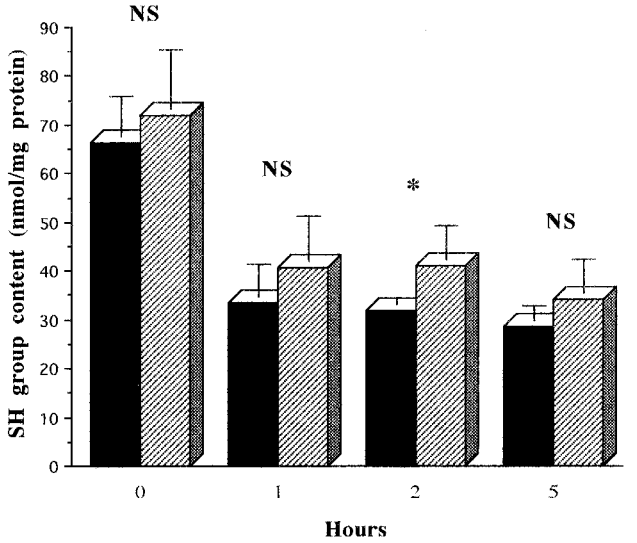

Figure 4. Thiol oxidation in myofibrillar proteins of LL (solid) and DP (striped) muscles induced by xanthine (2.5 mM) and xanthine oxidase (0.075 IU). Values are expressed as means of triplicate measurements. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

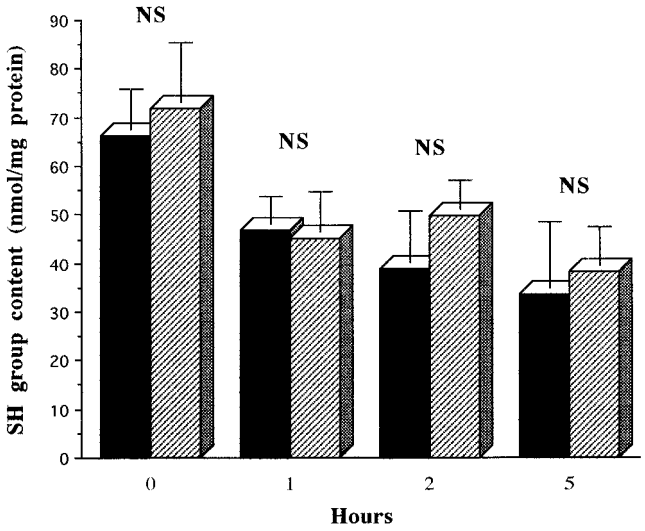

Figure 5. Thiol oxidation in myofibrillar proteins of LL (solid) and DP (striped) muscles induced by metmyoglobin (70 mM) and H_2O_2 (70 mM). Values are expressed as means of triplicate measurements. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

with the xanthine/xanthine oxidase system, iron was not added and, consequently, oxidation was very low. By incubating myofibrils from rat hearts with the xanthine/xanthine oxidase system, the creatine kinase activity (Kaneko et al., 1993) and the Ca^{2+}-stimulated ATPase activity (Suzuki et al., 1991) were depressed.

Storing meat for 10 days gives a carbonyl content near to that obtained after a 1 h incubation in the presence of the majority of the previously cited MCO systems, except with the Fe^{2+}/H_2O_2 system which may produce $OH^{\bullet}$ in greater quantities (Davies and Goldberg, 1987a). The mean values in carbonyl content, after 10 days storage were 5.1 for LL muscle (FOG) and 6.9 for DP muscle (SO). If the hypothesis of Starke-Reed and Oliver (1989) is correct, we have shown for the first time that, after 10 days storage, about 25 and 35% of myofibrillar proteins are oxidized in LL and DP beef muscles, respectively.

Effect of Different Oxidative Systems on Sulfhydryl Content. Regardless of the systems used, the sulfhydryl content before the action of different MCO systems was 66.2 ± 8.4 nmol/mg protein and 71.9 ± 12.2 nmol/mg protein for LL and DP muscles. These values were very near to those noted by Suzuki et al. (1991) from rat heart myofibrils.

Contrary to the previous results obtained from carbonyl contents, the xanthine/xanthine oxidase system was the most efficient of all systems in decreasing the SH content (Figure 4). After only a 1 h incubation, the SH content of myofibrillar proteins had decreased by about 50% for both observed muscles (Figure 4). Mercier (1994) had also previously indicated that the X/XO system was very effective in decreasing SH content (60% after a 2 h induction) on sarcoplasmic proteins. With contractile proteins from rat heart, Kaneko et al. (1993), with the X/XO, SH content decreased by 62% after a 1 h incubation. These results indicated that the X/XO system, which produced $O_2^{\bullet -} + H_2O_2$, is very efficient in the oxidation of SH content contrary to results obtained with carbonyl content. Liu and Xiong (1996) indicated that the loss of thiol groups in chicken meat could occur without formation of carbonyl derivatives.

With the MetMb/H_2O_2 system after a 1 h incubation, the SH content decreased by 30% to 46.7 ± 5.6 nmol/mg protein for LL muscle and by 37% to 45.3 ± 8.0 nmol/mg protein for DP muscle (Figure 5). Consequently, the system MetMb/H_2O_2 was capable of initiating lipid peroxidation (Anton et al., 1993) and was also able to oxidize myofibrillar proteins. After a 5 h incubation, and when compared to controls, the decrease in SH content was similar in both MetMb/H_2O_2 and Fe^{2+}/H_2O_2 systems. It was also noted that after only a 1 h incubation with the Fe^{2+}/H_2O_2 system, the decrease was about 50% for the two muscles (results not shown).

The lowest decrease (about 10%) of SH content was noted with the Fe^{3+}/ascorbate system (Figure 6). With the Fe^{2+}/EDTA system (results not shown), the decrease in SH content was low and only about 10% for LL muscle even after a 5 h incubation. During the same period, the decrease was more pronounced with the red and unstable muscle (DP muscle), compared to the LL muscle, and about 34% after a 5 h period, but the difference between muscles was not significant. Therefore, the different MCO systems had different effects on carbonyl or on SH contents. It must be also noted that the decrease in SH content during storage of meat

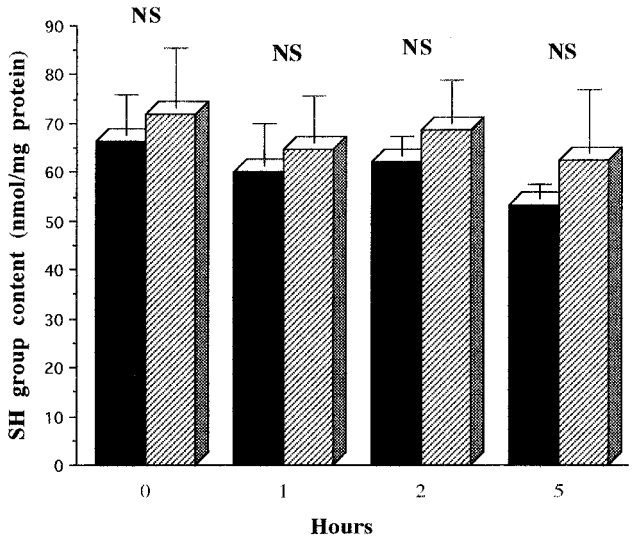

Figure 6. Thiol oxidation in myofibrillar proteins of LL (solid) and DP (striped) muscles induced by ferric iron (0.1 mM) and ascorbate (25 mM). Values are expressed as means of triplicate measurements. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

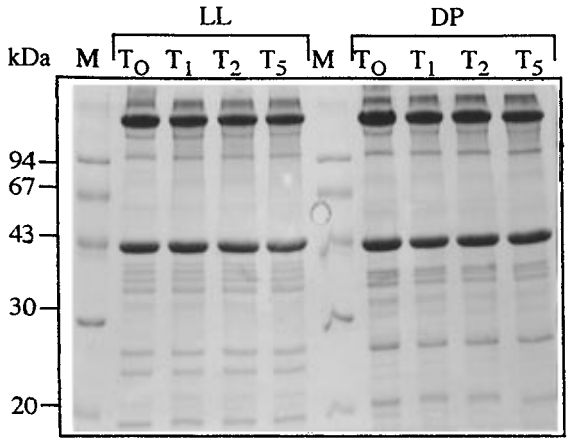

Figure 7. Gel electrophoresis patterns of myofibrillar proteins of LL and DP muscles oxidized by ferrous iron (4 mM) and EDTA (8 mM) for 0 h (T0), 1 h (T1), 2 h (T2) and 5 h (T5) at 37 °C.

for 10 days was similar to that observed after a 1 h incubation when the Fe^{2+}/EDTA and Fe^{3+}/ascorbate systems were about 60 nmol/mg protein.

Electrophoretic Studies. SDS-PAGE migration patterns with β-mercaptoethanol in the presence of the Fe^{2+}/EDTA system showed (Figure 7) the appearance of new bands below the myosin band, between C-protein and α-actinin corresponding to the formation of degradation products. Particularly with myofibrils of DP muscle, a band of about 32 kDa decreased from 1 (T0) to 5 h (T5) incubation (Figure 7). In DP muscle, this band could correspond to the oxidation of carbonic anhydrase as noted by Jeffery et al. (1986). Contrary to our previous observations (Mercier, 1994), a decrease in the troponin T and tropomyosin bands was not observed by the Fe^{2+}/EDTA system.

For the other systems (Fe^{2+}/H_2O_2; Fe^{3+}/ascorbate; xanthine/xanthine oxidase), depending on the muscle and/or oxidative system, there was a more or less rapid but low decrease in the myosin band and, concomitantly, in the appearance of myosin degradation products. For practical reasons, only the incubation with xanthine/xanthine oxidase was represented (Figure 8) where a degradation of myosin was particularly noted in DP (SO)

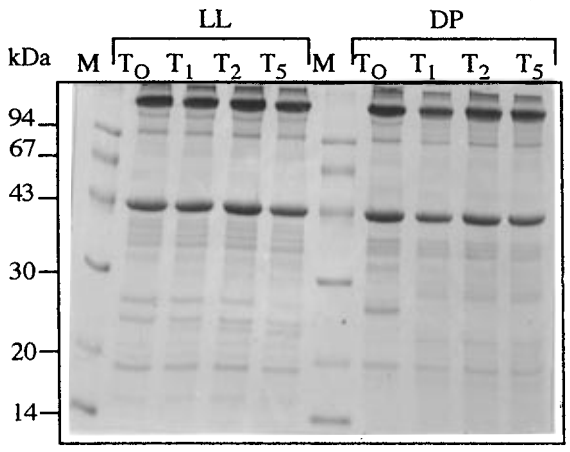

Figure 8. Gel electrophoresis patterns of myofibrillar proteins of LL and DP muscles oxidized by xanthine (2.5 mM) and xanthine oxidase (0.075 IU) for 0 h (T0), 1 h (T1), 2 h (T2) and 5 h (T5) at 37 °C.

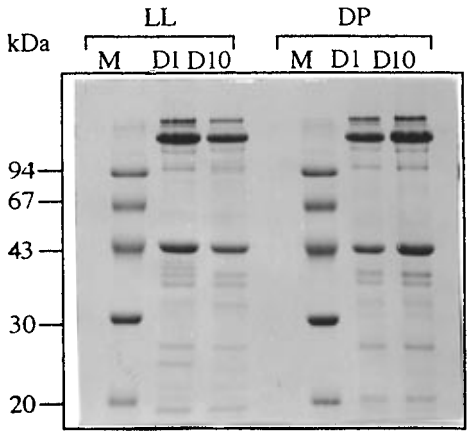

Figure 9. Gel electrophoresis patterns of myofibrillar proteins of LL and DP muscles at day 1 (D1) and day 10 post-mortem (D10).

muscle. Decker et al. (1993) showed, on turkey myofibrillar proteins, a decrease in the actin, myosin, and tropomyosin contents.

For DP muscle, C-protein was degraded and a new band appeared just below the myosin. In LL (fast oxidative glycolytic) muscle, slow myosin light chain (LC1sb) disappeared after a 5 h incubation, but in DP (slow oxidative) muscle, slow myosin light chains (LC1sa and particularly LC1sb) disappeared only after a 1 h incubation (Figure 8). In LL muscle, LC1f is very rapidly decomposed into two bands very similar in molecular weight. LC2s, in DP muscle, and LC2f and LC3f, in LL muscle, were not affected by the X/XO oxidative system (Figure 8). In our experiment, actin seemed protected against the oxidative process either because this protein is present as F-actin or there was no site of oxidation on this protein or the sites were masked by interaction with the chains of myosin. In cod muscle, by using a nonenzymic free-radical-generation system, Srinivasan and Hultin (1997) recently showed a diminution in the myosin heavy chain.

By comparison, after the maturation process where proteolysis is implicated, between D1 and D10 post-mortem (Figure 9), the troponin T band decreased, but the phenomena were more pronounced in myofibrillar proteins extracted from LL than for DP muscle (Figure 9). Moreover, a band, referred to as "the 30 kDa component" (which is now really known as a 32 kDa component), increased and could correspond to the

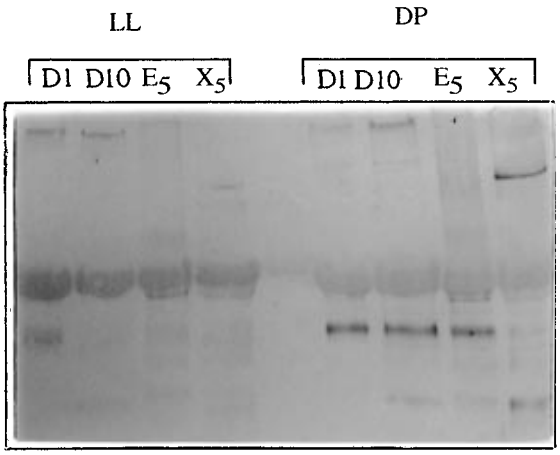

Figure 10. Comparison by Western blot analysis, of myofibrillar proteins oxidation induced by maturation (at day 1 (D1) and day 10 post-mortem (D10)) and by incubation, for 5 h at 37 °C, with Fe^{2+}/EDTA (E5) and xanthine/xanthine oxidase (X5) systems in LL and DP muscles.

degradation of troponin T (Yamamoto and Samejima, 1977; Ouali, 1990; Negishi et al., 1996) during meat conditioning, particularly in LL muscle. As the electrophoretic patterns obtained after 10 days meat maturation were not too different from those obtained after an oxidation with Fe^{2+}/EDTA and xanthine/xanthine systems, only these two systems will be considered in the last part of our study.

Western Blot Analysis. With the SDS-PAGE analysis, it was difficult to identify the protein particularly with meat matured for 10 days. For these reasons, Western blot analysis was used to better identify the different fragments. Polyclonal antimyosin antibodies from rabbit were developed and used against myofibrillar proteins extracted from LL and DP muscles, at day 1 (D0) and day 10 post-mortem (D10), and against oxidized proteins with the Fe^{2+}/EDTA (E5) and xanthine/xanthine oxidase (X5) systems after a 5 h incubation at 37 °C.

The examination of the Western blot analysis (Figure 10) showed that the antibody has identified several myosin fragments of high molecular weights after 10 days of meat storage and after an oxidation by different systems. At D10, aggregates of myosin were more evident in DP muscle than in LL, but after an incubation with the X/XO system (X5), aggregates of myosin were visible in LL and in DP muscles. Degradation products of myosin were also noted after 10 days of meat storage (D10) and after an incubation with the X/XO system (X5) (Figure 10) but were more visible in DP (SO) muscle.

In conclusion, oxidation of myofibrillar proteins is dependent on the different MCO systems. The carbonyl and SH contents after a beef maturation of 10 days are about equivalent to those produced after a 1 h incubation in using different chemical oxidative systems. Myosin, and perhaps troponin T, were the most oxidizible myofibrillar proteins.

ABBREVIATIONS USED

DETAPAC, diethylenetriaminepentaacetic acid; DMPO, 5,5-dimethyl-1-pyrroline 1-oxide; DNPH, 2,4-dinitrophenylhydrazine; DTNP, 2,2′-dithiobis (5-nitropyridine); ESR, electron spin resonance; LL, longissimus lumborum; DP, diaphragma pedialis; $LO^{\bullet}$, alkoxyl radical; $O_2^{\bullet -}$, superoxide anion; $OH^{\bullet}$, hydroxyl radical; LC1sa, slow myosin light chain; LC1sb, slow myosin light chain; LC2s, slow myosin light chain; LC1f, fast myosin light chain; LC2f, fast myosin light chain; LC3f, fast myosin light chain; PMSF, phenylmethanesulfonyl fluoride; X, xanthine; XO, xanthine oxidase.

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