Hydration Behavior of Heart Muscle Studied by Nuclear Magnetic Relaxation. Changes with Heat Treatment in Muscle Hydration and Water Distribution in Heart Muscle

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The ^1H nuclear magnetic relaxation behavior of heart muscle from chicken, pork, and beef was investigated in relation to composition and processing conditions. Nonlinear regression analysis of the transverse magnetization decay yielded a best fit with three exponential components that are likely to correspond to three proton populations of average T_2 values in the range 2 ms–0.7 s in three separate types of compartments in the heart muscle. The long T_2 relaxation component may correspond to the protons of a water population within relatively large spaces between muscle cells. Heat processing of heart muscle caused major changes in the value of the long T_2 relaxation component. Our findings have implications for quality control of meat products by nuclear magnetic resonance and indicate the possibility of accurate monitoring of water loss from meat as a result of heat processing or other types of processing. Potential applications in the meat industry are also in the area of monitoring meat quality related to hydration changes during storage of meat. Potential applications of our relaxation analysis to biomedical studies of the myocardium are also pointed out.

INTRODUCTION

Muscle can be classified as either striated or nonstriated. Striated muscle exhibits regularly spaced transverse bands along the length of the cell and can be further divided into skeletal and cardiac muscle. Smooth or nonstriated muscle is composed of cellular units that are not subject to voluntary control (Bechtel, 1986). Cardiac muscle cells are 50–120 μm in length and have an intercalated disk that connects adjacent cardiac cells. The intercalated disks of cardiac muscle are located on the opposing ends of cardiac muscle cells and have complex interdigitating structures that maintain cell–cell cohesion. At present, heart muscle is not considered to be a useful byproduct of the meat industry, and few studies have been carried out on heart muscle as a possible food product. However, the heart muscle is a potential source of protein and a functional ingredient in the food industry for new products such as beef heart surimi analogs (Yakubu et al., 1989, 1991).

Since striated muscle contains about 75% water (Bodwell and Anderson, 1986), the trapping and binding of water in meat are especially important. Furthermore, water strongly influences the functional properties and characteristics of meat products (Hamm, 1986). The distributions of water in muscle cells and water movements during muscle contraction are very important but insufficiently known. Almost all procedures for the storage and processing of meat, such as heating and freeze-drying, influence or are influenced by the trapping and binding of water in the tissue. Heat processing of meat is a common procedure that changes water trapping and binding in meat. Its effects have not been yet precisely quantitated.

NMR techniques are nondestructive and extremely "gentle" and provide some of the best means available for investigating binding of water, chemical structure of food biopolymers, and physicochemical changes induced by food processing. NMR allows, in principle, such a quantitation if rigorously applied. Pulsed NMR relaxation times have been measured for isolated muscle proteins in solution (Nakano and Yasui, 1979; Yasui et al., 1979), hydrated gels (Lioutas et al., 1988; Mora-Gutierrez, 1989; Baianu et al., 1991), intact skeletal muscle (Lee et al., 1991; Lillford et al., 1980; Currie et al., 1981; Suzuki, 1981; Resing et al., 1976; Hazlewood et al., 1969, 1974), and freeze-dried muscle (Yakubu et al., 1990).

There is little doubt that myofibrillar proteins are primarily responsible for the binding of water in muscle. It is also conceivable that different types of water binding exist in various parts of the tissue. The phenomenon of multiphase behavior in water proton relaxation has been observed in many systems such as striated muscle (Lee et al., 1991; Hazlewood et al., 1974; Lillford et al., 1980), myosin suspensions (Nakano and Yasui, 1979), and hydrated wheat flour dough (d'Avignon et al., 1990, 1991). The nonexponential transverse magnetization decay of the spin–echo signal as a function of the interpulse spacing (2τ) is caused by the existence of two or more water populations that do not exchange or are only in slow exchange on the NMR time scale compared to the relaxation times of bound water (Zimmerman and Brittin, 1957).

Our aim is to analyze the ^1H NMR relaxation behavior of heart muscle from chicken, pork, and beef, in relation to composition and processing conditions. Our findings have implications for the quality control of meat products by NMR measurements and also indicate the possibility of monitoring water loss from meat as a result of processing by heat or other means (e.g., freezing). The analysis of our NMR relaxation results for heart muscle is likely to have applications for both medical and pharmaceutical applications related to the myocardium.

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0021-8561/92/1440-2350$03.00/0

Table I. Chemical Composition of Beef, Pork, and Chicken Heart Muscle

	moisture, %	fat, %	protein, %
beef heart	78.7 ± 0.9	3.4 ± 0.2	17.4 ± 1.1
pork heart	79.2 ± 0.5	2.7 ± 0.1	16.0 ± 2.1
chicken heart	80.7 ± 0.5	4.5 ± 0.1	15.3 ± 0.6

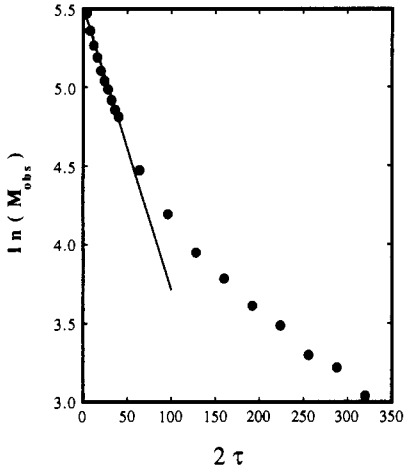

Figure 1. Semilogarithmic plot of the water proton transverse magnetization decay of beef heart muscle.

METHODOLOGY

Sample Preparation. Beef, pork, and chicken hearts were separated in the Muscle Biology Laboratory, Department of Animal Science, University of Illinois at Urbana–Champaign. These samples were carefully trimmed, and lean portions were ground. The moisture, fat, and protein contents are shown in Table I and were determined according to AOAC methods (AOAC, 1986). The samples were transferred into 10-mm NMR tubes and allowed to equilibrate at about 2 °C(on ice) before ^{1}H NMR measurements. For the heat treatment experiments, the samples were placed in 10-mm NMR tubes, equilibrated to 65 °C, and held at this temperature for 30 min on a water bath.

Pulsed ^{1}H NMR Measurements. Pulsed ^{1}H NMR measurements were carried out at 10 MHz and 20 ± 1 °C with a PC-10 NMR process analyzer (Bruker/IBM Instruments, Danbury, CT). The Carr–Purcell–Meiboom–Gill (CPMG) multipulse $90°-\tau[180°-2\tau-(\text{echo})]_n$ sequence (Carr and Purcell, 1954; Meiboom and Gill, 1958) was employed for measuring the decay of the transverse magnetization. The decay of the CPMG spin–echo maxima was monitored with a 30-MHz-bandwidth Tektronix storage oscilloscope (Model 5113, dual beam).

Relaxation Component Analysis. Figure 1 shows the plot of a transverse magnetization decay monitored by the dependence of the CPMG spin–echo maxima on 2 × interpulse spacing (2τ). T_2 values for three proton populations in muscle were estimated by a nonlinear regression analysis with

$$M(t) = \sum [M_{0i} \exp(-t/T_{2i})] \qquad i = 1, 2, 3 \qquad (1)$$

where $M(t)$ is the amplitude at the spin–echo maxima at $t = 2\tau$, M_{0i} is the transverse magnetization at $2\tau = 0.0$, and T_{2i} is a T_2 value of each component. The curve fitting was carried out with an iterating nonlinear regression program [SYSTAT (R) version 3.1 and 5.0] on an Apple Macintosh II microcomputer and involved the use of both Simplex and Quasi-Newton algorithms. This program determines values of the parameters that minimize the sum of the squares of the distances along the y axis of the data points to the curve. The goal of the least-squares method is to minimize the residual sum of squares (SS):

$$\text{SS} = \sum [(M_{\text{exptl}} - M_{\text{est}})^2] \qquad (2)$$

where M_{exptl} is the observed magnetization and M_{est} is the estimated value of magnetization. The root mean square (RMS) is also calculated as

$$\text{RMS} = [\text{SS}/(\text{no. of data points}) - (\text{no. of parameters})]^{1/2} \qquad (3)$$

The relative populations of the proton magnetization components can be calculated from the quantities M_{0i}, which are the initial spin–echo signal extrapolated at $2\tau = 0.0$, which is proportional to the number of protons in each of the components (Zimmerman and Lasater, 1958).

RESULTS AND DISCUSSION

Figure 1 represents the ^{1}H magnetization decays for ground beef heart samples after 1 h of equilibration at 20 ± 1 °C. The logarithm of the transverse magnetization decay is not linear, which indicates the presence of several slowly exchanging components in the beef heart. The nonexponential decay of the CPMG spin–echoes as a function of the pulse spacing (2τ) indicates the existence of two or more populations that do not exchange, or exchange only slowly, on the NMR time scale [that is, in comparison with bound water relaxation times (Zimmerman and Brittin, 1957)]. The relaxation curve can be resolved into three components characterized by short, medium, and long relaxation times, T_{2s}, T_{2m}, and T_{2l}, respectively. The T_{2s} component is dominated by the relaxation contribution of a less mobile proton fraction, whereas the T_{2l} component is dominated by a highly mobile water population, not unlike free water in several respects. Our multicomponent relaxation T_2 values were obtained by a simultaneous nonlinear regression analysis with eq 1. This method eliminates the drawbacks of semilogarithmic plots of magnetization decays. We obtained similar results with *intact* muscle, but the transverse relaxation decay was faster in the ground muscle samples (Lee, Baianu, and Bechtel, unpublished results).

Figure 2a shows the ^{1}H magnetization decays for the heart muscle and a comparison of the experimental data with the estimated values with the one-component model. There are discrepancies between calculated and experimental values at both short and long interpulse values (2τ). Figure 2b represents the calculated curve with the two-component model and exhibits a similar trend. The three-component model (Figure 2c) gives the best fit of the data. For the same sample, the value of the root mean square (RMS) for the three-component model is 1.38, whereas the RMS values for the one-component and two-component models are significantly higher (6.19 and 2.44, respectively). The RMS value of the four-component model is higher (1.42) than that of the three-component model because the former does not significantly improve the fit. Therefore, the three-component model analysis provides at present the best fit.

In an attempt to determine how many water proton magnetization components are in the heart muscle, a runs test was employed that involves residual plots for each set of data (Motulsky and Ransnas, 1987). The residual plot is a graphical representation of $(M_{\text{exptl}} - M_{\text{est}})$ vs 2τ. If the theoretical equation is consistent with the measurements, residuals will represent only experimental error without any systematic dependence on 2τ values, and the plot will have a random distribution of positive and negative residuals. A *run* is defined as a series of consecutive points with a residual of the same sign, either positive or negative. Parts a and b of Figure 3 represent the runs for the one- and two-component models, respectively, and indicate that there is a systematic error of the fit over the entire range. A cluster of residuals is apparent in Figure 3b at interpulse values between 0 and 40 ms, indicating the presence of a systematic error of the fit in the range of short times and probably a false estimate of the short T_2 value. Figure 3c does not show such systematic errors and residuals are randomly distributed; the number of runs for the three-component model is 16, whereas for the two-component model it is 8.

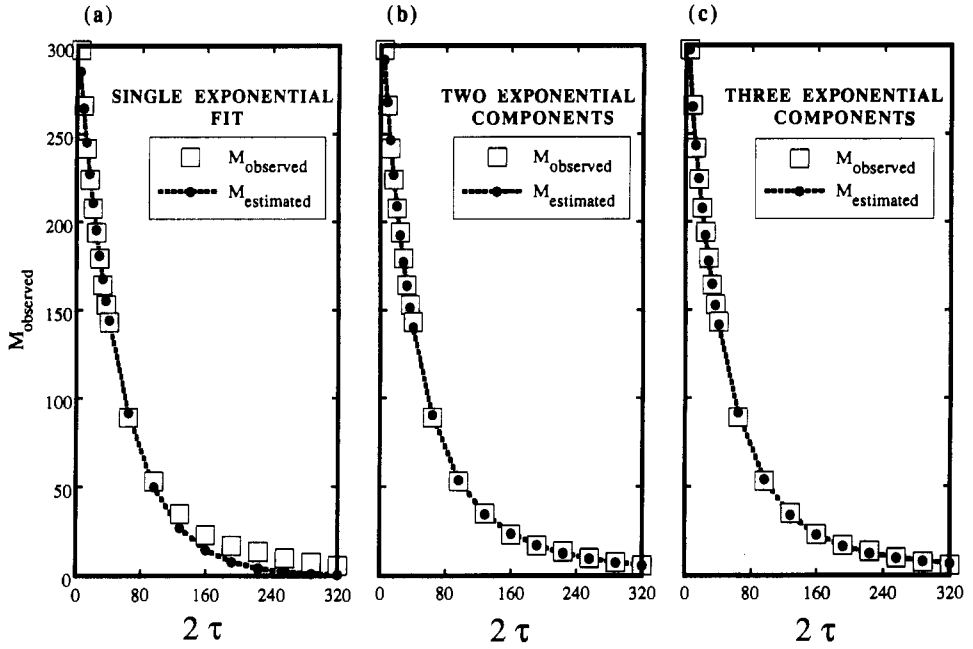

Figure 2. Proton transverse magnetization decays of beef heart muscle. Comparison of experimental and estimated values: (a) one-component relaxation model; (b) two-component relaxation model; (c) three-component relaxation model.

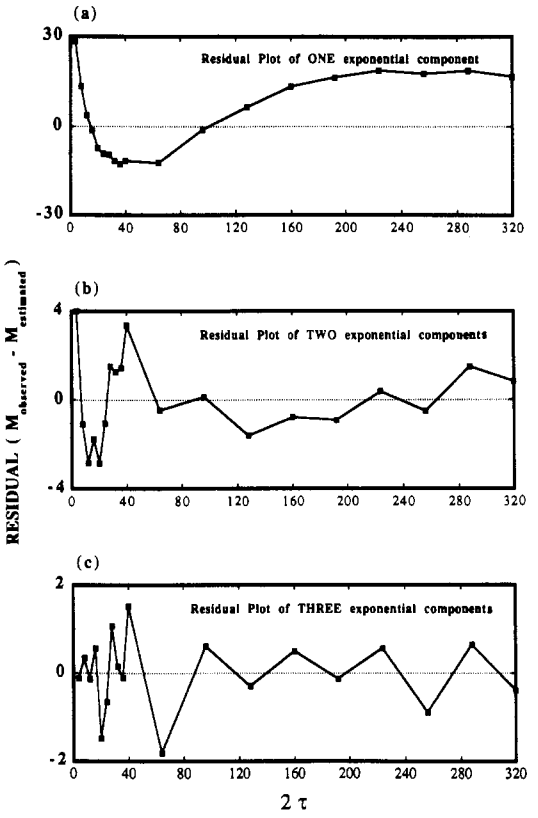

Figure 3. Residual plots of water proton transverse magnetization decays of pork heart muscle: (a) one-component relaxation model; (b) two-component relaxation model; (c) three-component relaxation model.

The ^{1}H NMR transverse relaxation of heart muscle from chicken, pork, and beef is multicomponent. Table II and Figure 4 summarize the results of our relaxation analysis for beef, pork, and chicken heart muscle. We find that the decays of the proton transverse magnetization of beef, pork, and chicken heart muscle can all be adequately described with the three-component model. The analysis of the beef heart muscle indicates that the relaxation is dominated by a relaxation component with T_2 = 45.5 ms and amplitude of 72.4%. Furthermore, approximately 19% of the decay is characterized by a T_2 value of 2.3 ms, whereas only 8.7% of the decay is characterized by a T_2 value of 197 ms. By analogy with previously reported results for other types of muscle [for example, Hazlewood et al. (1974)], the long T_2 value can be assigned to an extracellular water proton component and the intermediate T_2 value to the intracellular water protons. The ^{1}H NMR relaxation behavior of pork and chicken hearts is similar to that of beef heart muscle. However, the pork heart muscle has a higher T_2 value of the long T_2 component (335 ms) than the long T_2 component of the other species (chicken and beef; Figure 4), suggesting perhaps the presence of a larger fraction of loosely trapped water in the pork heart muscle. The correct value of long T_2 also may be influenced by diffusion of water during long time intervals between pulses.

The multiexponential relaxation behavior of water protons in the heart muscle indicates the existence of three water proton populations that do not exchange or are only in slow exchange on the NMR time scale compared to the relaxation times of bound water protons and the shortest relaxation time (T_{2s}) component (Zimmerman and Brittin, 1957). Each T_2 component is considered as an averaged T_2 value of the bound and free water protons within each separate water compartment. This averaged T_2 value can be explained by a two-state model with *fast exchange*. This relationship is based on a population-weighted average between bound and free water and can be expressed as

$$T_{2i}^{-1} = (P_{bi}/T_{2bi}) + (P_{fi}/T_{2fi}) \quad (4)$$

where P_{bi} and P_{fi} are the fractional populations of bound and free water protons in each T_2 component system, respectively (Derbyshire, 1982). Figure 5 represents a schematic diagram of the water compartments in heart muscle that determine water proton relaxation. The long T_2 represents an average over a free water population (F_L) and bound water sites (B_L), which are in fast exchange with each other. However, the free water population of the long T_2 compartment (F_L) is not in fast exchange with the free water population in the intermediate or short T_2 compartments (F_M or F_S). Therefore, this

Table II. Transverse Relaxation Times (T_2) and Fractional Populations of the Three Relaxation Components Observed in Beef, Pork, and Chicken Heart Muscle

	long T_2 relaxation component		medium T_2 relaxation component		short T_2 relaxation component		
	ms	%	ms	%	ms	%	RMS
beef heart	197 ± 37	8 ± 2	46 ± 1	72 ± 2	2.3 ± 0.3	19 ± 4	1.4
pork heart	335 ± 51	9 ± 1	55 ± 1	71 ± 1	4.6 ± 0.4	20 ± 2	2.2
chicken heart	226 ± 38	9 ± 2	54 ± 2	65 ± 2	4.9 ± 0.4	26 ± 1	3.2

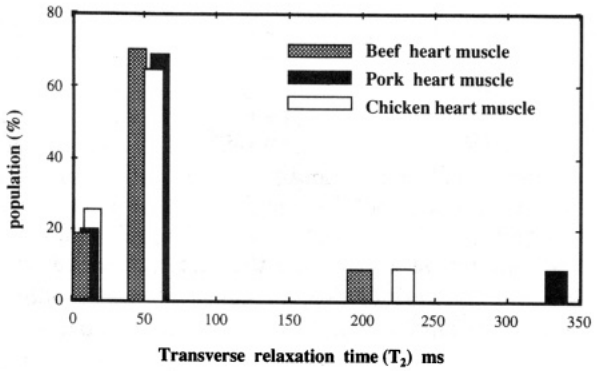

Figure 4. Distribution and fractional populations of three transverse relaxation components of water protons in beef, pork and chicken heart muscle.

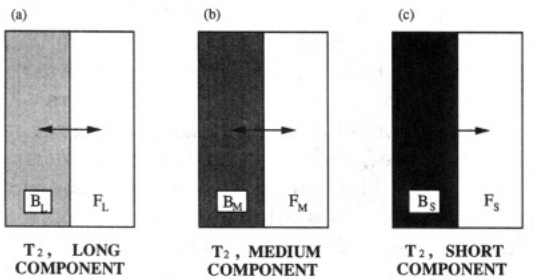

Figure 5. Schematic plot of the water compartments in heart muscle: (a) compartment responsible for the long T_2 component of the transverse relaxation curve [this component represents an average over a free water population (F_L) and bound water sites (B_L) which are in fast exchange with the free water population (F_L)]; (b) compartment responsible for the intermediate T_2 relaxation component; (c) water compartment responsible for the short T_2 relaxation component.

slow-exchange model gives the total proton magnetization as the sum of these compartments

$$M(t) = \sum\{M_{0i} \exp[-t/(P_{bi}/T_{2bi} + P_{fi}/T_{2fi})]\} \quad (5)$$

where $i = 1, 2, 3$. Equation 5 includes a weight-averaged fast-exchange relaxation time of bound and free water protons within each of the three water compartments, according to eqs 4 and 5.

It was previously suggested that three- or four-process fitting was appropriate for the transverse relaxation of skeletal muscle (L. dorsi of Lincolnshire Red Heifer; Lillford et al., 1980). In this case, the relaxation was dominated (~90%) by a proton relaxation component with $T_2 = 47$ ms, whereas approximately 6% of the decay is characterized by a process with $T_2 = 150$ ms. The four-process fit gave a better representation of the data than the three-process treatment. The difference between the three- and four-process treatments lies in the analysis of the rapid initial decay, which in a three-process treatment is described by a single exponential with $T_2 = 1.7$ ms but in a four-process treatment is described by two discrete relaxation components of 0.23 and 2.45 ms. A continuous relaxation time distribution function which could be derived from the transverse relaxation decay curve by mathematical deconvolution (Lillford, 1985) was also proposed for the multiexponential magnetization decay

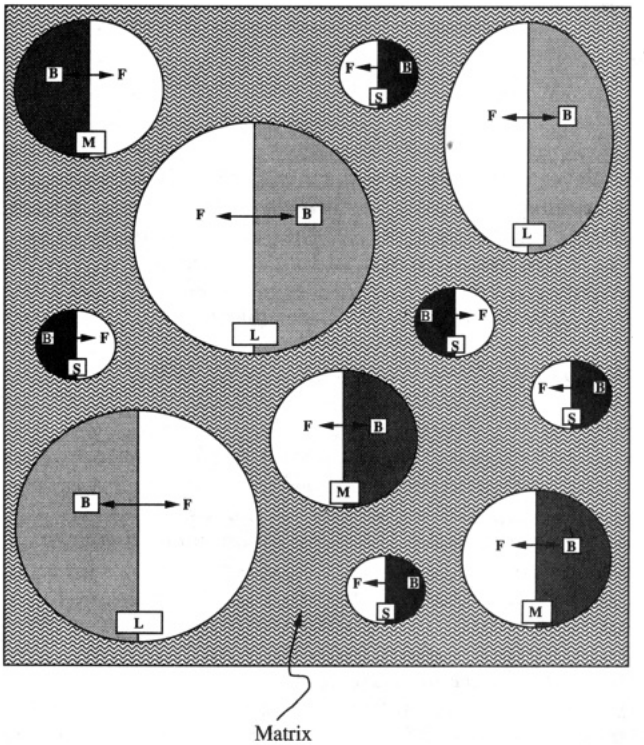

Figure 6. Schematic diagram of the water distribution within various size pores in heart muscle. This is essentially a three-compartment model for water distribution, involving two water populations (bound and free) in each compartment. The shortest T_2 compartment is likely to include a relaxation component from the matrix protons.

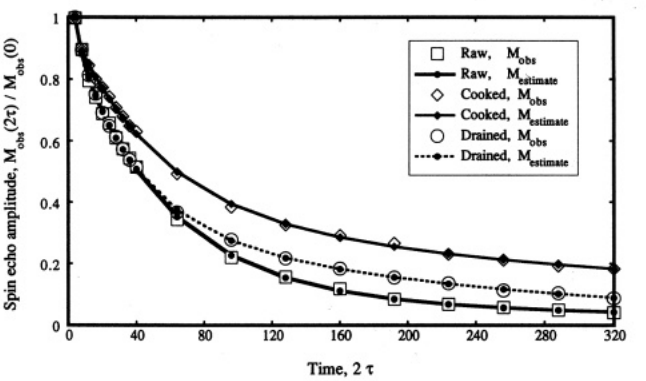

Figure 7. Proton transverse magnetization decay of pork heart muscle before and after heating (at 65 °C for 30 min) and after draining cooking juice. The open squares, rhombi, and circles represent the experimental data and solid ones with lines represent the estimated values after nonlinear regression of three T_2 components.

of skeletal muscle. This approach, however, seems to involve too many parameters and is not suitable for a number of data points less than ~500.

The differences between the transverse relaxation in heart and skeletal muscle are consistent with the presence of a larger fraction of water inside the skeletal muscle cells and larger *extracellular* regions containing water in the heart muscle.

Table III. Transverse Relaxation Times (T_2) and Fractional Population of the Three Relaxation Components Observed in Beef, Pork, and Chicken Heart Muscle before and after Heating at 65 °C for 30 min

		long T_2 relaxation component		medium T_2 relaxation component		short T_2 relaxation component		
		ms	%	ms	%	ms	%	RMS
beef heart	uncooked	197 ± 37	9 ± 2	46 ± 1	72 ± 2	2.3 ± 0.3	19 ± 4	1.4
	cooked	462 ± 27	24 ± 1	45 ± 1	44 ± 1	2.0 ± 0.2	32 ± 5	1.2
	drained	278 ± 24	20 ± 2	37 ± 2	54 ± 2	6.0 ± 0.8	26 ± 2	1.9
pork heart	uncooked	335 ± 51	9 ± 1	55 ± 1	71 ± 1	4.6 ± 0.4	20 ± 2	2.2
	cooked	458 ± 35	18 ± 1	52 ± 2	31 ± 1	1.4 ± 0.2	52 ± 1	1.9
	drained	242 ± 14	28 ± 2	39 ± 2	53 ± 1	4.7 ± 0.9	19 ± 2	0.9
chicken heart	uncooked	226 ± 38	9 ± 2	54 ± 2	65 ± 2	4.9 ± 0.4	26 ± 1	3.2
	cooked	614 ± 52	28 ± 1	44 ± 2	46 ± 1	5.7 ± 0.4	26 ± 1	2.2
	drained	265 ± 26	19 ± 2	39 ± 2	60 ± 7	3.9 ± 0.6	21 ± 9	1.7

Several authors have discussed the origin of the multiexponential transverse decay in skeletal muscle, and the current consensus is for the existence of physically separated water compartments that cause the multiple exponential decay (Lillford et al., 1980). Figure 6 represents schematically the water distribution in the heart muscle "matrix" (solid-like tissue mass). T_{2i} values can be related to pore sizes (Ablett et al., 1991), whereas an M_{0i} value corresponds to the fractions of water molecules present in a domain of equivalent pore sizes. Such an analysis suggests that there are three kinds of pore sizes in the heart muscle matrix; summing up the areas of an equivalent pore size gives the fractional population of that water compartment. The long T_2 component corresponds to the water compartment made of large size pores in which "bound" and free water populations are in fast exchange with each other. This schematic representation, or three-compartment model of water distribution, can be related to the existence of separate, extracellular (long T_2 component) and intracellular (T_2 component) water compartments in muscle. The short T_2 water compartment might correspond to the trapped water between myofibrillar proteins and would be most affected by *intermolecular dipolar interactions* with the matrix protons.

The action of heat (65 °C, 30 min) causes dramatic changes in the values of the water proton relaxation times. Figure 7 shows the proton magnetization decays of beef heart muscle with no treatment, after heat treatment, and after draining of cooking juice (containing free water). After the heat treatment, the long T_2 component had a larger value than that before draining the juice. For the cooked beef heart muscle about 25% of the decay exhibited a T_2 value of 460 ms. This long T_2 value is caused by the water released from the muscle upon heating as the juice separates visibly from the tissue mass after heating and can be readily removed. After draining the juice from the heated heart muscle, the T_2 values of the long and medium components decreased because some of the free water in the extracellular and intracellular compartments had been removed by draining. The water remaining within the bulk tissue still exhibited a complex relaxation curve. A large fraction of the proton population with a T_2 of 37 ms was, however, retained. Whereas the medium T_2 component (46 ms) dominates before heating (with a 72% fractional population), after heating, its relative amplitude decreased to 44% before draining the juice and to 54% after draining the juice. The short T_2 component showed a relative amplitude increase to 32% after heating (without draining the juice). The ^{1}H NMR relaxation components and their fractional populations for beef, pork, and chicken hearts after heat treatment are summarized in Table III. In all three types of muscle there is similar relaxation behavior, and similar changes occur upon heating. However, the cooked chicken heart muscle has a longer T_2 value (614 ms) for the long T_2 component than the other two species (before draining the juice), presumably as a result of releasing more water than the other two upon heating. However, the values of the long T_2 component became similar after draining the juice (265 ± 26 ms).

In summary, the ^{1}H NMR transverse relaxation of heart muscle from chicken, pork, and beef is adequately described by a three-component model with only slow or no exchange between the three water compartments. Intermediate chemical exchange rates within each compartment cannot be, however, ruled out completely. Deuterium exchange and oxygen-17 NMR experiments are in progress in our laboratory to check out this possibility and further investigate the validity of the three water compartment model of heart muscle that was proposed here. Major changes were observed in the values of the long relaxation component for beef, pork, and chicken heart muscle after heat processing. The longest relaxation component decayed slower in all cases after heating (with or without draining the juice) and had a significantly increased fractional population (~20–28%) in comparison with the uncooked heart muscle (9 ± 2%). On the other hand, the intermediate proton relaxation component decayed slightly faster after cooking (~38 ± 2 ms) than before cooking (46 ± 1 ms). The fractional population of the intermediate component decreased from about 70 ± 5% before cooking to about 50 ± 5% after cooking.

The analysis of our water NMR relaxation data for the heart muscle is likely to be of interest not only in the food industry for possible utilization of the heart muscle as a food ingredient but also in medical and pharmaceutical applications concerning the myocardium. This approach is likely to be useful for other types of muscle and meat and may provide a sensitive test for monitoring changes of water distribution during storage of meat. The techniques may also provide a new test for meat quality control since water release from the muscle can be monitored rapidly and accurately by pulsed ^{1}H NMR measurements of water proton transverse relaxation.

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Received for review June 4, 1992. Accepted September 17, 1992.

Registry No. Water, 7732-18-5.