Origin of Multiexponential T_2 Relaxation in Muscle Myowater

Hanne Christine Bertram, Anders Hans Karlsson, Marianne Rasmussen, Ole Dahl Pedersen, Sune Dønstrup, and Henrik Jørgen Andersen*

Department of Animal Product Quality, Danish Institute of Agricultural Sciences, Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, Denmark

To obtain a further understanding of the nature of the multiexponential T_2 relaxation seen in muscle tissue water (myowater), relaxation measurements were carried out on whole, minced, and homogenized pork of three different qualities with regard to water-holding capacity (normal, red soft exudative, and dark firm dry). Whole, minced, and homogenized pork all resulted in multiexponential T_2 relaxation (three components) independently of the quality, even though microscopic studies on homogenized meat revealed considerable disruption of the macroscopic structure. This states that the relaxation behavior in meat cannot be explained by intra-/extracellular compartmentalization of the water as suggested in earlier studies. Subsequent studies of T_2 relaxation in either whole meat, where the structure integrity was changed by the introduction of dimethyl sulfoxide (membrane disruption) or urea (protein denaturation), or minced meat with added NaCl (inter-/intraprotein interactions) lead to the suggestion that in whole meat (i) the fastest relaxation component reflects water tightly associated with macromolecules, (ii) the intermediate relaxation component reflects water located within highly organized protein structures, for example, water in tertiary and/or quaternary protein structures and spatials with high myofibrillar protein densities including actin and myosin filament structures, and (iii) the slowest relaxation component reflects the extra-myofibrillar water containing the sarcoplasmatic protein fraction. Finally, relaxation patterns in heat-set gels of superprecipitated actomyosin and bovine serum albumin similar to that identified in whole meat support the proposed nature of T_2 relaxation in muscle myowater.

Keywords: *NMR; T_2 relaxation; myowater; meat structure; muscle quality; water-holding capacity*

INTRODUCTION

Three decades ago, it was demonstrated that pulsed low-field NMR relaxation techniques (LF-NMR) can provide information about the state of water in muscle tissue, as relaxation data were found to indicate the existence of more than one type of water state in muscle tissue (*1*−*3*). Recently, a strong correlation between LF-NMR data obtained in meat samples 24 h post-mortem and the water-holding capacity (WHC) of the meat has been shown (*4*−*6*). Despite these convincing correlations, confusion still exists about how to interpret LF-NMR relaxation data in relation to the water distribution in the muscle tissue and meat. Three theories hypothesizing the relaxation behavior in muscle tissue have emerged. One hypothesis (i) is that the multiexponential behavior of the relaxation in muscle tissue is caused by a physical compartmentalization of water. This compartmentalization is most often interpreted as intra- and extracellular, as the cell membranes are expected to act as a physical barrier for the water molecules (*7*). The second theory (ii) suggests that intracellular water is more or less structured depending on the degree of contraction (*8*). The third theory (iii) explains the multiexponential behavior of the relaxation by fast exchange between free water and the wall of differently sized pores (spatial heterogeneity) (*9*).

* Author to whom correspondence should be addressed (telephone +45 89 99 12 41; fax +45 89 99 15 64; e-mail Henrik.Andersen@agrsci.dk).

Due to the dispute of how to interpret the multiexponential decay of the LF-NMR relaxation of myowater, the aim of the present study was to obtain additional data that may contribute to a further understanding of the nature of T_2 relaxation in myowater. For that purpose the macro- and microstructures of porcine meat of three qualities [normal, red soft exudative (RSE), and dark firm dry (DFD)] were changed by mincing, homogenization, and addition of sodium chloride with subsequent measurement of NMR relaxation patterns in the processed meat samples. Moreover, whole pork was treated with urea (enhanced protein denaturation) and dimethyl sulfoxide (DMSO) (membrane disruption) to measure their effect on the NMR relaxation pattern in the meat samples. Finally, NMR relaxation patterns were obtained from superprecipitated actomyosin and heat-set gels of actomyosin and bovine serum albumin (BSA) to make a comparison possible with those data found in the tested meat samples.

MATERIALS AND METHODS

Chemicals. Actomyosin [from rabbit muscle in 50% glycerol (pH 6.8) containing 0.6 M KCl], BSA (fraction V), DMSO (minimum = 99.5%), and urea (minimum = 99.5%) were obtained from Sigma Chemical Co. (St. Louis, MO). Nile red was obtained from Polysciences Inc. (Warrington, PA). Fluorescein 5-isothiocyanate (FITC) was obtained from Merck (Darmstadt, Germany). All other chemicals used were of analytical grade, and double-deionized water was used throughout.

Animals, Treatments, and Meat Samples. Thirty-nine pigs were used in this study (three female littermates from

10.1021/jf001402t CCC: $20.00
Published on Web 05/12/2001

13 litters of the crossbreed between Danish Duroc boars and Danish Landrace × Large White sows) with a live weight of ~100 kg at slaughter. To receive a large variation in meat quality, the three female littermates were assigned randomly to three different treatment groups the day before slaughter as described in ref *10*. Group A served as the control animals without special treatment (subsequently referred to as pigs resulting in meat of *normal* quality). Group B animals were exercised on a treadmill at a speed of 3.8 km/h for 10 min immediately prior to stunning (subsequently referred to as pigs resulting in meat of *RSE* quality). Group C animals were administered 0.2 mg of adrenaline/kg of live weight 18−20 h preslaughter (subsequently referred to as pigs resulting in meat of *DFD* quality).

The pigs were slaughtered in the experimental abattoir at Research Centre Foulum over a period of 3 days. Animals in groups A and C were stunned by 80% CO_2 for 3 min, whereas the animals in group B were electrically stunned (220 V for 15 s). After stunning, the pigs were exsanguinated and scalded at 62 °C for 3 min. Cleaning and evisceration of the carcasses were completed within 30 min post-mortem. Wtihin 2−6 h post-mortem the carcasses were transferred to the chilling room, where they were stored at 4 °C until further assessments were performed 24 h post-mortem.

pH Measurements. The pH was measured at the last rib curvature of M. longissimus dorsi 45 min and 24 h post-mortem with a pH-meter (Metrohm AG CH-9101 Herisau, Switzerland) equipped with an insertion glass electrode (LL glass electrode, Metrohm). At the measurement 45 min post-mortem the calibration temperature was 35 °C, and at the measurement 24 h post-mortem the calibration temperature used was 4 °C. A two-point calibration was performed, and the pH of the calibration buffer used was 7.000 and 4.005 at 25 °C (Radiometer, Copenhagen, Denmark).

Water-Holding Capacity (WHC). Measurement of WHC was performed using the Honikel bag method (*11*), which is a gravimetric method. A slice of M. longissimus dorsi from the last rib curvature with a weight of ~100 g was trimmed and weighed 24 h post-mortem. Subsequently, the sample was placed in a net and then hung in an inflated plastic bag for 48 h at 4 °C, after which time it was weighed again. Drip loss was calculated as the difference in weight before and after hanging. Determination of WHC was carried out only on two of the three slaughter days; therefore, WHC was determined only on pork from a total of 30 pigs.

Manipulation of Micro- and Macrostructures of Pork Meat. Low-field NMR relaxation (LF-NMR) measurements were performed 24 h post-mortem on whole meat samples from 30 slaughtered pigs (10 per quality group). Subsequently the samples were minced for 30 s using a mini-mincer (Speedy Pro from Krups), and the LF-NMR measurements were repeated. Finally, the samples were homogenized for 30 s on ice using an Ultra Turrax homogenizer (model T25 from IKA, speed 13.000 L/min, 8 mm spindle), and LF-NMR measurements were repeated once more.

Samples of meat (normal quality, ~5 cm long and 1 × 1 cm in sectional area, weight ~5 g) were cut along the fiber direction by a scalpel 24 h post-mortem and placed with the fiber direction vertical in a cylindrical glass tube containing either DMSO known to disintegrate membrane structures (*12, 13*) or 8 M urea known to denature protein structures (*14−16*). To promote the migration of DMSO or urea through the samples, filter papers were placed on top of the samples and replaced regularly. After 17 h at 4 °C, the samples were removed from the cylindrical glass tubes, and LF-NMR relaxation measurements were performed on the samples thermostated to 25 °C.

Finally, to change the electrostatic interactions and the subsequent local protein interactions in meat, three independent samples of meat of normal quality were minced using the same procedure as previously described. After mincing, each sample was divided into two samples, and 5% (w/w) sodium chloride (NaCl) was added and mixed into one subsample, the other remaining as a control. After 24 h at 4 °C, LF-NMR relaxation measurements were performed on the samples thermostated to 25 °C.

Preparation of Superprecipitated Actomyosin and Heat-Set Actomyosin and BSA Gels. Actomyosin was concentrated by centrifugation (3000 rpm) and subsequently washed three times with 50 mM phosphate buffer (pH 7.4) containing 0.15 M NaCl. This resulted in ~150 mg of superprecipitated actomyosin. The superprecipitated actomyosin was subsequently thermostated at 25 °C, and LF-NMR relaxation measurements were performed.

BSA was dissolved in distilled water to a final concentration of 24% (w/w). Subsequently, two heat-set gels were prepared by heating BSA and superprecipitated actomyosin for 12 min at 85 °C in a water bath. These gels were then thermostated at 25 °C, and LF-NMR relaxation measurements were performed.

Confocal and Light Microscopy. Light microscopy was carried out on whole, minced, and homogenized pork samples. Whole, minced, and homogenized pork samples were mounted in an embedded medium, frozen in isopentane, cooled in liquid nitrogen, and mounted for cryostat sectioning. All samples were stored at −80 °C until use. Cryostat sections (10 μm) were immuno-histochemically stained for collagen IV (undiluted monoclonal mouse anti-human collagen IV, code M785, DAKO Co., CA) for 30 min at 37 °C. Biotinylated rabbit anti-mouse IgG secondary antibody (diluted 1:300) (DAKO A/S, Glostrup, Denmark) was added for 15 min at 37 °C. Horseradish peroxidase-labeled streptavidin (diluted 1:500) (DAKO A/S) was added for 15 min at 37 °C followed by 3,3′-diaminobenzidine tetrahydrochloride (DAKO A/S) for 5 min at room temperature. Between steps, the sections were rinsed with Tris-buffered saline (pH 7.6). Microscopy evaluation were performed with an Olympus BH-2 microscope (Olympus Optical Co., Hamburg, Germany), allowing a linear magnification of 8−20 times, fitted with a CCD black and white video camera and a computer, based on an SB 1024-SA and SB 1024-RAM board, provided with a high-resolution monitor.

Heat-set gels of superprecipitated actomyosin, BSA, and pork samples (normal quality) were sliced with a scalpel and placed on microscopic slides. Subsequently, gel samples were covered with coverslips, FITC dissolved in acetone was settled, and the acetone was evaporated before mounting, whereas pork samples were covered with coverslips, Nile red dissolved in acetone was settled, and the acetone was evaporated before mounting. Finally, samples were examined with a laser-scanning confocal fluorescence microscope (TCS NT, Leica Microsystems, Wetzlar, Germany).

LF-NMR Relaxation Measurements. The relaxation measurements were performed on a Maran benchtop pulsed NMR analyzer (Resonance Instruments, Witney, U.K.) with a magnetic field strength of 0.47 T and a corresponding resonance frequency for protons of 23.2 MHz. The NMR instrument was equipped with an 18 mm temperature-variable probe. Transverse relaxation, T_2, was measured using the Carr−Purcell−Meiboom−Gill sequence (CPMG; *17, 18*). The T_2 measurements were performed with a τ value (time between 90° pulse and 180° pulse) of 150 μs. Data from 4096 echoes were acquired; they were obtained as 16 scan repetitions. The repetition time between two succeeding scans was 2 s. All relaxation measurements were performed at 25 °C.

Biexponential fitting analysis of T_2 relaxation data was performed using the RI Winfit program (version 1.2.3, Resonance Instruments Ltd.). Biexponential fitting analysis is performed by fitting the absolute value of the CPMG data to a linear combination of two exponentials

$$g = f_{21}\, e^{-t/T_{21}} + f_{22}\, e^{-t/T_{22}} + DC \qquad (I)$$

where g is the magnetization amplitude. T_{21} and T_{22} are the two characteristic transverse relaxation time constants, and f_{21} and f_{22} are the corresponding fractions of the magnetization amplitude that can be described by the two time constants, respectively. DC is a constant term included to fit the noise from the absolute value data.

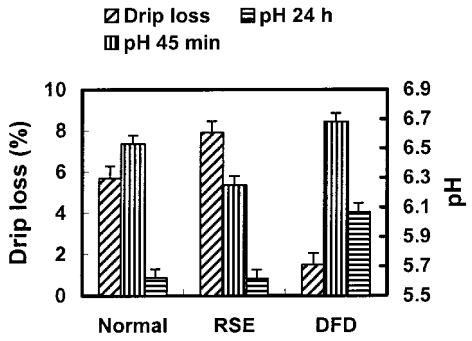

Figure 1. Meat quality traits for the animal material used: least-square means and standard errors of pH measured 45 min post-mortem ($n = 39$), pH measured 24 h post-mortem ($n = 39$), and drip loss ($n = 30$). Normal (group A) animals served as control, red soft exudative, RSE (group B), animals were exercised for 10 min on a treadmill immediately prior to slaughter, and dark firm dry, DFD (group C) animals were administered 0.2 mg of adrenaline/kg of live weight at 18−20 h preslaughter.

The reason for fitting only a biexponential decay rather than a triexponential decay was that the RI Winfit program preferred to split the T_{21} component in two instead of adding a fast component.

Furthermore, the RI Win-DXP program (version 1.2.3, Resonance Instruments Ltd.) was used for data analysis. The RI Win-DXP program performs distributed exponential curve fitting. A continuous distribution of exponentials for a CPMG experiment may be defined by the equation

$$g_i = \sum_{j=1}^{m} f_j \, e^{-t_i/T_j} \qquad \text{(II)}$$

where g_i are the values of the exponential distribution at time t_i, f_j are the pre-exponential multipliers of the distribution, and T_j are the exponential time constants (the T_2 values). The RI Win-DXP program solves this equation by minimizing the function

$$(g_i - \sum_{x=1}^{m} f_x \, e^{-\tau_i/T_x})^2 + \lambda \sum_{x=1}^{m} f_x^{\,2} \qquad \text{(III)}$$

where $\lambda \Sigma_{x=1}^{m} f_x^{\,2}$ is a linear combination of functions, added to the equation to stabilize the equation system, as described in ref *19*. The λ is the weight, and we used a value of 0.005. Moreover, before fitting was performed, the data were pruned from 4096 to 64 points to increase the signal to noise ratio and get reasonable calculation times.

Statistical Analysis. Analysis of variance was carried out using the MIXED procedure from the Statistical Analysis System (SAS; *20*). The statistical model used included the fixed effects of meat quality (normal, RSE, and DFD) and treatment (mincing, homogenization, and salting) and the random effect of litter. Interactions between the main effects were included if significant.

RESULTS

The different treatments included in the study resulted as expected in a large variation in the meat quality as seen in Figure 1. The pH measured at 45 min post-mortem varied from 5.55 to 6.92 with averages of 6.68, 6.53, and 6.25 for the three meat qualities groups (DFD, normal, and RSE), respectively, and pH measured at 24 h post-mortem varied from 5.45 to 6.41 with averages of 6.06, 5.60, and 5.57 for the three qualities, respectively. Morever, WHC varied from 0.5 to 11.7% with average drip losses of 1.5, 5.7, and 7.9% for the three qualities (Figure 1).

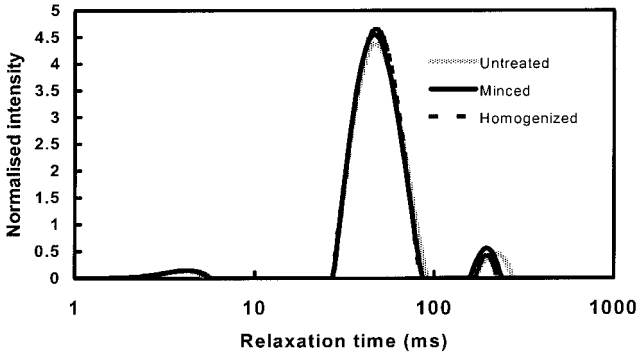

Figure 2. Representative distribution of low-field transverse relaxation (T_2) times for whole, minced, and homogenized meat. T_2 data were performed with a τ value (time between 90° pulse and 180° pulse) of 150 μs. Measurements were performed at 25 °C, and data were acquired from 4096 echoes as 16 scan repetitions with a repetition time of 2 s between two succeeding scans.

Table 1. Least-Square Means and Standard Errors (SE) of the Two Relaxation Components T_{21} and T_{22} Found by Biexponential Analysis of CPMG Data Measured in Meat of Normal, RSE, and DFD Quality[a]

	mean (SE)		
whole meat	normal ($n = 10$)	RSE ($n = 10$)	DFD ($n = 10$)
T_{21} (ms)	44.05[a] (0.61)	41.63[a] (0.54)	42.82[a] (0.61)
T_{22} (ms)	111.44[a] (4.00)	126.21[b] (3.95)	84.91[c] (4.00)
pT_{21} (%)	80.68[a] (1.23)	81.32[a] (1.22)	78.65[a] (1.23)
pT_{22} (%)	19.32[a] (1.23)	18.68[a] (1.23)	21.35[a] (1.23)

[a] Different superscript letters in a row indicate significant differences ($P < 0.05$) between meat quality categories. T_{21} and T_{22} represent relaxation time constants, and pT_{21} and pT_{22} equal the corresponding populations.

Figure 2 shows typical examples of results obtained upon distributed exponential fitting on relaxation data from whole, minced, and homogenized meats. Three components, in the following referred to as T_{2b}, T_{21}, and T_{22}, were detected in all meats, a minor component between 1 and 10 ms, a major component between 40 and 60 ms, and finally a third component between 150 and 400 ms.

Traditional biexponential analysis (two-component model) of T_2, measured in whole fresh meat samples, revealed that the slowest time constant (T_{22}) differed significantly ($p = 0.0001$, $p = 0.0001$, and $p = 0.0297$) among the three meat qualities. DFD (group C) had the fastest T_{22} and RSE (group B) the slowest T_{22} (Table 1). In contrast, no significant differences were found in the fastest time constant (T_{21}) among the three meat qualities. The T_{21}/T_{22} ratio among the three meat quality groups was not significantly different. However, compared with data obtained from meat of normal quality, DFD and RSE meats tended to have smaller and higher percentages, respectively, described by the myowater fraction characterized by the slow time constant (Table 1).

The results of the microscopic analysis carried out on whole, minced,and homogenized meat are shown in Figure 3. Mincing resulted in disintegration of the overall structure, however, with maintenance of intact myofilaments (Figure 3b). In contrast, homogenization resulted in further disintegration with a much more homogeneous appearance where only minor fractions could be identified as intact myofibrills (Figure 3c).

Table 2 includes the results of distributed exponential analysis of the T_2 decay, measured on whole, minced,

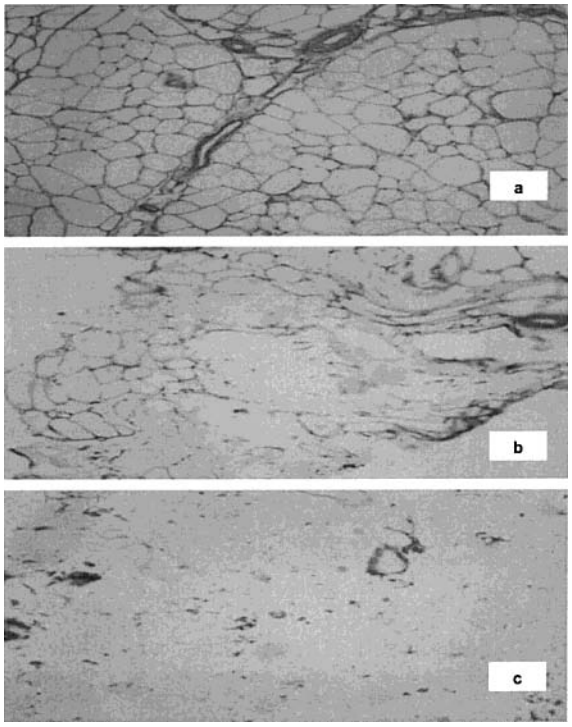

Figure 3. Light microscopy of (a) whole, (b) minced, and (c) homogenized meat.

Table 2. Least-Square Means and Standard Errors (SE) of the Three Relaxation Components T_{2b}, T_{21}, and T_{22} Found by Distributed Exponential Curve Fitting of CPMG Data Measured in Fresh, Minced, and Homogenized Meat, Respectively (Normal, RSE, and DFD Represent the Three Different Meat Quality Categories)[a]

	mean (SE)		
	normal ($n = 10$)	RSE ($n = 10$)	DFD ($n = 10$)
Whole Meat			
T_{2b} (ms)	4.09^{ax} (0.07)	4.29^{bx} (0.07)	3.74^{cx} (0.07)
T_{21} (ms)	47.06^{ax} (0.73)	44.90^{bx} (0.73)	47.72^{ax} (0.73)
T_{22} (ms)	191.78^{ax} (5.87)	196.76^{ax} (5.87)	215.95^{bx} (5.87)
pT_{2b} (%)	2.24^{ax} (0.16)	2.13^{ax} (0.16)	1.44^{bx} (0.16)
pT_{21} (%)	93.49^{axy} (0.55)	92.38^{ax} (0.55)	96.99^{bx} (0.55)
pT_{22} (%)	4.27^{ax} (0.52)	5.49^{ax} (0.52)	1.57^{bx} (0.52)
Minced Meat			
T_{2b} (ms)	4.11^{ax} (0.07)	4.11^{ay} (0.07)	3.84^{bx} (0.07)
T_{21} (ms)	46.74^{ax} (0.73)	43.37^{by} (0.73)	46.27^{ay} (0.73)
T_{22} (ms)	191.87^{ax} (5.87)	187.30^{ax} (5.87)	185.91^{ay} (5.87)
pT_{2b} (%)	2.19^{ax} (0.16)	2.02^{ax} (0.16)	1.47^{bx} (0.16)
pT_{21} (%)	92.24^{ax} (0.55)	92.49^{ax} (0.55)	96.51^{bx} (0.55)
pT_{22} (%)	5.57^{ax} (0.52)	5.52^{ax} (0.52)	2.02^{bx} (0.52)
Homogenized Meat			
T_{2b} (ms)	4.61^{ay} (0.07)	4.68^{az} (0.07)	4.39^{by} (0.07)
T_{21} (ms)	49.70^{ay} (0.73)	47.42^{bz} (0.73)	47.76^{abx} (0.73)
T_{22} (ms)	204.21^{abx} (5.87)	187.99^{ax} (5.87)	213.01^{bx} (5.87)
pT_{2b} (%)	3.47^{aby} (0.16)	3.25^{bcy} (0.16)	2.94^{cy} (0.16)
pT_{21} (%)	94.69^{ay} (0.55)	94.43^{ay} (0.55)	95.80^{ax} (0.55)
pT_{22} (%)	1.84^{ay} (0.52)	2.31^{ay} (0.52)	1.26^{ax} (0.52)

[a] Different superscript letters (a−c) in a row indicate significant differences ($P < 0.05$) between meat quality categories. Different superscript letters (x−z) in a column indicate significant differences ($P < 0.05$) between treatments (whole, minced, and homogenized). T_{2b}, T_{21}, and T_{22} denote relaxation time constants, whereas pT_{2b}, pT_{21}, and pT_{22} equal the corresponding populations.

and homogenized pork of the three meat qualities. Independently of the quality, mincing of the meat did not result in any distinct change in the T_2 components.

Homogenization of the meat tended to result in a minor, insignificant increase in the slowest time constant (T_{22}) without any change in the intermediate time constant (T_{21}). However, a significant increase and decrease appeared in the population of the intermediate and slow water fraction in meat of normal and RSE qualities, respectively (Table 2).

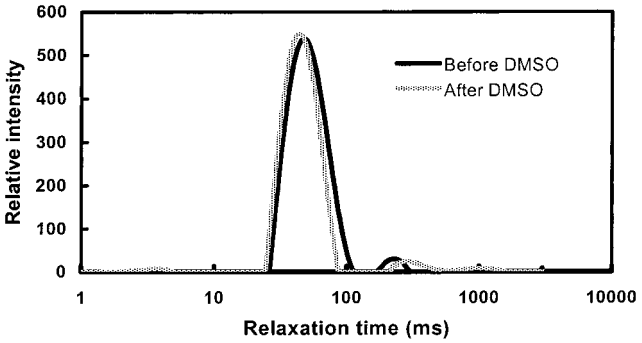

Figure 4. Distribution of low-field transverse relaxation (T_2) times for meat samples before and after treatment with DMSO. T_2 data were performed with a τ value (time between 90° pulse and 180° pulse) of 150 μs. Measurements were performed at 25 °C, and data were acquired from 4096 echoes as 16 scan repetitions with a repetition time of 2 s between two succeeding scans.

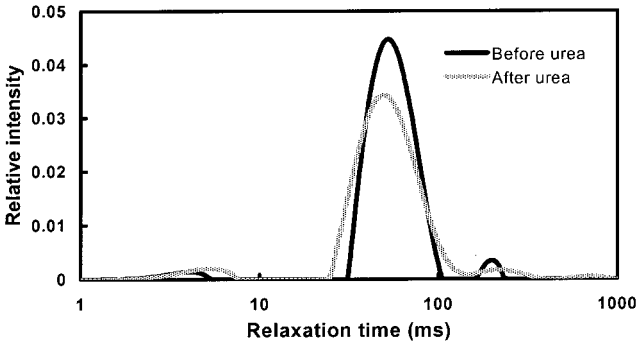

Figure 5. Distribution of low-field transverse relaxation (T_2) times for meat samples before and after treatment with 8 M urea. T_2 data were performed with a τ value (time between 90° pulse and 180° pulse) of 150 μs. Measurements were performed at 25 °C, and data were acquired from 4096 echoes as 16 scan repetitions with a repetition time of 2 s between two succeeding scans.

Figure 4 shows the influence of DMSO treatment. The presence of DMSO did not eliminate the characteristic multiexponential behavior of the meat. However, DMSO caused a minor change of the fastest relaxation component (T_{21}) toward faster relaxation and, more noticeably, a less well-defined slow relaxation component (T_{22}) compared with whole meat without DMSO.

The influence of urea and hereby denaturation of the protein structures is shown in Figure 5. The intermediate and slowest water fractions normally corresponding to T_{21} and T_{22} tended to broaden upon treatment with urea, whereupon these started to merge and thereby change the characteristic relaxation pattern seen in whole meat toward a relaxation pattern of a more biexponential character.

Figure 6 shows the characteristic effect of the addition of NaCl on relaxation data from minced pork of normal quality. Addition of NaCl caused an increase in the fraction of water characterized by the fast time constant (T_{21}) with a simultaneous shift toward a slower mean time constant. Concurrently, a decrease in the water fraction characterized by the fast time constant (T_{22}) was observed with a shift toward higher mean time

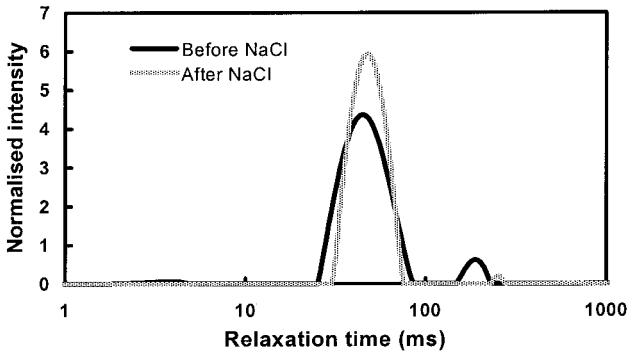

Figure 6. Representative distribution of low-field transverse relaxation (T_2) times for minced meat of normal quality before and after addition of 5% (w/w) NaCl. T_2 data were performed with a τ value (time between 90° pulse and 180° pulse) of 150 μs. Measurements were performed at 25 °C, and data were acquired from 4096 echoes as 16 scan repetitions with a repetition time of 2 s between two succeeding scans.

Table 3. Least-Square Means and Standard Errors (SE) of the Three Relaxation Components T_{2b}, T_{21}, and T_{22} Found by Distributed Exponential Curve Fitting of CPMG Data Measured in Minced Meat of Normal Quality before and after Addition of 5% NaCl[a]

	mean (SE)	
minced meat	without NaCl ($n = 3$)	with NaCl ($n = 3$)
T_{2b} (ms)	5.15[a] (1.29)	3.63[a] (1.59)
T_{21} (ms)	46.71[a] (0.62)	49.84[b] (0.62)
T_{22} (ms)	196.98[a] (9.94)	235.93[b] (9.94)
pT_{2b} (%)	1.78[a] (0.30)	1.02[a] (0.30)
pT_{21} (%)	92.77[a] (0.63)	96.66[b] (0.63)
pT_{22} (%)	5.44[a] (0.50)	2.72[b] (0.50)

[a] Different superscript letters in a row indicate significant differences ($P < 0.05$) between before and after NaCl addition. T_{2b}, T_{21}, and T_{22} denote relaxation time constants, whereas pT_{2b}, pT_{21}, and pT_{22} represent the corresponding populations.

constant values, as also seen on the obtained mean value for T_{22} (Table 3).

Figures 7 and 8 show typical relaxation patterns after distributed exponential fitting analysis of T_2 decays in superprecipitated actomyosin, heat-set actomyosin gels, and heat-set BSA gels. Multiexponential relaxation patterns were found in all of these matrices with the superprecipitated actomyosin having four components ($T_a = 8-20$ ms; $T_b = 50-200$ ms; $T_c = 700-900$ ms; $T_d = 1000-2000$ ms), the heat-set actomyosin gel having three components ($T_a = 8-90$ ms; $T_b = 90-200$ ms; $T_c = 250-600$ ms), and the heat-set BSA gel having three components ($T_a = 5-8$ ms; $T_b = 50-90$ ms; $T_c = 800-850$ ms).

Figure 9 shows typical structural characteristics of heat-set gels of actomyosin and BSA together with structural characteristics of pork of normal quality, as found by confocal microscopy.

DISCUSSION

Multiexponential transverse (T_2) decay of muscle water (myowater) first reported by Belton and coworkers (*21*, *22*) and Hazlewood et al. (*23*) has since gained attention due to its potential for a better understanding of water distribution in muscle during its conversion to meat and the role of different water fractions in relation to the WHC of meats. The existence of three different water fractions in fresh meat found in the present study is in agreement with data obtained for muscle tissue (*21*, *23*). Moreover, the time constants

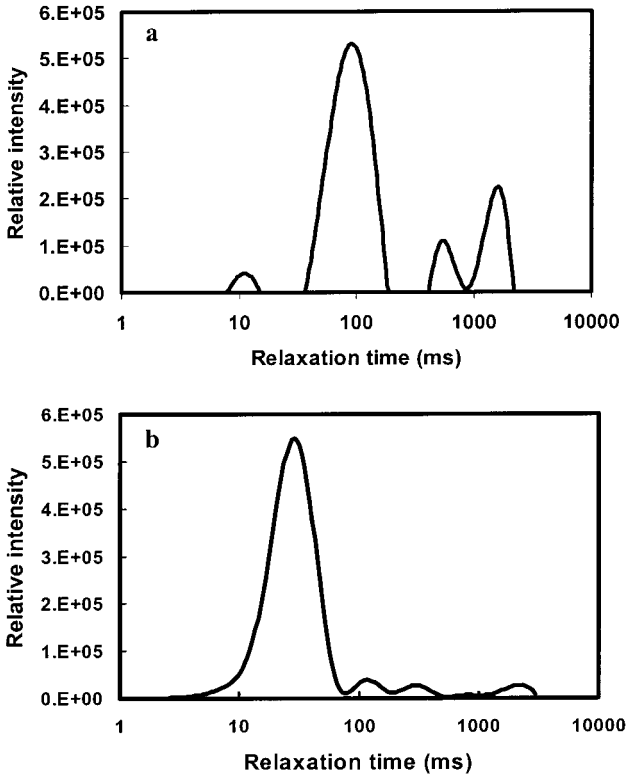

Figure 7. Typical distribution of low-field transverse relaxation (T_2) times in actomyosin gels (a) before and (b) after heat-setting. T_2 data were performed with a τ value (time between 90° pulse and 180° pulse) of 150 μs. Measurements were performed at 25 °C, and data were acquired from 4096 echoes as 16384 scan repetitions with a repetition time of 2 s between two succeeding scans.

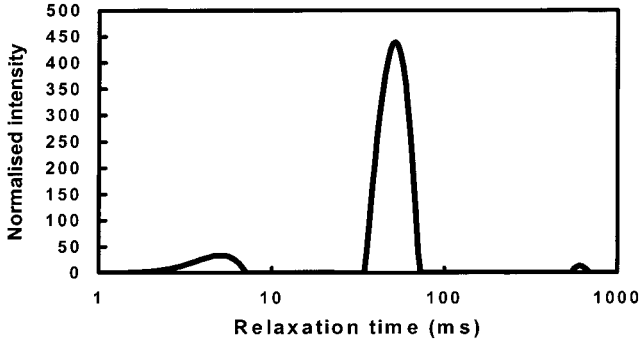

Figure 8. Typical distribution of low-field transverse relaxation (T_2) times for heat-set BSA gels. T_2 data were performed with a τ value (time between 90° pulse and 180° pulse) of 150 μs. Measurements were performed at 25 °C, and data were acquired from 4096 echoes as 16 scan repetitions with a repetition time of 2 s between two succeeding scans.

for the three water fractions found by Belton et al. (*21*) and Hazlewood et al. (*23*) in muscle tissue are comparable to those found within this study on pork meat. It is generally agreed that the fastest fraction (with $T_2 \sim 1-10$ ms), T_{2b}, is mainly assigned to water which is closely associated with macromolecules (*21*, *23*) with a population of 1−4%. However, up to one-third of the total T_{2b}-associated proton relaxation may be assigned to protons of larger molecules (*24*, *25*). In contrast, the nature of the intermediate fraction (with $T_2 \sim 40-60$ ms), T_{21}, and the slowest fraction (with $T_2 \sim 150-400$ ms), T_{22}, with populations of 85−96 and 2−10%, respectively, is still a matter of dispute.

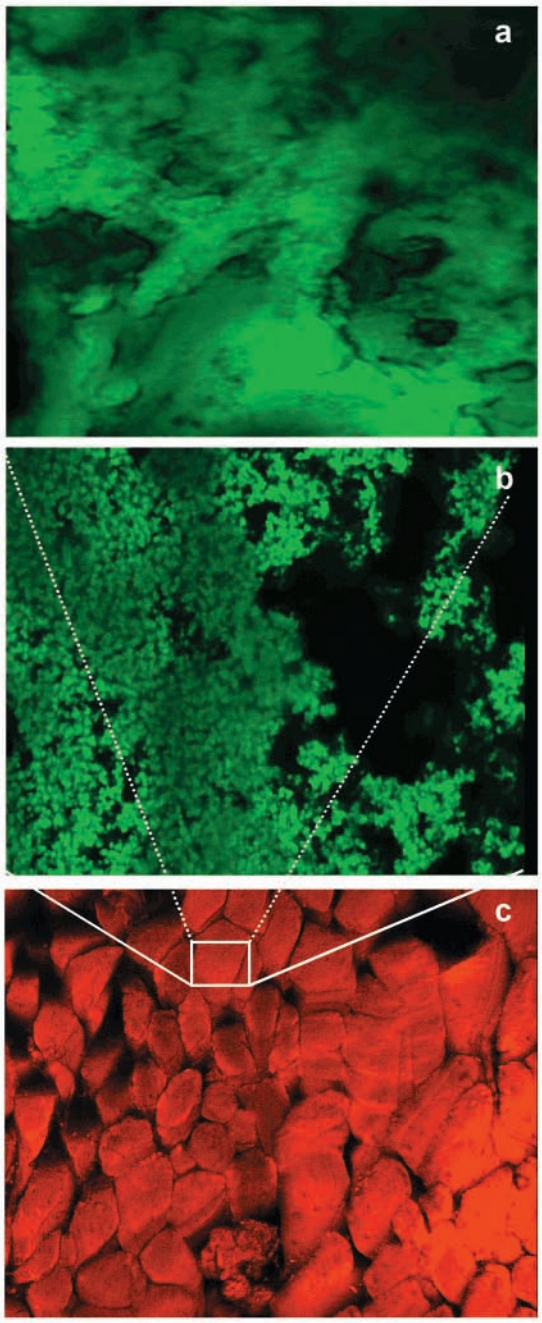

Figure 9. Confocal microscopy of (a) heat-set actomyosin gel (10× magnification), (b) heat-set BSA gel (100× magnification), and (c) pork at normal quality 24 h post-mortem (10× magnification). The window in (c) is marked to indicate the resemblance of this well-known myofibrillar structure with the structure fund in the heat-set BSA gel (b). (Figure is reproduced here at 75% of its original size.)

Despite the fact that the T_2 decay is recognized to be multiexponential in muscle tissue, nearly all previous work has focused on a mechanism of biexponential T_2 relaxation in myowater, as >90% of the relaxation signal may be described by two exponential rates representing T_{21} and T_{22}, respectively (*7*, *26*−*28*). Likewise, earlier studies regarding a correlation between the T_2 relaxation in muscle and the WHC of meat have been based solely on a mechanism of biexponential T_2 in myowater (*4*−*6*). Using this generally accepted approach on the T_2 decay in myowater from meat of different meat qualities in the present study shows, as also reported by the above authors, that the slowest time constant (T_{22}) differentiates among the three tested meat quality classes (Table 1). Consequently, T_{22} must reflect factors of importance for the WHC of the meat. However, to extract all of the important information from the relaxation data obtained on muscle tissue or meat related to the WHC of the meat, a further understanding of the individual parameters associated with the different water fractions is needed.

As mentioned in the Introduction, three theories explaining the relaxation behavior of myowater in muscle and meat tissue have been proposed over the years. One theory (i) proposes the existence of two components reflecting intra- and extracellular water, where the cell membrane is believed to act as a physical barrier (*7*). The second theory (ii) suggests that intracellular water may be more or less structured depending on structure changes in the actomyosin system, for example, degree of contraction (*8*). The third theory (iii) given by Lillford et al. (*9*) proposes that the multiexponential relaxation can be explained by the existence of a continuum of distances from water molecules to macromolecular surfaces or the local substrate density in the microenvironment (theory of spatial heterogeneity). Finally, the relaxation behavior of myowater has also been proposed to be explained by a combination of the above-mentioned theories i and ii (*4*). A consequence of the first theory (i) is that removal of the cell membrane should lead to monoexponential relaxation behavior of the myowater. However, neither mincing, giving rise to partial destruction of the macroscopic anatomical structure, nor homogenization, resulting in severe damages to the anatomical structure of the meat as revealed by microscopic investigations of the meats, changed the multiexponential relaxation pattern of the meats (Figure 3). Consequently, the above-mentioned theory explaining the biexponential T_2 decay in postmortem muscles does not seem to be valid, as both mincing and homogenization must be expected to disrupt the present membrane systems. This was further supported by additional experiments in which meat samples were soaked in DMSO. DMSO is known to penetrate biological membranes (*12*, *13*) and increase membrane permeability; such a treatment should change the multiexponential T_2 decay pattern, if membranes display a role as physical barriers in meat. However, no change in the multiexponential T_2 decay of myowater was observed when pork meat was exposed to DMSO in the present study (Figure 4). This supports earlier data reported by Fung and Puon (*29*) and Cole et al. (*7*), which showed that mouse and rat muscle, respectively, soaked in DMSO/glycerin still showed multiexponential T_2 decay. Moreover, cooking of meat has earlier been reported not to eliminate the multiexponential T_2 decay (*9*). Overall, the available data argue against a theory in which membranes cause multiphasic relaxation.

To investigate the effect of structural alterations at the protein level on the relaxation pattern in meat, meat samples were soaked in urea, which is known to unfold proteins through disruption of noncovalent interactions (*14*−*16*). This changes both the tertiary and quaternary structures in the usually highly structured myofibrillar systems. Such a global unfolding of the muscle proteins is expected to cause an evenly distributed protein matrix within the meat as also demonstrated in raw meat batters (*30*). Figure 5 clearly shows that this general disruption of muscle proteins in fact changes the

relaxation pattern where the two slow components tend to merge, which equals a more evenly distributed water environment. Consequently, the pattern of the T_2 decay in muscle and meat is associated with anatomically fixed structures, and thereby it provides spatial information, as also proposed in other food systems (*31*).

Because disruption of the macroscopic structure of fresh meat did not change the relaxation pattern in meat, NaCl was added to minced meat of normal quality to investigate whether intra- and intermolecular changes caused by electrostatic alterations within the meat matrix had an effect on the T_2 decay and the components therein. Addition of NaCl resulted in a significant increase in the population of T_{21} (~4%) with a simultaneous decrease in the population of T_{22} (~3.5%), without a significant change in the population of T_{2b} (Table 3). NaCl is known to cause solubilization of myosin (*32*, *33*) and enlarged electrostatic repulsion within myofilaments, which increases myofilament spacing. Accordingly, the observed increase in the population of the intermediate relaxation component (T_{21}) can be ascribed to an increase in the water fraction associated/trapped within the myofibrillar matrix, whereas the observed decrease in the population of the slowest relaxation component (T_{22}) must mainly be due to a reduction in the water fraction outside the highly structured myofibrillar matrix. Moreover, the addition of NaCl increases myofilament spacing (*34*). This corresponds to the registered significant change in the intermediate relaxation component (T_{21}) toward higher relaxation values, that is, slower relaxation. Concurrently, the slowest relaxation component (T_{22}) also moved toward higher relaxation. This indicates that addition of salt to meat causes more water to be tightly associated with the myofibrillar matrix while the remaining water becomes less tightly associated, that is, having a T_2 value more like the one found in drip from meat (~200−300 ms, unpublished data). At present no studies on the effect of NaCl addition on different water fractions in meat have been reported in the literature. Therefore, further LF-NMR studies on the effect of NaCl addition can probably lead to a better understanding of the effect on distribution and mobility of water during the curing of meat.

The multiexponential T_2 decay in aggregated (superprecipitated) actomyosin displayed a major water fraction characterized by a mean time constant, T_b, of ~90 ms (Figure 7a), which is intermediate to T_{21} and T_{22} found in whole meat. Superprecipitated actomyosin is reported to form interfilament interactions analogous to those found in contracting muscle, that is, rigor complexes (*35*, *36*). The other main water fraction characteristic for the aggregated actomyosin system, T_c, resembles free water, which is known to have a time constant of ~2 s (*31*). Heating of the superprecipitated actomyosin gave rise to a gel with a rather amorphous structure (Figure 9a), which resulted in a T_2 decay represented by a major water fraction with a somewhat ill-defined mean time constant. In contrast, heat-setting of BSA gave rise to highly ordered protein gel structures similar to known exceedingly ordered intra-myofibrillar structures (Figure 9b). The T_2 decay in these BSA gels was dominated by a water fraction (T_b = 50−90 ms) (Figure 8) very much identical to the T_{21} fraction identified as intra-myofibrillar water in whole meat in the present study. Altogether, this supports the assumption that the individual relaxation times in a multiexponential T_2 decay describe the distribution of distances from water molecules to surfaces, or the local substrate density in the microenvironment, as suggested by Lillford et al. (*9*). Moreover, the above data showing that the amorphous protein structure in BSA gels give rise to a relaxation pattern as in meat exclude the proposed theory ii, which suggests that the water in muscle tissue is structured as a function of a highly organized actomyosin structure.

Assuming that each component of the multiexponential relaxation represents water experiencing different correlation times for motion, we suggest in accordance with our data that the fastest relaxation component (T_{2b}) reflects a water fraction that is independent of the micro- and macrostructural changes in the meat matrix. This would resemble water that is closely associated with the macromolecules, as also proposed in most other studies (*21*, *23*). Moreover, in accordance with the observed susceptibility of the intermediate relaxation component (T_{21}) to (i) urea treatment, (ii) the presence of salt, and (iii) the analogy to a similar water fraction in highly structured heat-set BSA gels, we suggest that T_{21} reflects water located inside tertiary and quaternary protein structures and other highly structured parts of the muscle protein matrix with high myofibrillar protein densities, for example, actin and myosin filament structures, in whole meat. Finally, the effect of the addition of salt on the slowest relaxation component (T_{22}) in fresh meat makes us suggest that this component represents water between fiber bundles together with intermyofibrillar water containing sarcoplasmatic proteins. The addition of NaCl is known to cause lateral expansion of the filament lattice within myofibrils (*34*), which will minimize intermyofibrillar spaces (reduce the T_{22} population) and expulse the remaining T_{22} fraction to the bigger spaces between fiber bundles (T_{22} move toward longer relaxation times). Overall such an interpretation is consistent with the above-mentioned theory iii proposed by Lillford et al. (*9*).

To verify the hereby suggested nature of the different water fractions found by T_2 relaxation studies on meat, we compared data on whole, minced, and homogenized meat of the three meat qualities included in the study (Table 2), as the specific structural differences within and between some of these meat qualities are well-described.

The structural differences between meats of normal and RSE qualities are only poorly understood; however, RSE has been considered a mild form of PSE (*37*). Compared with normal meat, much more disruption of the myofibrils occurs in PSE meat (*38*) due to the denaturation of the myofibrillar proteins (*39*). Such a disruption leads to increased lateral myofilament shrinkage compared with normal meat, as shown by X-ray diffraction studies (*40*). Assuming that RSE resembles a mild form of PSE, meat lateral myofilament shrinkage should also proceed in this meat quality even though to a minor degree as compared with PSE meat. Due to our interpretation of the three water fractions found by T_2 relaxation studies on meat, shrinkage in lateral myofilament spacing should result in a reduction in the T_{21} time constant with a simultaneous decrease in the T_{21} population and an increase in the T_{22} population compared with the same parameters obtained from normal meat. This is exactly what we found in our relaxation studies on meat of RSE and normal qualities (Table 2). In contrast to meat of normal and RSE

qualities, the lateral myofilament spacing is larger in DFD meat because the pH (>6.2) increases the electrostatic repulsion between proteins compared with the pH (pH ≈5.6) in normal meat, which is close to the isoelectric point of the myofibrillar proteins (pH ≈5.2). According to our interpretation of T_2 relaxation, meat with larger lateral myofilament spacing should give rise to an increase in both the intermediate time constant T_{21} and the corresponding population described by this component. Moreover, as the amount of water inside the meat is constant, a concurrent decrease in the population of T_{22} is a consequence. These predictions are likewise in accordance with our relaxation studies of the different meat qualities (Table 2).

Mincing partly disrupts the overall structural integrity of the meat, but the structural integrity is maintained in smaller units (Figure 3b). With the suggested interpretation of the T_2 decay in meat we would not expect any major changes in the different T_2 components and the corresponding populations. This is also what we observed for minced meat of normal and RSE qualities (Table 2). However, in meat of DFD quality we found a significant decrease in the T_{22} time constant. This might be explained by the fact that partial disruption of the overall structural integrity of the meat reduces the number of gaps existing between fiber bundles, which are expected to contribute to the T_{22} time constant.

In contrast to mincing, homogenization results in a more evenly distributed protein matrix (Figure 3c). According to the given interpretation of the T_2 decay in meat, one would expect that structures giving rise to the three registered water fractions should vanish upon homogenization. Subsequently, two water fractions should appear, one corresponding to water closely associated with the macromolecules (T_{2b}) and another fraction characterized by a time constant equal to that of a highly concentrated protein solution, as also described for fine skim milk powder suspensions (*31*). A close analysis of the data on homogenized meat of all three qualities supports a situation in which the matrices approach a two-component system, as the contribution from the T_{22} component accounts for only 2% or less.

If T_{2b}, as suggested, represents water closely associated with macromolecules, no difference in this component should be found between meat qualities and degree of processing. This is exactly what was found in this study, and that is why the present data support the nature, earlier stated, for this water component.

Nevertheless, the above verification of the nature of the T_2 decay in meat also resulted in an interesting observation. The T_2 decay found in whole meat of DFD quality resembled an identical transverse relaxation pattern in homogenized DFD meat. This indicates that the water fraction characterized by T_{21} does not necessarily resemble water within a well-defined structured integrity with high protein density such as the myofibrils, but more probably reflects the diffusive exchange between the proteins and the solution phase. Moreover, the relaxation pattern in minced meat with added NaCl (Table 3), in which the myofilaments are known to be depolymerized (*41*), was also found to be comparable to the one found in whole and minced meat of DFD quality. This confirms that water within differently structured microenvironments with high protein density can display equal T_2 decay.

The results obtained in the present study support the conclusions that with the existing structures in whole meat (i) the fastest relaxation component reflects water tightly associated with macromolecules; (ii) the intermediate relaxation component reflects water located within highly organized protein structures, for example, water in tertiary and/or quaternary protein structures and spatials with high myofibrillar protein densities including actin and myosin filament structures; and (iii) the slowest relaxation component reflects extramyofibrillar water containing the sarcoplasmatic protein fraction. However, due to the results found in processed meat and other protein matrices, relaxation measurements may not give useful information about the state of water, as also stated by Hills et al. (*31*), but rather information about the state of the protein integrity in the systems together with some information about morphology.

ACKNOWLEDGMENT

Bettina Mikkelsen, Arla Foods amba R&D, Brabrand, Denmark, is gratefully acknowledged for performing confocal microscopy.

LITERATURE CITED

(1) Cope, F. W. Nuclear magnetic resonance evidence using D_2O for structured water in muscle and brain. *Biophys. J.* **1969**, *9*, 303−319.
(2) Hazlewood, C. F.; Nichols, B. L.; Chamberlain, N. F. Evidence for the Existence of a Minimum of Two Phases of Ordered Water in Skeletal muscle. *Nature* **1969**, *222*, 747−750.
(3) Finch, E. D.; Harmon, J. F.; Muller, B. H. Pulsed NMR Measurements of the Diffusion Constant of Water in Muscle. *Arch. Biochem. Biophys.* **1971**, *147*, 299−310.
(4) Tornberg, E.; Andersson, A.; Göransson, Å.; von Seth, G. Water and Fat Distribution in Pork in Relation to Sensory Properties. In *Pork Quality: Genetic and Metabolic Factors*; Puolanne, E., Demeyer, D. I., with Ruusunen, M., Ellis, S., Eds.; CAB International: Oxford, U.K., 1993.
(5) Brøndum, J.; Munck, L.; Henckel, P.; Karlsson, A.; Tornberg E.; Engelsen, S. B. Prediction of Water-holding Capacity and Composition of Porcine Meat with Comparative Spectroscopy. *Meat Sci.* **2000**, *55* (2), 177−185.
(6) Bertram, H. C.; Andersen, H. J.; Karlsson, A. H. Comparative study of low-field NMR relaxation measurements and two traditional methods in the determination of water-holding capacity of pork. *Meat Sci.* **2001**, *57*, 125−132.
(7) Cole, W. C.; LeBlanc, A. D.; Jhingran, S. G. The Origin of Biexponential T2 Relaxation in Muscle Water. *Magn. Reson. Med.* **1993**, *29*, 19−24.
(8) Yamada, T. ^{1}H NMR spectroscopy of the intracellular water of resting and rigor frog skeletal muscle. In *Mechanisms of Work Production and Work Absorption in Muscle*; Sugi, Pollack, Eds.; Plenum Press: New York, 1998; pp 145−155.
(9) Lillford, P. J.; Clark, A. H.; Jones, D. V. Distribution of Water in Heterogeneous Food and Model Systems. In *Water in Polymers*; Rowland, S. P., Ed.; ACS Symposium Series 127; American Chemical Society: Washington, DC, 1980; pp 177−195.
(10) Henckel, P.; Karlsson, A.; Oksbjerg, N.; Petersen, J. S. Control of post mortem decrease in pig muscles: experimental design and testing of animals models. *Meat Sci.* **2000**, *55*, 131−138.
(11) Honikel, K. O. Reference Methods for the Assessment of Physical Characteristics of Meat. *Meat Sci.* **1998**, *49*, 447−457.

(12) van Hoek, A. N.; de Jong, M. D.; van Os, C. H. Effects of dimethylsulfoxide and mercurial sulfhydryl reagents on water and solute permeability of rat kudney brush border membranes. *Biochim. Biophys. Acta* **1990**, *1030*, 203−210.
(13) Anchordoguy, T. J.; Carpenter, J. F.; Crowe, J. H.; Crowe, L. M. Temperature-dependent perturbation of phospholipid bilayers by dimethylsulfoxide. *Biochim. Biophys. Acta* **1992**, *1104*, 177−122.
(14) Stark, G. R. Recent developments in chemical modification and sequential degradation of proteins. *Adv. Protein Chem*. **1979**, *24*, 261−308.
(15) Nakai, S. Structure−Function Relationships of Food Proteins with an Emphasis on the Importance of Protein Hydrophobicity. *J. Agric. Food Chem*. **1983**, *31*, 676−683.
(16) Whiting, R. C. Solute-Protein Interactions in a Meat Batter. *Reciprocal Meat Conf. Proc.* **1988**, *41*, 53−56.
(17) Carr, H. Y.; Purcell, E. M. Effects of Diffusion on Free Precession in Nuclear Magnetic Resonance Experiments. *Am. J. Phys.* **1954**, *94*, 630−638.
(18) Meiboom, S.; Gill, D. Modified spin−echo method for measuring nuclear times. *Rev. Sci. Instrum*. **1958**, *29*, 688−691.
(19) Press, W. H.; Teukolsky, S. A.; Vetterling, W. T.; Flannery B. P. Intregral Equations and Inverse Theory. In *Numerical Recipes in Fortran*, 2nd ed.; Cambridge University Press: Cambridge, U.K., 1992; Chapter 18.
(20) SAS Institute Inc. *SAS User's Guide: Statistics*, version 6, 4th ed.; SAS Institute: Cary, NC, 1991.
(21) Belton, P. S.; Jackson R. R.; Packer, K. J. Pulsed NMR studies of water in striated muscle: I. Transverse nuclear spin relaxation times and freezing effects. *Biochim. Biophys. Acta* **1972**, *286* (1), 16−25.
(22) Belton P. S.; Parker K. J. Pulsed NMR studies of water in striated muscle: III. The effects of water content. *Biochim. Biophys. Acta* **1974**, *354* (2), 305−314.
(23) Hazlewood, C. F.; Chang, D. C.; Nichols, B. L.; Woessner, D. E. Nuclear Magnetic Resonance transverse relaxation times of water protons in skeletal muscle. *Biophys. J.* **1974**, *14*, 583−606.
(24) Peemoeller, H.; Pintar, M. M. Nuclear magnetic resonance multiwindow analysis of proton local fields and magnetization distribution in natural and deuterated mouse muscle. *Biophys. J.* **1979**, *28*, 339−355.
(25) Peemoeller, H.; Pintar, M. M.; Kydon, D. W. Nuclear magnetic resonance analysis of water in natural and deuterated mouse muscle above and below freezing. *Biophys. J.* **1980**, *229*, 427−436.
(26) Mauss, Y.; Grucker, D.; Fornasiero, D.; Chambron, J. NMR compartmentalization of free water in the perfused heart. *Magn. Reson. Med.* **1985**, *2*, 187−194.
(27) Sobel, W. T.; Cameron, L. G.; Inch, W. R.; Pintar, M. M. Modeling of proton spin relaxation in muscle tissue using nuclear magnetic resonance spin grouping and exchange analysis. *Biophys. J.* **1986**, *50* (1), 181−191.
(28) Rumeur, E. C.; De Certaines, J.; Toulouse, P.; Rochcongan, P. Water phases in rat striated muscles as determined by T2 proton NMR relaxation times. *Magn. Reson. Imaging* **1987**, *5* (4), 267−272.
(29) Fung, B. M.; Puon, P. S. Nuclear magnetic resonance transverse relaxation in muscle water. *Biophys. J.* **1981**, *33*, 27−38.
(30) Gordon, A.; Barbut, S. Effect of chemical modifications on the microstructure of raw meat batters. *Food Struct.* **1991**, *10*, 241−253.
(31) Hills, B. P.; Takacs, S. F.; Belton, P. S. A new interpretation of proton NMR relaxation time measurements of water in food. *Food Chem.* **1990**, *37*, 95−111.
(32) Parsons, N.; Knight, P. Origin of Variable Extraction of Myosin from Myofibrils Treated with Salt and Pyrophosphate. *J. Sci. Food Agric.* **1990**, *51*, 71−90.
(33) Xiong, Y. L.; Lou, X.; Wang, C.; Moody, W. G.; Harmon, R. J. Protein Extraction from Chicken Myofibrils Irrigated with Various Polyphosphate and NaCl Solutions. *J. Food Sci.* **2000**, *65*, 96−100.
(34) Offer, G.; Trinick, J. On the mechanism of water-holding in meat: The swelling and shrinkage of myofibrils. *Meat Sci.* **1983**, *8*, 245−281.
(35) Levy, H. M.; Moy, W. W. Studies on the special properties of actomyosin in the gel form. II. An analysis of the turbidity changes seen in gel suspensions. *Biochim. Biophys. Acta* **1981**, *658* (2), 318−326.
(36) Strzelecka-Golaszewska, H.; Piwowar, U.; Pliszka, B. Changes in the ultrastructure of actomyosin gel during hydrolysis of ATP under various conditions. *Eur. J. Cell Biol*. **1981**, *24* (1), 116−123.
(37) Warner, R. D.; Kauffman, R. G.; Greaser, M. L. Muscle Protein Changes Post Mortem in Relation to Pork Quality Traits. *Meat Sci.* **1997**, *45*, 339−352.
(38) Dutson, T. R.; Pearson, A. M.; Merkel, R. A. Ultrastructural postmortem changes in normal and low quality porcine muscle fibers. *J. Food Sci.* **1974**, *39*, 32−37.
(39) Greaser, M. L. Conversion of muscle to meat. In *Muscle as Food*; Bechtel, P. J., Ed.; Academic Press: Orlando, FL, 1986; pp 37−102.
(40) Offer, G.; Knight, P. The structural basis of water-holding in meat Part 2: Drip Losses. In *Developments in Meat Science*, 4th ed.; Lawrie, R., Ed.; Elsevier Applied Science: London, U.K., 1988; pp 172−243.
(41) Offer, G.; Knight, P.; Jeacocke, R.; Almond R.; Cousins, T.; Elsey, J.; Parsons, N.; Sharp, A.; Starr R.; Purslow, P. The structural basis of the water-holding, appearance and toughness of meat and meat products. *Food Microstruct.* **1989**, *8*, 151−170.

Received for review November 21, 2000. Revised manuscript received March 20, 2001. Accepted March 22, 2001. We are grateful to the Danish Veterinary and Agricultural Research Council (SJVF 9702805) and to the Danish Directorates for Development (Product Quality Project to H.J.A.) for financial support of this project.

JF001402T