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# Does Oxidation Affect the Water Functionality of Myofibrillar Proteins?

HANNE CHRISTINE BERTRAM,*,† METTE KRISTENSEN,‡ HENRIK ØSTDAL,§ CAROLINE P. BARON,|| JETTE F. YOUNG,† AND HENRIK JØRGEN ANDERSEN⊥

Research Center Foulum, Department of Food Science, Faculty of Agricultural Sciences, University of Aarhus, P.O. Box 50, DK-8830 Tjele, Department of Human Nutrition, The Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Cereal Food, Novozymes A/S, Krogshøjvej 36, DK-2880 Bagsvaerd, Department of Seafood Research, Danish Institute for Fisheries Research, DTU, Building 221, DK-2800 Kgs. Lyngby, and Corporate R&D, Arla Foods amba, Skanderborgvej 277, DK-8260 Viby J, Denmark

Water-binding properties of myofibrils extracted from porcine muscle, and added hemoglobin with and without exposure to $H_2O_2$, were characterized using low-field proton NMR $T_2$ relaxometry. The effects of pH and ionic strength in the samples were investigated as pH was adjusted to 5.4, 6.2, and 7.0 and ionic strength was adjusted to 0.29, 0.46, and 0.71 M, respectively. The formation of dityrosine as a measure of oxidative protein cross-linking revealed a significant increase in dityrosine concentrations upon $H_2O_2$ activation. The formation of dityrosine was strongly pH-dependent and increased with decreasing pH. In addition, increased levels of thiobarbituric acid reactive substances were observed upon addition of $H_2O_2$, implying that lipid oxidation was enhanced, however, with a different oxidation pattern as compared to the myofibrillar proteins. Low-field NMR relaxation measurements revealed reduced $T_2$ relaxation times upon $H_2O_2$ activation, which corresponds to reduced water-holding capacity upon oxidation. However, a direct relationship between degree of oxidation and $T_2$ relaxation time was not observed with various pH values and ionic strengths, and further studies are needed for a complete understanding of the effect of oxidation on myofibrillar functionality.

**KEYWORDS: NMR; $T_2$ relaxation; water mobility; di-tyrosine; cross-linking; meat; water-holding capacity; pork**

## INTRODUCTION

Water is the predominant constituent of meat, comprising approximately 75% of the meat based on weight. The majority of the water is located in the spaces between the myofibrillar filaments, and myofibrillar proteins are of great importance for the water-holding capacity (WHC) of the meat. Several biochemical and biophysical factors such as pH (for a review, see *1*), ionic strength (*2*, *3*), and degree of muscle contraction (*4*, *5*) are known to affect the WHC of meat. Moreover, it has also been shown that WHC changes during storage (*6*−*10*).

Oxidation of muscle-based food is of great interest due to its role in the development of products with inferior perception properties, e.g., development of warmed-over flavor (*11*−*13*). Cross-linking of myosin as a result of oxidation has been found to take place (*14*). However, how oxidation affects the functional properties of meat, in particular in relation to WHC, has only been studied on a very limited basis (*15*). This is despite the fact that losses in myosin upon iron- and copper-catalyzed oxidation have been demonstrated in turkey myofibrillar proteins (*16*) and that effects of free radical generation and disulfide formation on protein solubility were found in cod muscles (*17*).

Nuclear magnetic resonance (NMR) relaxometry enables characterization of water mobility and distribution, and NMR relaxometry has proven an excellent tool for determination of WHC in fresh meat (for a review, see *18*). This can be explained by the fact that NMR transverse relaxation ($T_2$) also provides information about meat structure (*4*), thereby making the technique highly sensitive to water mobility and distribution as a function of changes at the microstructural level. This was recently demonstrated on extracted myofibrils, where the effects of pH and ionic strength on the water mobility and distribution were investigated using low-field NMR $T_2$ relaxometry (*19*). Using low-field NMR $T_2$ relaxometry, the aim of the present study was to investigate if oxidation induced by $H_2O_2$-activated hemoglobin (Hb) (*20*−*22*) affects the functionality of myo-

* To whom correspondence should be addressed. Tel: +45 89 99 15 06. Fax: +45 89 99 15 64. E-mail: HanneC.Bertram@agrsci.dk.
† University of Aarhus.
‡ The Royal Veterinary and Agricultural University.
§ Novozymes A/S.
|| Danish Institute for Fisheries Research.
⊥ Arla Foods amba.

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fibrillar proteins at various pH values and ionic strengths before and after heat treatment.

## MATERIALS AND METHODS

**Extraction of Myofibrils.** The meat used in the present study was porcine *M. longissimus dorsi* from a single animal. The meat was frozen 24 h postmortem and stored at −20 °C until extraction. Extraction of myofibrillar proteins was carried out essentially as described by Martinaud et al. (*23*). Seven grams of crushed frozen muscle tissue was homogenized in 25 mL of ice-cold myofibrillar extraction buffer (MFE) [100 mM KCl, 20 mM potassium phosphate (pH 7.0), 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 1 mM $Mg_2Cl_2$, and 1 mM $NaN_3$] for 30 s using an Ultra Turrax $T25_{BASIS}$ (IKA Labortechnik, Germany) with a speed of 11500 revolutions/min. Solutions were kept on ice throughout the extraction. The homogenate was centrifuged at 1500*g* for 15 min at a temperature of 4 °C. The pellet was washed with 25 mL of ice-cold MFE buffer, and centrifugation was repeated. Thereafter, the pellet was suspended in 12.5 mL of MFE buffer and mixed on a Whirley mixer (Scientific Instrumentation, U.K.). Connective tissue was eliminated by filtration on a nylon net (pore size, 400 μm). The protein extracts were frozen in aliquots and stored at −80 °C until further use. Before further analyses, the protein extracts were thawed and centrifuged at 1500*g* for 15 min at a temperature of 4 °C after which they were washed twice with 25 mL of citrate buffer (see next) followed by centrifugation at 1500*g* for 15 min at a temperature of 4 °C. The protein extracts were then resuspended in 25 mL of ice-cold citrate buffer and stored at a temperature of 4 °C for 24 h before further analysis. A total of nine citrate buffers (50 mM) that were combinations of three different pH values (pH 5.4, 6.2, and 7,0) and three different ionic strengths (0.2856, 0.4567, and 0.7133 M) were used. Three replicates were prepared per condition. The buffers were prepared using 50 mM citric acid, $H_2O$ (10.507 g/L), and trisodium citrate, $2H_2O$ (14.707 g/L). The pH was adjusted with 4 M NaOH or HCl, and the ionic strength was adjusted by addition of NaCl according to eq I:

$$I = 1/2 \times \sum c_i z_i^2 \quad \text{(I)}$$

where $c_i$ is the concentration and $z_i$ is the charge. In the present study, this corresponded to eq II:

$$I = 1/2 \times ([H_2A] \times 1 + [HA^{-2}] \times 2^2 + [A^{-3}] \times 3^2 + 2 \times [NaCl]) \quad \text{(II)}$$

Prior to NMR measurements, the protein concentration was determined in the pellet and adjusted using Bio-Rad protein assay kit (Bio-Rad, Hercules, CA) by measurement of absorbance at 595 nm with a Hewlett-Packard 8453 UV−vis spectrophotometer (Hewlett-Packard, United States). Before adjustment, the protein content ranged from 4.50 to 4.69 mg/mL in the protein extracts.

**Oxidation Assay.** Oxidation of the extracted myofibrils was initiated by Hb (oxy-Hb) and $H_2O_2$ in a 1:5 ratio, which generates high oxidation states of Hb (ferryl- and perferryl-Hb) (*24*, *25*).

Hb was extracted from freshly collected heifer blood as described by Fyhn et al. (*26*). The Hb concentration was determined and adjusted to 1.47 mM by measurement of the absorbance at 525 nm with a HP 8453 UV−vis spectrophotometer (Hewlett-Packard). An extinction coefficient of $2.58 \times 10^4$ L cm$^{-1}$ M$^{-1}$ was used to quantify the Hb concentration (*27*).

The concentration of $H_2O_2$ was determined and adjusted to 0.39 mM by measurement of the absorbance at 240 nm with a HP 8453 UV−vis spectrophotometer (Hewlett-Packard). An extinction coefficient of 39.4 L cm$^{-1}$ M$^{-1}$ at 240 nm was used to quantify the $H_2O_2$ concentration (*28*).

The reaction mixture consisted of 25 mL of myofibrillar protein extract in buffer (see description in the section on extraction of myofibrils), 5 mL of the Hb, and 0.375 mL of $H_2O_2$. Control samples without $H_2O_2$ were also prepared. All reaction mixtures were incubated at 37 °C for 1 h to accelerate oxidation and thereafter stored at 4 °C overnight. Thereafter, the reaction mixtures were centrifuged for 15 min at 2000*g* and subsequently centrifuged twice at 12500*g* for 30 min.

Subsequently, NMR measurements were carried out (see below); then, heat treatment of the samples was carried out in a water bath at 70 °C for 10 min, and NMR measurements were repeated on heat-treated samples after tempering (see below).

**NMR Transverse Relaxation ($T_2$) Measurements.** NMR relaxation measurements were performed on a Maran Benchtop Pulsed NMR analyzer (Resonance Instruments, Witney, United Kingdom) with a magnetic field strength of 0.47 T and with a corresponding resonance frequency for protons of 23.2 MHz. The NMR instrument was equipped with an 18 mm variable temperature probe. Approximately 2 g of sample (pellet) was placed in a cylindrical glass tube and inserted in the NMR probe. Transverse relaxation, $T_2$, was measured using the Carr−Purcell−Meiboom−Gill sequence (*29*, *30*). The $T_2$ measurements were made with a $\tau$ value (time between 90° pulse and 180° pulse) of 150 μs. Data from 4096 echoes were acquired as 64 scan repetitions. The repetition time between two subsequent scans was 2 s. The NMR relaxation measurements were performed on both nonheat-treated samples and heat-treated samples. All relaxation measurements were performed at 25 °C, after the samples had been tempered to this temperature.

**Postprocessing of NMR $T_2$ Data.** Transverse ($T_2$) relaxation data were analyzed using distributed exponential fitting analysis using the RI Win-DXP program (software release 1.2.3) released from Resonance Instruments Ltd. (United Kingdom). A continuous distribution of exponentials for a CPMG experiment may be defined by eq III:

$$g_i = \int_0^\infty A(T) \times e^{-ti/T} \, dT \quad \text{(III)}$$

where $g_i$ is the intensity of the decay at time $t_i$ and $A(T)$ is the amplitude of the component with transverse relaxation time $T$. The RI Win-DXP program solved this equation by minimizing the function (eq IV):

$$(g_i - \int_{x=1}^{m} f_x e^{-\tau i/T} x)^2 + \lambda \sum_{x=1}^{m} f_x^2 \quad \text{(IV)}$$

where $f_x = \int_{Tx}^{Tx+1} A(T) \, dT$.

$\lambda \sum_{x=1}^{m} f_x^2$ is a linear combination of functions added to the equation in order to perform a zero-order regularization as described by Press et al. (*31*). The data were pruned from 2048 to 256 points using linear pruning, which on synthetic data was found to give robust solutions. This analysis resulted in a plot of relaxation amplitude for individual relaxation processes vs relaxation time. From such analyses, the time constant for each peak was calculated from the peak position, and the corresponding water fractions were determined by cumulative integration. All calculations were carried out using an in-house program written in Matlab (The Mathworks Inc., Natick, MA).

**Determination of Water Content.** The total water content of all (both nonheated and heat-treated) samples was determined by freeze drying for 22 h of approximately 100 mg of sample (pellet). The samples were weighted before and after freeze drying, and the water content was calculated as the weight change percentage. Weighing of the samples after freeze drying was made on a scale placed at −18 °C.

**Determination of Thiobarbituric Acid Reactive Substances (TBARS).** TBARS were determined using a microplate method (*15*). Approximately 100 mg of protein pellet was incubated in 0.5 mL of 50% trichloroacetic acid containing 1.3% (wt/vol) thiobarbituric acid (dissolved at 60 °C) and heated to 60 °C for 1 h, followed by determination of the absorbance of supernatant at 532 nm. Tetraethoxypropane (TEP) (Sigma, St. Louis, MO), which spontaneously decomposes in an aqueous environment to form malondialdehyde (MDA), was used as a standard, and absorbance was expressed as MDA equivalents. MDA equivalents were calculated after subtraction of a blank (water) and correction for turbidity measured at 650 nm. The standard curve obtained with TEP standards ranged from 0.0313 to 2 mM TEP.

**Determination of Dityrosine.** Determination of dityrosine was performed using the method from Daneshvar et al. (*32*) after adaptation. Approximately 100 mg of protein pellet was mixed with 1 mL of 6 M HCl, purged with argon, and hydrolyzed overnight at 105 °C. The

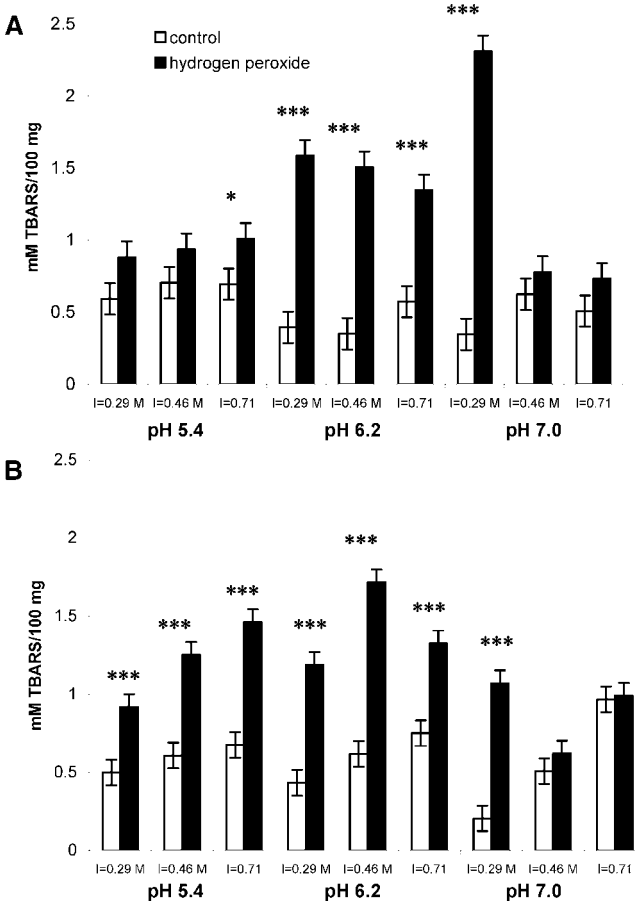


**Figure 1.** TBARS formation for different combinations of pH and ionic strength in control myofibrillar protein extracts and $H_2O_2$-activated samples. LSMeans are given. Bars show standard errors. (**A**) Before heat treatment and (**B**) after heat treatment. *, **, and *** indicate statistically significant differences at $P < 0.05$, 0.01, and 0.001, respectively, between control and $H_2O_2$-activated samples.

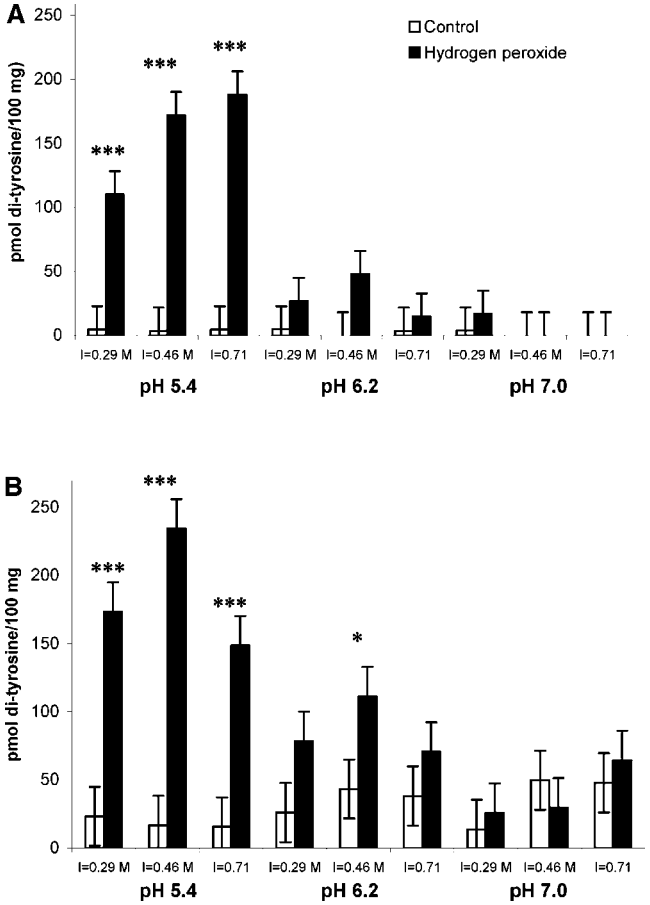


**Figure 2.** Dityrosine formation for different combinations of pH and ionic strength in control myofibrillar protein extracts and $H_2O_2$-activated samples. LSMeans are given. Bars show standard errors. (**A**) Before heat treatment and (**B**) after heat treatment. *, **, and *** indicate statistically significant differences at $P < 0.05$, 0.01, and 0.001, respectively, between control and $H_2O_2$-activated samples.

samples were neutralized with 700 $\mu$L of 6 M NaOH and filtered using 0.45 $\mu$m filters. Thereafter, 20 $\mu$L of the sample was injected onto a C-18 column (Microsorb 100-s 250 × 4.6, Varian, CA) equilibrated with 4% acetonitrile in 0.1 M citric acid at a flow of 1 mL/min. Chromatographic separation was performed on a high-performance liquid chromatography (HPLC) system consisting of a Varian 9012 HPLC pump connected to a Varian 9100 autosampler and a Varian 9075 fluorescence detector (excitation, 283 nm; emission, 410 nm). Assignment was performed by using synthesized dityrosine as the standard as also described by Daneshvar et al. (*32*).

**Statistical Analyses.** Statistical analyses were carried out with the Statistical Analysis System (SAS), using analysis of variance (Proc Mixed). The statistical model included the fixed effect of oxidation (i.e., $H_2O_2$ activiation), pH, and ionic strength. Three replicates were present for each condition corresponding to a total 54 samples (3 × 2 × 3 × 3). A $P$ value of <0.05 was used as the criterion for statistical significance.

## RESULTS

**Lipid Oxidation—TBARS.** TBARS results for all combinations of pH and ionic strength are illustrated in **Figure 1A** (nonheated) and **B** (heat-treated). In all samples, a significant increase in TBARS levels was found upon $H_2O_2$ activation of Hb as compared with nonactivated Hb (control samples). In nonheated samples, an effect of pH on oxidation was evident, as the TBARS levels in the samples added $H_2O_2$ in general were highest at pH 6.2 (**Figure 1A**). At this pH, the TBARS level was almost three times higher for activated Hb as compared to nonactivated Hb. The effect of ionic strength on oxidation was less pronounced than the effect of pH; however, at pH 7.0 and ionic strength $I = 0.29$ M, the TBARS level was dramatically higher than at higher ionic strengths. In heat-treated samples, interactions between ionic strength and pH were observed. At pH 5.4, TBARS levels increased with increasing ionic strength whereas this was not evident at higher pH (**Figure 1B**).

**Formation of Dityrosine.** Dityrosine concentrations in samples of the different combinations of pH and ionic strength are shown in **Figure 2A** (nonheated) and **B** (heat-treated). Dityrosine concentrations were highly influenced by pH, and in nonheated samples, a strong significant increase in dityrosine levels was found upon $H_2O_2$ activation of Hb as compared with control samples at pH 5.4 (**Figure 2A**). At pH 6.2, a significant effect of $H_2O_2$ addition was also observed; however, dityrosine levels were much lower than at pH 5.4. At pH 7.0, dityrosine levels were negligible in both nonoxidized and oxidized samples (**Figure 2A**). A similar pH dependence on dityrosine concentrations was observed in heated samples; that is, dityrosine levels decreased with increasing pH (**Figure 2B**). In addition, the formation of dityrosine was dependent on ionic strength, as a significantly higher amount of dityrosine was found at ionic strength 0.46 M as compared with both lower ($I = 0.29$ M) and higher ($I = 0.71$ M) ionic strengths; however, this tendency was not evident at pH 7.0. Dityrosine levels increased upon heat treatment, and this increase was more pronounced for pH 6.2 and 7.0.

**Table 1.** Water Content (%) for the Different Combinations of pH and Ionic Strength in Myofibrillar Protein Extracts in Control and $H_2O_2$-Activated Samples before and after Heat Treatment ($n = 108$)[a]

| | control samples | | | $H_2O_2$-activated samples | | |
|---|---|---|---|---|---|---|
| | 0.29 M | 0.46 M | 0.71 M | 0.29 M | 0.46 M | 0.71 M |
| **before heat treatment** | | | | | | |
| pH 5.4 | 79.7(0.35) bx | 80.1 (0.35) cx | 81.9 (0.35) dx | 78.0(0.35) ax | 79.9 (0.35) bcx | 81.6 (0.35) dx |
| pH 6.2 | 83.4 (0.35) ay | 91.2 (0.35) cy | 92.2 (0.35) dy | 83.2 (0.35) ay | 89.4 (0.35) by | 91.6 (0.35) cdy |
| pH 7.0 | 85.8 (0.35) bz | 92.2 (0.35) cy | 92.8 (0.35) cdy | 84.8 (0.35) az | 93.0 (0.35) dz | 93.0 (0.35) dz |
| **after heat treatment** | | | | | | |
| pH 5.4 | 70.68 (0.35) ax | 73.49 (0.35) dx | 71.97 (0.35) bx | 71.88 (0.35) bx | 73.48 (0.35) dx | 72.22 (0.35) cx |
| pH 6.2 | 79.26 (0.35) by | 87.91 (0.35) dy | 88.74 (0.35) dey | 77.67 (0.35) ay | 85.59 (0.35) cy | 89.52 (0.35) ey |
| pH 7.0 | 82.85 (0.35) az | 90.63 (0.35) bz | 90.28 (0.35) bz | 82.48 (0.35) az | 90.86 (0.35) bz | 90.44 (0.35) by |

[a] LSMeans are given; values in parentheses show standard errors. Letters a–e indicate significant differences ($P < 0.05$) within rows, and letters x–z indicate significant differences ($P < 0.05$) within columns.

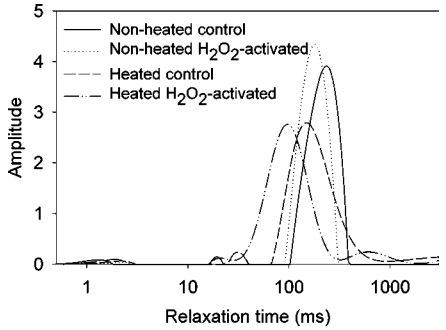


**Figure 3.** Representative distributions of $T_2$ relaxation times in nonheated and heat-treated myofibrillar protein extracts with added Hb or Hb/$H_2O_2$.

**Water Content.** The water content in the samples of the different combinations of pH and ionic strength is shown in **Table 1**. The water content increased significantly with both increasing pH and increasing ionic strength for both nonheated and heated samples; however, the effect of increasing ionic strength from $I = 0.29$ to 0.46 M was higher than the effect of increasing ionic strength from $I = 0.46$ to 0.71 M. However, this was not the case for nonheated samples at pH 5.4. The water content was significantly lower upon oxidation as compared with control samples ($p = 0.0002$) and lower after heat treatment. Samples with higher pH seemed to retain their water better after heat treatment as compared with the other two pH values.

**NMR $T_2$ Relaxation.** In **Figure 3**, distributed $T_2$ relaxation times in control and $H_2O_2$-activated samples before and after heat treatment are displayed (pH 6.2, $I = 0.46$ M). The $T_2$ relaxation was characterized by two minor populations with relaxation times around 1−5 and 20 ms and a major population with a relaxation time ranging from 60 to 300 ms. Upon heat treatment, the major component broadened and shifted toward faster relaxation times. In both nonheated and heated samples, the major population was clearly shifted toward faster relaxation times in oxidized samples as compared with control samples.

Mean $T_2$ values at the different combinations of pH and ionic strength are shown in **Figure 4A** (nonheated) and **B** (heat-treated). The $T_2$ values increased significantly with both increasing pH and increasing ionic strength. However, interactions between pH and ionic strength were observed, and the increase in $T_2$ values was higher when ionic strength was increased from $I = 0.29$ to 0.46 M than from $I = 0.46$ to 0.71 M. In general, the $T_2$ relaxation times were slightly lower in oxidized as compared with control samples, and this was most pronounced at pH 6.2.

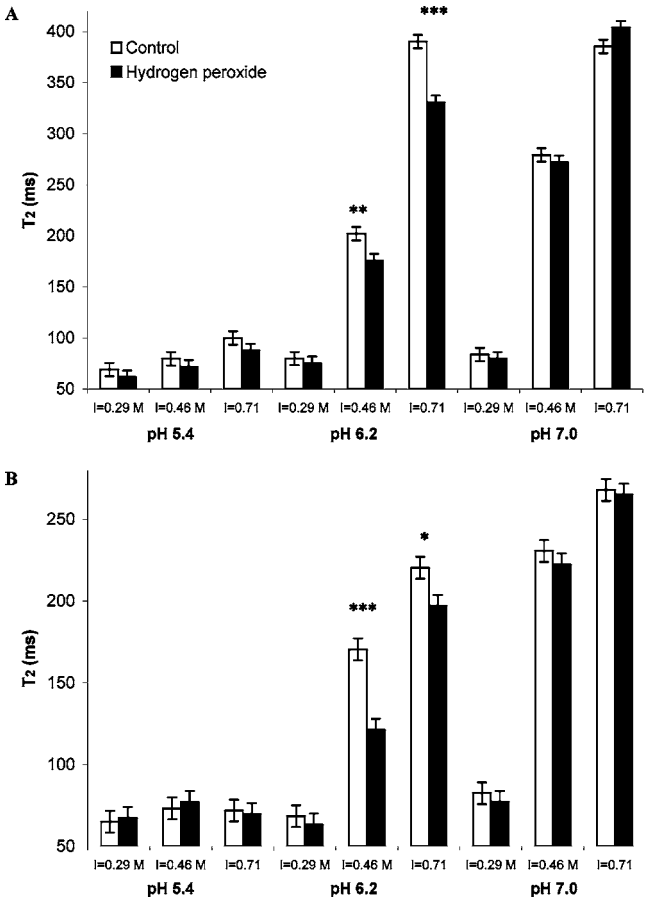


**Figure 4.** Mean $T_2$ relaxation times for the different combinations of pH and ionic strength in control myofibrillar protein extracts and $H_2O_2$-activated samples. LSMeans are given. Bars show standard errors. (**A**) Before heat treatment and (**B**) after heat treatment. *, **, and *** indicate statistically significant differences at $P < 0.05$, 0.01, and 0.001, respectively, between control and $H_2O_2$-activated samples.

## DISCUSSION

Oxidative processes are known to be the major cause for development of inferior meat products resulting in the development of off-flavor, e.g., warmed-over flavor (*11*−*13*). However, research has concentrated mainly on lipid oxidation, whereas oxidation of the protein fraction is less studied, and work conducted on the effect of oxidation on the functional properties of muscle-based foods is limited (*14*−*17*, *33*, *34*). Nevertheless, it is now recognized that proteins are a major target for free radical attack (*35*). In the presence of transition metals such as Fe(II) or Cu(I) and oxygen, a wide range of metal-catalyzed

oxidation systems, which generates OH radicals in Fenton type reactions or hypervalent oxidation states, are able to oxidize amino acid residues of proteins (*36*). The present study reports for the first time the use of low-field NMR proton $T_2$ relaxometry to investigate functional properties of extracted porcine myofibrils oxidized by $H_2O_2$-activated Hb.

Determination of TBARS revealed increased levels of TBARS upon addition of $H_2O_2$ as expected. Lipid oxidation generates free radicals, which have the potency to oxidize proteins. In addition, secondary lipid oxidation products are reported to react with muscle proteins (*37*). Moreover, a high correlation between lipid and protein oxidation has previously been demonstrated in a model system (*38*). In addition, it has also been reported that severe oxidative damage to protein can be initiated by $H_2O_2$-activated heme protein such as Hb and myoglobin (*18*−*20*). Heat treatment is known to induce oxidative changes in the lipid fraction (*39*). However, this could not be deduced from the TBARS determinations in the present study, since no increase in TBARS was observed upon heat treatment. This may be a result of (i) formation of volatile aldehydes not "detected" by the TBARS method; (ii) that the fluid expelled during heat treatment, which possibly could contain a considerable amount of TBARS, was removed; or (iii) a limited amount of lipid in the myofibril preparation.

Formation of dityrosine, reflecting cross-linking in proteins, was also measured in the myofibrillar samples. Strong significant increases in dityrosine concentrations were found upon oxidation, which is in agreement with previous studies on myosin and other proteins (*25*, *40*, *41*). The formation of dityrosine showed a strong pH dependence. A plausible explanation is that $H_2O_2$ activation of oxy-Hb is pH-dependent. The repeated cycles of $H_2O_2$ activation of oxy-Hb involve autoreduction of ferryl-Hb to Met-Hb, which is accelerated at a lower pH (*42*). However, it is possible that a pH-induced denaturation of Hb resulted in iron release and thereby initiated a Fenton-like mechanism contributing to dityrosine formation. Finally, it cannot be ruled out that the electrostatic repulsion caused by elevated pH limits the cross-linking of the tyrosine groups due to longer distances between the reactive amino acids and the individual tyrosine residues, hereby hindering the necessary electron transfer. Increased dityrosine formation was found upon heat treatment, implying that protein oxidation and cross-linking take place during heating of myofibrillar proteins, which probably contributes to the heat-induced gelation process.

Myofibrillar proteins are of great importance for functional properties of meat, in particular WHC and water-binding capacity. In the present study, water properties in myofibrillar systems with different pH values, ionic strengths, and oxidative statuses were characterized using NMR $T_2$ relaxation measurements. Increasing $T_2$ relaxation times were observed with increasing pH values and ionic strengths (**Figure 4**), which is in agreement with earlier studies (*17*, *43*). Brownstein and Tarr (*44*) proposed that the relaxation rate is determined by exchange processes between water and macromolecules, where the latter can be considered as surfaces and are described as relaxation sinks. In the case of fast exchange, the distance from the molecule to the surface determines the relaxation rate, and accordingly, an increase in the distance between the thin and the thick filaments is expected to result in an increase in the relaxation time. These ideas are consistent with empirical results, which have demonstrated a positive correlation between myofilament lattice spacing and $T_2$ relaxation time in meat (*4*). Consequently, the present data can be interpreted as a swelling of the myofilament lattice with increasing pH and ionic strength. This is also in agreement with the water content of the samples (**Table 1**), which also increased with pH and ionic strength. Reduced $T_2$ relaxation times were observed upon oxidation (**Figure 4**), corresponding to reduced myofilament lattice spacing. The amount of water confined in the myofibrils determines WHC (*2*), and accordingly, the reduced $T_2$ relaxation times in oxidized samples concur with lower water contents in these samples (**Table 1**) and indicate that oxidation reduces the ability of myofibrillar proteins to retain water. These findings may explain the reduced protein solubility and reduced gel strength previously reported in oxidized myofibrillar systems (*16*, *17*, *33*, *34*) and would be consistent with cross-linking between sarcoplasmatic proteins and the myofibrillar protein matrix, hereby decreasing the contribution from the sarcoplasmatic protein fraction to WHC (*45*) and impairing gel formation by formation of rigid structures. Contrary results showing improved emulsifying properties of oxidized cod proteins (*17*) and increased gel strength of surimi products in which the proteins were exposed to oxidation (*46*) may be explained by either the narrow pH range applied in these studies, as a higher pH might have resulted in greater protein unfolding known to have negative consequences for WHC, or simply by different amino acid composition of mammalian and fish proteins and hereby different isoeletric points of these systems. In the present study, it was clearly demonstrated that different pH values and ionic strengths are critical factors for the degree of protein oxidation and consequences for simultaneous structural changes in muscle systems, as the formation of dityrosine was markedly higher at pH 5.4 as compared to higher pH values, while the largest reduction in $T_2$ values upon oxidation was observed at pH 6.2. Thus, the data in the present study indicate divergent effects of oxidation and that the effects are strongly influenced by other biochemical characteristics such as pH and ionic strength. Further studies are needed to fully understand the role of oxidation on the functionality of myofibrillar proteins and to determine a potential optimum of oxidation most favorable for improving the functionality of meat proteins.

In conclusion, the present study has demonstrated that $^1H$ NMR relaxometry can be applied to study the effects of oxidation on water properties and thereby improve the understanding of how functionality is affected by oxidation. Reduced $T_2$ relaxation times were observed upon $H_2O_2$ activation of Hb, implying reduced WHC upon oxidation. However, a direct relationship between degree of oxidation and $T_2$ relaxation time was not observed with various pH and ionic strength values, and further studies are needed for a complete understanding of the effect of oxidation on myofibrillar functionality.

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