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Magnetic Resonance Imaging, Rheological Properties, and Physicochemical Characteristics of Meat Systems with Fibrinogen and Thrombin

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Magnetic resonance imaging (MRI) and textural and physicochemical analyses were carried out to evaluate the effect of fibrinogen and thrombin (Fibrimex) addition to meat systems formulated with and without NaCl. For this purpose, different model systems were elaborated: fibrinogen and thrombin (FT), meat emulsion (ME), and meat emulsion with fibrinogen and thrombin (MEFT), with 0, 1, and 2% of NaCl. The addition of fibrinogen–thrombin to meat emulsions results in a gel network with modified physicochemical and textural characteristics, increasing the hardness and springiness. The addition of NaCl at 2% to FT and MEFT systems reduced the gel hardness. MRI parameters (T_2, T_1, and apparent diffusion coefficient) indicated that systems with fibrinogen and thrombin (FT and MEFT) presented a structure with many and large pores, bulk water, and higher translational motion of water. Significant correlations were found between MRI, texture, and physicochemical parameters.

KEYWORDS: Meat systems; fibrinogen; thrombin; magnetic resonance imaging (MRI); texture

INTRODUCTION

Traditionally, the elaboration of meat products depends on the hot-set binding of myofibrillar proteins extracted from meat by the combined effects of sodium chloride, phosphates, and mechanical actions (*1–4*). Moreover, there is a demand for healthy meat products elaborated without adding phosphate and with reduced salt content, because a high salt content has been related to cardiovascular disease and excessive dietary phosphate can increase the risk of bone diseases (*5, 6*). An alternative to hot-set binding systems to elaborate meat products is to use cold-set binders. Several cold-set binding systems (alginate–calcium, transglutaminase of different origins, fibrinogen, and thrombin) have been developed to produce meat products that can be used in the chilled and raw state, reducing or eliminating the need to add sodium chloride and phosphates (*7–12*). A cold-set binding agent that has given good results for meat product formulation is the combination of fibrinogen and thrombin (fibrinogen–thrombin). It is possible to obtain fibrinogen and thrombin from porcine or bovine blood plasma. Fibrinogen is an elongated protein and is made up of globular domains at each end connected by α-helical coiled coils to a globular region in the middle. Thrombin converts soluble fibrinogen to insoluble fibrin polymer by cleaving fibrin peptides from the central domain, exposing knobs that can then interact with holes that are always exposed at the ends of the molecule, giving rise to a half-staggered structure called the protofibril. When the protofibrils grow long enough, they aggregate laterally to form fibers, which then branch to yield a three-dimensional network fibrin clot or stable fibrin gel (*13–17*). It is known that the variation of fibrin parameters, such as fibrinogen concentration, thrombin concentration, and ionic strength, generates gels with different appearances, mechanical properties, and stabilities (*13, 15*). Frozen independent solutions of fibrinogen and thrombin are commercialized as Fibrimex (FNA Foods, Harimex Inc.) to be mixed just before use, for the elaboration of meat products. Fibrimex has been used to manufacture several restructured meat products (*10–12*). The effects of Fibrimex addition on consumer acceptance and textural properties of restructured meat products (*10–12*) have been studied. However, structural information relating to aspects of how the fibrinogen and thrombin affect the components (proteins, water, etc.) of the finished product is required to increase the use of this cold-set binding agent.

In this respect, magnetic resonance imaging (MRI) is a noninvasive and nondestructive technique that provides structural information of biological tissue. The use of MRI permits the quantification of magnetic resonance parameters such as spin–lattice (T_1) and spin–spin (T_2) relaxation times, magnetic

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Table 1. Elaboration Procedure (Components, Additon Order, and Quantity Added) of the Different Systems Used

system[a]	mixture name[b]	meat emulsion (g)	NaCl aqueous solution		fibrinogen (mL)	thrombin (mL)
			NaCl %[c]	mL		
FT	FT00	0	0	0	100	10
	FTW0	0	0	11	100	10
	FTW1	0	11	11	100	10
	FTW2	0	22	11	100	10
ME	ME00	100	0	0	0	0
	MEW0	100	0	11	0	0
	MEW1	100	10	11	0	0
	MEW2	100	20	11	0	0
MEFT	MEFT00	100	0	0	10	10
	MEFTW0	100	0	11	10	10
	MEFTW1	100	12	11	10	10
	MEFTW2	100	24	11	10	10

[a] FT, fibrinogen and thrombin, both together commercially called Fibrimex (FNA Foods, Harimex Inc.). [b] ME, meat emulsion; MEFT, meat emulsion with fibrinogen and thrombin. [c] NaCl (%, w/v) in the NaCl aqueous solution.

transfer ratio (MTR), and apparent diffusion coefficient (ADC). These parameters are potentially sensitive to local variations of water mobility resulting from the modification of water macromolecule interactions and changes in tissue structure. In food science, MRI techniques allow images of the interior of foods to be obtained. These images permit the quantification of T_1, T_2, and ADC parameters to obtain information about several processes and material properties, such as mass and heat transfer, fat and ice crystallization, gelation, and water mobility. In this way, several applications of MRI in food such as to determine chemical composition, internal structure, and quality have been developed (*18–20*). The potential of MRI to characterize and understand meat characteristics and muscle structure has been studied by some authors (*21–25*). MRI has been used in meat to study carcass composition, adipose tissue distribution, connective tissue, and muscle fiber type (*22, 23, 25*). Some authors also showed that MRI parameters are correlated with meat properties such as pH, water-holding capacity, water content, and sensory attributes (*26–29*).

The first aim of this work was to study the textural properties and the MRI parameters of three model systems: fibrinogen–thrombin (FT) gels, meat emulsions (ME), and meat emulsion supplemented with fibrinogen–thrombin (MEFT). The second aim was to study the effect of water and NaCl addition on the structure of these model systems. Finally, the potential of FT to cold gelify meat systems was considered.

MATERIALS AND METHODS

Experimental Design. Three different types of model systems (**Table 1**) were considered: (1) fibrinogen and thrombin (FT), (2) meat emulsion (ME), and (3) meat emulsion with fibrinogen and thrombin (MEFT).

Fibrinogen and thrombin, both together commercially called Fibrimex, were obtained from FNA Foods, Harimex Inc. Fresh pork (M. biceps femoris) was purchased from a local abattoir at 48 h post-mortem.

Preparation of the Fibrinogen and Thrombin Model System (FT). Independent fibrinogen (F) and thrombin (T) were thawed in plastic containers immersed in agitated water at 25 °C for 60 min. For each experience with fibrinogen and thrombin, 10 parts of fibrinogen was mixed with 1 part of thrombin (FT, 10:1, v/v) and shaken for 30 s. To obtain the different gels, mixtures of F and T with or without water and 0, 1, or 2% NaCl were prepared as indicated in **Table 1**: (a) 100 mL of fibrinogen and then 10 mL of thrombin were added (mixture named FT00); (b) 100 mL of fibrinogen was mixed with 11 mL of water and then 10 mL of thrombin was added (mixture FTW0); (c) 100 mL of fibrinogen was mixed with 11 mL of an aqueous saline solution with 11% of salt and then 10 mL of thrombin was added (samples with FT 10:1 and 1% of salt, mixture FTW1); and (d) 100 mL of fibrinogen was mixed with 11 mL of a NaCl aqueous solution with 22% of salt and then 10 mL of thrombin was added (samples with FT 10:1 and 2% of salt, mixture FTW2).

Preparation of Meat Emulsion Model System (ME). The visible fat and connective tissue were removed from the meat. Then, meat was added to a cutter (Robot Coupe R 8 V.V., Vincennes Cedex, France), where the material was cut and, after that, mixed with water or NaCl aqueous solution to obtain a homogeneous mixture. The process was performed by controlling the temperature to maintain the product at 0–2 °C. With this procedure four different (**Table 1**) meat emulsions were elaborated: (a) 100 g of meat (mixture with 0% of water and no salt, named ME00); (b) 100 g of meat was mixed with 11 mL of water (meat emulsion with 0% salt, mixture MEW0); (c) 100 g of meat was mixed with 11 mL of NaCl aqueous solution at 10% (meat emulsion with 1% of NaCl, mixture MEW1); and (d) 100 g of meat was mixed with 11 mL of NaCl aqueous solution at 20% (meat emulsion with 2% of NaCl, mixture MEW2).

Preparation of Meat Emulsion Supplemented with Fibrinogen and Thrombin Model System (MEFT). Four different types of mixture of meat emulsion, fibrinogen (F), and thrombin (T) (w/v/v) were elaborated (**Table 1**). The mixtures were (a) a mixture named MEFT00 composed of 100 parts of meat with 10 parts of F and 10 parts of T; (b) a mixture named MEFTW0 composed of 100 g of meat, 11 mL of water, 10 mL of F, and 10 mL of T; (c) a mixture named MEFTW1 composed of 100 g of meat with 11 mL of NaCl aqueous solution at 12% and then mixed with 10 mL of F and 10 mL of T (mixture with 1% of NaCl); and (d) a mixture named MEFTW2 composed of 100 g of meat with 11 mL of NaCl aqueous solution at 24% and then mixed with 10 mL of F and 10 mL of T (mixture with 2% of NaCl). These mixtures were gently prepared in a mixer by adding the ingredients in the following order: meat emulsion, NaCl aqueous solution, F, and T (**Table 1**).

Each mixture of fibrinogen and thrombin with or without NaCl (FT00, FTW0, FTW1 and FTW2), of meat emulsion with and without NaCl (MEW0, MEW1 and MEW2), and of meat emulsion with or without NaCl and FT (MEFTW0, MEFTW1 and MEFTW2) was extended (0.5 cm thick) in circular plastic containers (diameter = 9 cm) and immediately after were individually packed in plastic bags (Cryovac) in a high-vacuum machine (750/400 model, Vapta, SL, Spain). They were then stored for 24 h at 4 °C until analysis.

Physicochemical Analysis. The pH was determined in a homogenate of the sample with distilled water (1:10) (w/v), using a Crison Digit-501 pH-meter (Crison Instruments Ltd., Barcelona, Spain). Water activity (a_w) was measured using a Decagon CX1 hygrometer (Decagon Devices Inc., Pullman,WA) at 25 °C. The water (oven air-drying method) content from the three model systems was analyzed following the AOAC (*30*) procedure. The protein (Kjeldhal nitrogen) and fat contents of the ME mixtures were determined using the methods of AOAC (*30*) and Bligh and Dyer as described by Hanson and Olley (*31*), respectively.

Water-holding capacity (WHC) was measured by using the Carver press method (*32*). This method used a 0.3 g sample pressed onto an oven-dried Whatman 125 mm filter paper. The WHC values were calculated as the percentage of water retained based on water content in the product before pressing. Four replicates of each sample were determined.

Textural Analysis. Texture profile analysis (TPA) was performed at about 22 °C using a TA.XT2i SMS Stable Micro Systems Texture Analyzer (Stable Microsystems Ltd., Surrey, U.K.) with the *Texture Expert* program. In general, this procedure involved the preparation of four cylinders of 1.5 cm height and 2 cm width from each sample. A double-compression cycle test was performed up to 50% compression of the original portion height with an aluminum cylinder probe P/25. A time of 5 s was allowed to elapse between the two compression cycles. Force–time deformation curves were obtained with a 25 kg load cell applied at a cross-head speed of 2 mm/s. The following parameters were quantified (*33*): hardness (N), maximum force required to compress the sample; springiness (m), ability of the sample to recover

its original form after deforming force was removed; adhesiveness (N × s), area under the abscissa after the first compression; and cohesiveness, extent to which the sample could be deformed prior to rupture.

MRI Analysis. All MRI measurements were performed using a Bruker BIOSPEC 47/40 spectrometer (Bruker GmbH, Ettlingen, Germany) operating at 4.7 T (200 MHz) equipped with a 12 cm diameter actively shielded imaging gradient capable of reaching 200 mT/m along all of the axes.

The samples for imaging were cut approximately 4 cm long, 3 cm wide, and 1.5 cm thick per sample. The samples were placed in a home-built shaped surface coil with dimensions of 4 × 4 cm. A first global shimming was performed, and then three scout spin–echo experiments in axial, sagital, and coronal direction were acquired to localize the slices in the desired place.

For measurements of T_2, separate images were acquired at 10 echo times (TE = 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 ms). All other imaging parameters remained constant between images (NA = 1; TR = 3046.1 ms; MTX = 256 × 256; FOV = 4 × 4 cm; slice thickness = 1 mm; number of slices = 10). The data are then fitted to a single-exponential decay, according to the equation

$$S(\mathrm{TE}) = S_0 \exp^{-\mathrm{TE}/T_2} \quad (1)$$

where S(TE) is the signal at this echo time (TE) and S_0 is the image signal when TE is equal to zero.

For measurements of T_1 separate images were acquired at six recovery times (TR = 310, 510, 1210, 2010, 3010, and 6010 ms); all other imaging parameters remained constant between images (NA = 1; TE = 20 ms; MTX = 256 × 128; reconstructed 256 × 256; FOV = 4 × 4 cm; slice thickness = 1 mm; number of slices = 8). The data are then fitted to an exponential equation

$$S(\mathrm{TR}) = S_0(1 - \exp^{-\mathrm{TR}/T_1}) \quad (2)$$

where S(TR) is the signal at this recovery time (TR) and S_0 is the image signal when TR is equal to zero.

To measure the ADC, separate images were acquired at four diffusion weightings (b value = 62.8, 370.8, 1131.4, and 2309.5 s/mm^2). All other imaging parameters remained constant between images (NA = 1; TR = 2000 ms; TE = 75 ms; Δ = 50 ms; δ = 10 ms; MTX = 256 × 128; reconstructed 256 × 256; FOV = 4 × 4 cm; slice thickness = 1 mm, between slice gap of 0.5 mm; number of slices = 5). For ADC the echo signal intensity is fitted to the equation

$$\mathrm{ADC} = \log(S/S_0)b \quad (3)$$

where S is the signal diffusion weighted and S_0 is the image signal when the value of diffusion weightings (b) is equal to zero.

Statistical Analysis. An individual mixture was the experimental unit for all data analysis. To check the normal distribution (90% confidence) of samples, the Shapiro–Wilks test was applied. When samples fitted the normal distribution, one-way ANOVA analysis was performed. When samples did not fit the normal distribution, the Kruskal–Wallis test was used to test the null hypothesis that the medians of the variable within each of the levels of samples were the same. Duncan's test was used for multiple mean comparisons, Pearson product moment correlation (R), and multiple regression analysis (R^2), using a Durbin–Watson statistical tests, at 95% of confidence level, were performed to determine the relationships between data obtained by TPA, physicochemical analysis, and MRI parameters. The statistical analysis was carried out using Statgraphics Plus version 5.0. Data were presented as the means and the standard deviations (SD) of each mixture.

RESULTS AND DISCUSSION

Physicochemical Characteristics. The meat emulsions (ME) used had similar chemical compositions with percentages of protein and fat of about 19 ± 1 and 5 ± 0.3, respectively (data not shown). The water activity (a_w), water content (water %), pH value, and water-holding capacity (WHC) of the different model systems analyzed are listed in **Table 2**. The highest (p < 0.05) pH and water content values corresponded to the fibrinogen–thrombin (FT) system.

Table 2. pH Values, Water Content (%), Water Activity (a_w), and Water-Holding Capacity (WHC %, Calculated as the Percentage of Water Retained Based on Water Content in the Product) of the Different Types of Systems Analyzed: Fibrinogen and Thrombin (FT System), Meat Emulsion (ME System), and Meat Emulsion with Fibrinogen and Thrombin (MEFT System)[a]

	system		
mixture[b]	FT	ME	MEFT
	pH		
00	8.49 ± 0.08 a,α	5.74 ± 0.04 a,γ	5.98 ± 0.04 a,β
W0	8.42 ± 0.09 a,α	5.68 ± 0.02 b,β	5.67 ± 0.01 b,β
W1	8.48 ± 0.02 a,α	5.72 ± 0.01 a,γ	5.92 ± 0.03 a,β
W2	8.47 ± 0.04 a,α	5.79 ± 0.03 a,γ	6.01 ± 0.03 a,β
	Water Content (%)		
00	87.2 ± 0.4 b,α	77.8 ± 0.4 b,β	78.4 ± 0.5 b,β
W0	88.1 ± 0.3 a,α	79.5 ± 0.3 a,β	80.6 ± 0.1 a,β
W1	88.4 ± 0.1 a,α	79.7 ± 0.9 a,β	80.4 ± 0.8 a,β
W2	88.2 ± 0.1 a,α	79.6 ± 1.1 a,β	80.3 ± 0.5 a,β
	a_w		
00	0.996 ± 0.001 a,α	0.991 ± 0.001 a,β	0.992 ± 0.002 a,β
W0	0.991 ± 0.001 a,b,α	0.981 ± 0.002 c,β	0.985 ± 0.001 b,β
W1	0.992 ± 0.001 a,b,α	0.987 ± 0.003 a,b,β	0.986 ± 0.001 b,β
W2	0.984 ± 0.001 b,α	0.985 ± 0.001 b,c,α	0.984 ± 0.003 b,α
	WHC (%)		
00	94.9 ± 0.6 a,β	96.4 ± 0.3 a,α	97.3 ± 0,9 a,α
W0	90.8 ± 0.1 c,β	95.6 ± 0.2 a,α	91.7 ± 0.7 c,β
W1	92.1 ± 0.2 b,β	95.9 ± 0.1 a,α	94.5 ± 0.2 b,α
W2	92.2 ± 0.4 b,β	96.2 ± 0.1 a,α	96.5 ± 0.7 a,α

[a] Different letters in the same column (a–c) indicate significant differences (p < 0.05). Different letters in the same row (α–γ) indicate significant differences (p < 0.05). [b] Mixtures analyzed: without water and NaCl (00), with water (W0), with water and 1 and 2% of NaCl, respectively (W1 and W2), as indicated in **Table 1**.

The mixtures with the highest a_w (p < 0.05) were those of system FT, except when NaCl at 2% was added (mixtures W2). With this NaCl concentration all of the systems presented similar a_w values.

The WHC was significantly higher (p < 0.05) in the mixtures elaborated with meat emulsions (ME) and in the MEFT system, although in the latter the addition of water without NaCl (mixture W0) caused a significant decrease (p < 0.05) in WHC up to values close to that observed in the FT system (FTW0). The addition of NaCl and water significantly decreased (p < 0.05) the WHC of the FT, whereas no differences were observed in the ME system. The WHC values of the mixture of MEFT with added NaCl (2%) increased to the values of the samples without water and NaCl (MEFT00). Also, a significant negative correlation (R = −0.747, p < 0.0001) was observed between the FT concentration of the mixtures and the WHC, which could explain the lower WHC of the samples with higher FT concentration.

Textural Properties. Textural properties of samples analyzed are shown in **Table 3**. Results showed that the highest (p < 0.05) hardness and cohesiveness values corresponded to the FT system, independently of the water or NaCl concentration. The high hardness and cohesiveness of the FT system could be attributed to the three-dimensional network formed throughout the process of fibrin polymerization responsible for the mechanical properties of the fibrin gels or clots (*15–17*). These results are in agreement with those of other authors (*13*, *15*) who have described high rigidity and stiffness in fibrin clots. The high values of hardness and cohesiveness of the FT gels could indicate an important potential of the FT mixtures to give stability to different systems, that is, meat products. The ME

Table 3. Texture Profile Analysis (TPA) Parameters of the Different Types of Systems Analyzed: Fibrinogen and Thrombin (FT Systems), Meat Emulsion (ME Systems), and Meat Emulsion with Fibrinogen and Thrombin (MEFT Systems)[a]

	system		
mixture[b]	FT	ME	MEFT
	Hardness (N)		
00	70.1 ± 1.0 a,α	5.7 ± 0.1 a,γ	14.0 ± 1.4 a,β
W0	67.2 ± 1.7 a,α	4.1 ± 0.3 c,γ	14.3 ± 1.0 a,β
W1	68.1 ± 2.4 a,α	4.9 ± 0.3 b,γ	7.7 ± 1.3 b,β
W2	58.1 ± 2.6 b,α	4.3 ± 0.1 c,γ	5.3 ± 0.6 c,β
	Adhesiveness (Ns)		
00	−0.68 ± 0.06 a,β	−1.14 ± 0.24 b,α	−0.48 ± 0.32 a,β
W0	−0.65 ± 0.04 a,β	−0.97 ± 0.27 b,α	−0.36 ± 0.25 a,β
W1	−0.70 ± 0.06 a,β	−1.72 ± 0.22 a,α	−0.58 ± 0.30 a,β
W2	−0.68 ± 0.03 a,β	−1.70 ± 0.19 a,α	−0.59 ± 0.24 a,β
	Cohesiveness		
00	0.66 ± 0.01 a,α	0.46 ± 0.01 c,γ	0.54 ± 0.01 a,β
W0	0.68 ± 0.01 a,α	0.47 ± 0.03 c,γ	0.53 ± 0.02 a,β
W1	0.67 ± 0.01 a,α	0.49 ± 0.01 b,β	0.48 ± 0.01 b,β
W2	0.68 ± 0.02 a,α	0.52 ± 0.01 a,β	0.45 ± 0.03 c,γ
	Springiness (m)10^{-2}		
00	0.35 ± 0.01 a,β	0.32 ± 0.03 b,β	0.51 ± 0.01 a,α
W0	0.34 ± 0.01 a,β	0.37 ± 0.01 a,β	0.48 ± 0.04 a,b,α
W1	0.34 ± 0.02 a,γ	0.39 ± 0.02 a,β	0.46 ± 0.02 b,α
W2	0.36 ± 0.02 a,β	0.38 ± 0.03 a,β	0.45 ± 0.04 b,α

[a] Different letters in the same column (a–c) indicate significant differences ($p < 0.05$). Different letters in the same row (α–γ) indicate significant differences ($p < 0.05$). [b] Mixtures analyzed: without water and NaCl (00), with water (W0), with water and 1 and 2% of NaCl, respectively (W1 and W2), as indicated in **Table 1**.

system showed the highest ($p < 0.05$) adhesiveness and the lowest ($p < 0.05$) hardness and cohesiveness. These results indicated a pseudoplastic fluid behavior of this system because of a lack of myofibrillar protein gelification. The highest ($p < 0.05$) springiness values were those of the MEFT system. It could be observed that the MEFT system presented lower adhesiveness and higher ($p < 0.05$) hardness, cohesiveness, and springiness than the ME system (**Table 3**). These results indicated that fibrinogen–thrombin modifies the textural properties of meat batters, giving a behavior similar to that of a gel. A positive correlation was obtained between hardness ($R = 0.989$, $p < 0.0001$) and cohesiveness ($R = 0.953$, $p < 0.0001$) and the presence of fibrinogen–thrombin. The hardness and cohesiveness values obtained for the MEFT system are in the range reported in restructured meat products formulated with Fibrimex (*12*).

There are significant differences ($p < 0.05$) in textural properties as a function of the addition of salt in the different systems analyzed. In the ME system there are significant differences ($p < 0.05$) in hardness as a function of water and NaCl addition. In contrast, in MEFT significant differences ($p < 0.05$) were observed only in hardness as a function of NaCl content ($R = -0.928$, $p < 0.0001$). In the FT system the lowest hardness ($p < 0.05$) was observed when NaCl 2% was added. These results are in agreement with those of De Spirito et al. (*34*). These authors indicated that when the salt concentration is increased the fibrin, fibers are more branched and thinner. Some authors have identified a role for chloride ions as a basic physiological modulator of fibrin polymerization and therefore in the formation of fibrinogen gel (*17, 35, 36*). It has been described that Cl^- specifically binds to the fibrin fibers and opposes the lateral aggregation of protofibrils, resulting in thinner fibers, impeding fibrin polymerization and the formation of the gel or clot three-dimensional network (*17, 37*).

There are no significant differences ($p > 0.05$) in adhesiveness, cohesiveness, and springiness as a function of water without salt and water and NaCl addition in FT system. In the MEFT system a significant negative correlation was observed between cohesiveness ($R = -0.888$, $p < 0.0001$) or springiness ($R = -0.427$, $p < 0.05$) versus NaCl addition. The adhesiveness ($R = 0.795$, $p < 0.05$) and cohesiveness ($R = 0.624$, $p < 0.05$) of the ME system increased significantly when the NaCl concentration increased.

MRI Analysis. An example of T_1, T_2, and ADC maps obtained by the MRI technique of FT00, ME00, and MEFT00 is presented in **Figure 1**. Regardless of their weighting (T_1, T_2, or diffusion), all images display good signal homogeneity across the surface. The differences in map signal intensity of the three systems allow higher T_2 values to be attributed to the FT00 and MEFT00 mixtures than to ME00. Similar comments can be made for the T_1 values. A decrease in signal intensity was observed for ADC maps with FT00 > MEFT00 > ME00. In addition, **Figure 2** shows the exponential curves obtained for the calculation of the T_1, T_2, and ADC parameters of samples FT00, ME00, and METF00. The best fit and the regression coefficients (R^2) are also shown in **Figure 2**. The regression coefficient values ranged between 0.980 and 0.997. Similar R^2 values were found in the mixtures with added water and NaCl (W0, W1, and W2). The high R^2 values indicated that the data points give a good fit. Five regions of interest (ROIs) were randomly selected on the central slice of each experiment to calculate the magnetic resonance parameters (T_1, T_2, and ADC). **Table 4** shows the mean values of T_1, T_2, and ADC parameters for the different mixtures.

Transverse or Spin–Spin Relaxation Time. Transverse relaxation time (T_2) is a measure of the relaxing effect of spin–spin interactions, which can be considered as the effect of water protons on other water or protein systems (FT, ME, and MEFT) protons. These spin–spin interactions are amplified by the forced proximity of the spins and the increased fraction of bound water. As a result, T_2 is particularly sensitive to the porosity of the sample matrix. T_2 values correlate most strongly with the bulk water content of a tissue and therefore changes in total water content, the degree of binding, and water compartmentalization will result in alterations in T_2 (*38*). **Table 4** shows T_2 values for all of the mixtures analyzed. The FT system presented the highest T_2 values and therefore the highest values of bulk water content. Weisel (*15*) described a small part of this type of gel or clot as being constituted by fibrin, the remainder being liquid occupying the space between the protein polymer. These authors showed that there are large spaces between the protofibrils in the fibers and much larger spaces between the fibers (*15–17, 39*). In this respect, it has been described (*29, 40, 41*) that there is a highly significant correlation between the T_2 values obtained by MRI and the major component of T_2 named T_{21} from low-field nuclear magnetic resonance, which is associated with water within the highly organized protein structures, for example, water in tertiary and/or quaternary protein structures. Therefore, the high T_2 values obtained in the FT samples could indicate that the FT system presented more bulk water organized within large spaces between their protein polymers. In contrast, ME samples showed lower T_2 values than FT and MEFT, probably because this is not an organized structure (such as a pseudoplastic fluid). The increase in relaxation time T_2, associated with the presence of fibrin (FT and MEFT systems), could indicate the formation of

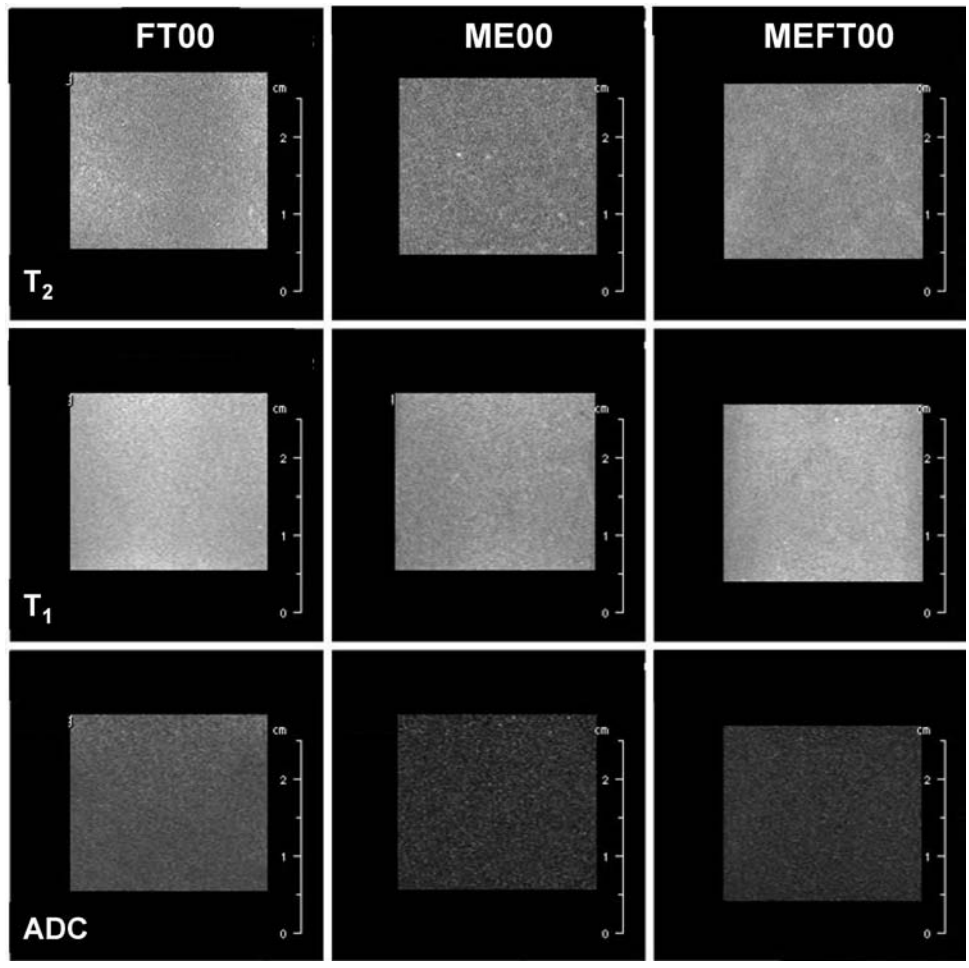

Figure 1. Representative MRI maps of T_2, T_1, and ADC of mixture of fibrinogen and thrombin (FT00), meat emulsion (ME00), and meat emulsion supplemented with fibrinogen and thrombin (MEFT00) without NaCl and water.

a three-dimensional network with large spaces or pores (pools) increasing their size with the FT concentration in the system. As and Lens (*42*) reported that T_2 is influenced by the pore size of the material in which water is examined. In principle, longer T_2 times correspond to large pores in the alginate matrix (*43*). Simple linear regression analyses showed a significant positive correlation between the added fibrinogen concentration and the T_2 values ($R = 0.849$, $p < 0.0001$). A significant positive correlation ($R = 0.898$, $p < 0.0001$) was found between T_2 and water content and a negative one ($R = -0.685$, $p < 0.0005$) between T_2 and WHC. These results are in agreement with previous works (*44, 46*) which indicated that the WHC values of meat are correlated to the T_2 values, and some authors have suggested that T_2 values are physically related to the state (bound or free) of the water embedded within the meat fibers (*44*). There were significant ($p < 0.05$) differences in T_2 values as a function of NaCl content in the different model systems analyzed (**Table 4**). The results indicated that NaCl addition produces an increase in T_2 values in the three systems ($p < 0.05$). These results could suggest that NaCl addition influences the structure of the different systems studied, probably increasing the pore size and the bulk water content. In myofibrils extracted from porcine muscle some authors have reported an increase in T_2 relaxation times obtained by low-field NMR with increasing ionic strength by the addition of NaCl (*47*). Structural changes should be reflected in the rheological properties. In this way, a significant multiple linear regression ($R^2 = 0.903$, $p < 0.0001$) was found using the T_2 values as the dependent variable and as independent variables the hardness, cohesiveness, and NaCl concentration of the different mixtures. However, the structural changes associated with NaCl addition should be different for each system. In the FT and MEFT systems, a significant negative correlation between T_2 values and hardness ($R = -0.733$, $p < 0.05$ and $R = -0.853$, $p < 0.0001$, respectively) was observed. These results may be related with those of other authors (*17, 35*) who indicated that fibrin polymerization is inhibited by the presence of Cl^-, resulting in a less dense and more branched fibrin matrix. Hence, in the MEFT system the springiness of the gel increased significantly when the T_2 values decreased ($R = -0.622$, $p < 0.05$). However, in the ME system a significant positive correlation was found between cohesiveness and T_2 values ($R = 0.596$, $p < 0.05$). In this system the addition of NaCl at 2% originated a marked ($p < 0.05$) increase in T_2, probably because the addition of NaCl to meat batter causes changes in the conformation and stability of meat proteins, which increases the solubility of meat proteins and the myofibrilar structure opening (*45*). Both increases could improve the molecular interactions (giving rise to greater cohesiveness) and bulk water content (higher T_2).

Longitudinal or Spin–Lattice Relaxation Time. Longitudinal relaxation time (T_1) is a measure of the relaxing effect of the external environment on the spins. The T_1 describes the mobility of the molecules (particularly water molecules) and hence the binding of water molecules, for instance, to macromolecules. Free water presents a high T_1 because of its mobility; when water is bound to macromolecules, there is a loss of mobility with easier energy release and a decrease in the T_1 value. An increase in water mobility implies a higher T_1 value (*48*).

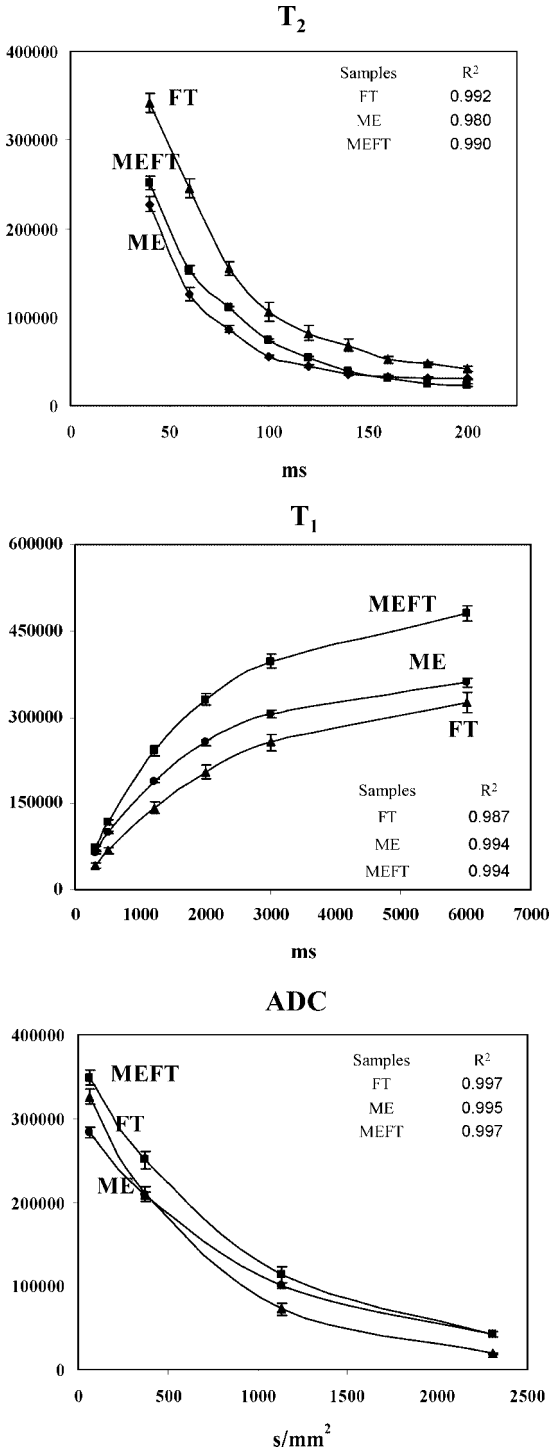

Figure 2. Representative graphs of the exponential time decay of the echo amplitude for selected points of T_1, T_2, and ADC parameters and the regression coefficient values of the curve fit of mixture of fibrinogen and thrombin (FT00), meat emulsion (ME00), and meat emulsion supplemented with fibrinogen and thrombin (MEFT00) without NaCl and water.

The FT gel showed the higher T_1 values ($p < 0.05$). A significant ($p < 0.05$) (**Table 4**) increase in the transverse relaxation time T_1 of the samples with fibrinogen–thrombin (FT and MEFT) was observed when compared to the samples without fibrinogen–thrombin (ME). The results indicated that fibrinogen addition produces an increase in T_1 ($R = 0.846$, $p < 0.0001$). These results could indicate that when fibrinogen–thrombin is forming the gel network, the water presents a free

Table 4. MRI Parameters of the Different Types of Systems Analyzed: Fibrinogen and Thrombin (FT Systems), Meat Emulsion (ME Systems), and Meat Emulsion with Fibrinogen and Thrombin (MEFT Systems)[a]

	system		
mixture[b]	FT	ME	MEFT
	T_2[c] (ms)		
00	70.4 ± 1.7 d,α	54.8 ± 0.5 c,γ	58.6 ± 0.6 d,β
W0	74.5 ± 0.8 c,α	53.3 ± 0.8 c,γ	62.3 ± 0.8 c,β
W1	82.4 ± 1.6 b,α	56.9 ± 0.7 b,γ	64.2 ± 0.7 b,β
W2	92.3 ± 1.7 a,α	64.0 ± 1.3 a,γ	66.1 ± 0.7 a,β
	T_1[d] (ms)		
00	2325.9 ± 48 a,α	1664.9 ± 37 c,γ	1888.8 ± 53 b,β
W0	2363.2 ± 55 a,α	1982.4 ± 19 b,γ	2104.9 ± 30 a,β
W1	2302.9 ± 95 a,α	1702.9 ± 31 c,γ	1769.2 ± 40 c,β
W2	2355.9 ± 73 a,α	2070.6 ± 55 a,β	1845.5 ± 26 b,c,γ
	ADC[e] ($mm^2\ s^{-1}$)10^{-3}		
00	1.37 ± 0.03 b,α	0.91 ± 0.01 c,γ	1.01 ± 0.04 b,β
W0	1.21 ± 0.06 c,α	0.95 ± 0.02 b,γ	0.99 ± 0.02 b,β
W1	1.44 ± 0.04 a,α	0.96 ± 0.03 b,γ	1.05 ± 0.03 a,β
W2	1.49 ± 0.04 a,α	1.02 ± 0.02 a,β	1.04 ± 0.03 a,β

[a] Different letters in the same column (a–c) indicate significant differences ($p < 0.05$). Different letters in the same row (α–γ) indicate significant differences ($p < 0.05$). [b] Mixtures analyzed: without water and NaCl (00), with water (W0), with water and 1 and 2% of NaCl, respectively (W1 and W2), as indicated in **Table 1**. [c] T_1 = longitudinal relaxation time (milliseconds). [d] T_2 = transversal relaxation time (milliseconds). [e] ADC = apparent diffusion coefficient.

distribution (water retained in microspaces without interactions with structural macromolecules and with more mobility).

There were no significant differences ($p > 0.05$) between the T_1 values of the FT mixture with different water and NaCl contents. Because around 88% of the gel is composed of water (**Table 2**), the fibrin has a negligible effect on water protons, and the T_1 is not influenced by the fibrin matrix. In mixtures of the ME and MEFT systems, significant ($p < 0.05$) variations of T_1 were observed in relation to water and NaCl addition. The highest T_1 in the ME system corresponded with mixtures with 10% added water and 2% of NaCl (MEW2). This salt concentration is the usual one in meat products to facilitate the myofibrillar protein partial solubilization (*49*). In this condition, the α-helix structure is opened with a decrease in the water present in the hydratation layers and, because of this, the water acquires more mobility and T_1 should increase. The presence of 1% NaCl should not be sufficient to modify the myofibrillar protein structure, and the T_1 of these gels is similar to that of ME00 mixtures. In the MEFT system, the effect of NaCl on T_1 was not as clear, probably because of the effect of the fibrin matrix. The mixtures of the ME and MEFT systems with water but without NaCl (MEW0 and MEFTW0) showed T_1 values higher than in mixtures without water (ME00 and MEFT), which could indicate that the water added is not bound by the macromolecular structure of the gels.

A simple linear regression analysis was used to determine the relationship between T_1 (**Table 4**) and different physicochemical characteristics (**Table 2**). The significant negative correlation obtained between T_1 values and the WHC of the different systems ($R = -0.738$, $p < 0.0001$) can be explained by the fact that an increase in water freedom implies a higher T_1 value. The significant positive correlation ($R = 0.848$, $p < 0.0001$) between T_1 and water content of the different systems could indicate a water incorporation without interactions with macromolecules. In the three systems studied, cohesiveness was observed to increase when the T_1 value increased ($R = 0.905$, $p < 0.0001$), which could indicate that cohesiveness is depend-

ent on the development of interactions between macromolecules (protein–protein) maintaining the water in the free form.

In the ME system, the hardness increased when the T_1 values decreased ($R = -0.836$, $p < 0.0001$), which could indicate that hardness is related to the macromolecules' density. A significant negative correlation was also observed between hardness and water content of the ME system ($R = -0.866$, $p < 0.01$). However, the hardness of the FT and MEFT systems increased significantly with T_1 values ($R = 0.912$, $p < 0.0001$) which, as in the case of cohesiveness, could indicate that this rheological property mainly depends on the establishment of inter/intramolecular interactions. In these systems, no correlation was found ($p > 0.05$) between water content and TPA properties. These observations are closely related with those of T_2 and suggest that fibrin could modify the meat emulsion conformation, conferring a gel network behavior with the presence of large spaces (high T_2) where water could move freely (high T_2 and T_1).

ADC. The ADC is a measure of the average translational motion of water and reflects the mobility of the molecules in their microenvironment. Water in a free environment can diffuse easily in all directions. In biological tissue there are barriers (such as cell walls and large-chain proteins) that reduce the ability of water to diffuse, and the destruction of these biological barriers is reflected by an increase in the ADC (*50*). **Table 4** shows that the ADC values of the mixtures with fibrinogen–thrombin (FT and MEFT) are higher ($p < 0.05$) than those of system ME, with the highest corresponding to fibrin gels (FT). In alginate gels ADC values close to that of FT (**Table 4**) have been described (*43*). These high ADC values could be attributed to the fact that the matrix formed by the gelifying agent (fibrinogen–thrombin) does not restrict or compartmentalize water (*43*).

A global analysis of the three MRI parameters indicates that the fibrin matrix presents a structure with many and large pores, bulk water, and higher translational motion of water. These characteristics of the MEFT system came from FT, although the MEFT system showed less mobility of the water molecules in their microenvironment than FT ($p < 0.05$). On the contrary, meat emulsions (ME) showed the lowest ADC ($p < 0.05$), indicating a compact matrix with restricted diffusive motion of water, which could be due to the pseudoplastic fluid character of this system.

In the different mixtures of the three systems studied (FT, ME, and MEFT), there were significant variations ($p < 0.05$) in ADC values associated with water and NaCl addition. The addition of 2% NaCl produces a significant increase in ADC ($p < 0.05$). These results are related with the effects of NaCl on the ME and FT systems. A NaCl solution at 2% partially solubilizes the myofibrillar proteins and opens the myofibrillar structure (*45*), with higher T_1 and T_2 and less water restriction (higher ADC). In this way, the cohesiveness of the ME system was found to rise significantly when the ADC values increased ($R = 0.817$, $p < 0.0001$). This correlation could be due to the establishment of interactions (mainly hydrophobic and electrostatic bounds) between portions of salt-solubilized α-helix, which should increase the system cohesion (*45*). In this microenvironment, the water mobility was less restricted.

The increase in ADC in the FT system with 2% NaCl could be related to inhibition of the fibrin polymerization by the Cl^- effect (*17, 35*). It could be possible that these systems present less compartmentalized water and therefore a higher ADC. In relation to these results, a significant correlation between ADC values and FT hardness ($R = -0.536$, $p < 0.05$) has been determined. Possibly, in the MEFT system the increase in ADC associated with NaCl addition was related to the two salt effects, previously indicated for the ME and FT systems. No significant ($p > 0.05$) correlation in the MEFT system was found between ADC and the TPA properties.

Conclusions. From the global analysis of the fibrin gels and meat systems with and without fibrinogen–thrombin addition it is possible to conclude the following:

The fibrin gels were characterized with high water content, water activity, hardness, and cohesiveness. The MRI analysis reflected a structure with large pores (high T_2), high bulk water content (high T_1), and low content of compartmentalized water (high ADC).

The meat emulsions were characterized by high adhesiveness and low hardness. The MRI analysis showed a less organized matrix, low porosity (low T_2), high binding of water molecules (low T_1), and restricted diffusive motion of water (low ADC).

The addition of fibrinogen–thrombin to meat emulsions produces gel network behavior increasing the springiness and hardness. The MRI parameters were higher than those of meat emulsions showing a higher content of bulk water organized within a porous structure (higher T_1 and T_2) and higher diffusive motion of water (high ADC).

The addition of NaCl at 2% to fibrinogen–thrombin and meat emulsion fibrinogen thrombin systems caused a reduction in the gel hardness. In these systems a significant negative correlation was found between hardness and transverse relaxation time (T_2) or apparent diffusion constants (ADC).

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