^{1}H NMR Studies of Water in Chicken Breast Marinated with Different Phosphates

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ABSTRACT: Samples of boneless, skinless chicken breasts were vacuum tumbled in 7% salt and 1.2% of either disodium pyrophosphate, tetrasodium pyrophosphate, tetrapotassium pyrophosphate, sodium tripolyphosphate, or glass phosphate, and studied using ^{1}H NMR. Spin-lattice (T_1) values ranged from 568 to 642 ms, with highest values seen for phosphate-treated samples. Increases with treatment were attributed to changes in moisture. Cooking reduced T_1 values due to loss of moisture and setting of the protein gel. Compression produced further reductions in T_1 values. Little dispersion in R_2 with pulse spacing (τ) was observed for uncooked samples.

Key Words: chicken, NMR, water, phosphates, marination

Introduction

THE ABILITY OF PROCESSED MEAT AND POULTRY PRODUCTS TO retain water is a key quality characteristic. Salt and phosphates in meat marinade enhance water-holding capacity (WHC), overall moisture, juiciness, and yield while limiting cooking and purge loss. In addition, processing procedures such as tumbling and injection are used to enhance the penetration of marinade ingredients (Yasui and others 1979; Schmidt 1986). Sodium chloride causes meat systems to swell and increases the WHC of meat products (Hamm 1986; Schmidt 1986). There is a synergistic effect between salt and phosphates on reducing cooking loss in poultry meat (Hellendoorn 1962; Puolanne and Ruusuned 1980; Froning and Sackett 1985) and in improving sensory properties of cooked products (Maki and Froning 1986; Chambers and others 1992). However, different chemical forms of phosphates do not necessarily produce identical effects in the product, and processors may use various forms or combinations of phosphates in the marinade.

The terms "water-holding capacity" or "water binding" are defined primarily by the ways in which they are measured (Trout 1988). In general, a compressive force is applied, and the amount of expressed water is determined by weight difference. The mechanisms behind WHC at the molecular and microstructural levels are more complex. Several researchers have investigated these mechanisms, including Lillford and others (1980), Fung and Puon (1981), and Nola and Brosio (1983). There is still much to be known about the nature of water binding and how macromolecular structures influence the retention of water within biological tissues (Baianu and others 1991).

Nuclear magnetic resonance (NMR) has been used to study water dynamics and physical structures of foods through analysis of spin magnetization relaxation. While the initial signal intensity is proportional to the number of nuclei present, the decay rate is related to the mobility of such nuclei. In particular, the mobility of protons in water molecules may be hindered through bulk phase changes, increased viscosity, binding to other molecules, chemical exchange, or restricted diffusion (Schmidt and Lai 1991; Baianu and others 1991). Free induction decay (FID), spin-lattice (T_1) relaxation, and spin-spin (T_2) relaxation have all been used to study water properties in foods. For example, in biological materials the signal intensity at 60 to 70 μs on the FID trace is directly proportional to protons associated with the liquid component (Ruan and Chen 1998). Chang and others (1976) and Currie and others (1981) observed that the longitudinal relaxation time constant (T_1) was very sensitive to the early postmortem changes of muscle. Hazlewood and others (1974) used transverse relaxation times (T_2) to show that water associated with macromolecules was approximately 8% of total tissue water.

The goal of this work was to study water properties in chicken breast marinated with different phosphates in combination with salt. First, we examined whether rapid FID measurements could be used to determine overall moisture content in such products. Next, we used spin-lattice and spin-spin measurements to study water dynamics in treated samples. Finally, we studied water binding and expressible moisture in compressed products and attempted to relate these findings to NMR relaxation values.

Results and Discussion

pH, pickup, and yield

Table 1 shows the pH of the marinade solution and treated meat, as well as the weight gain, yield, and inorganic phosphate content of the treated meat. For the solutions, the pH ranged from 4.22 to 9.06. For the marinated meat, pH values ranged from 5.60 to 6.19. Treatment with DSPP resulted in lower pH (5.60) as compared to control (5.97). STPP, TSPP, and TKPP produced higher pH (6.01, 6.10, and 6.19, respectively), while glass phosphate produced no change in pH. The inorganic phosphate content of phosphate-treated samples ranged from 1.05% to 1.14% of the muscle weight. For a given treatment, the phosphate level did not vary significantly from samples taken at different locations in the meat.

Table 1—pH of marinade solutions and pH, weight gain, yield, and total monophosphate in cooked meat[C]

Treatment[A]	Soln pH	Meat pH[D]	%Weight gain (uncooked)	%Yield (cooked)	%Pi[B]
	n = 3	n = 30	n = 30	n = 30	n = 3
No treatment	—	5.97^{b}		77.73^{f}	—
NaCl only	6.22^{c}	5.97^{b}	17.52^{b}	84.82^{e}	—
DSPP	4.22^{a}	5.60^{a}	19.45^{a}	86.29^{de}	1.14^{a}
TSPP	9.00^{e}	6.10^{cd}	18.10^{a}	90.79^{ab}	1.13^{a}
TKPP	9.06^{e}	6.19^{d}	19.71^{a}	92.36^{a}	1.10^{a}
STPP	7.96^{d}	6.01^{c}	18.97^{ab}	89.58^{bc}	1.05^{b}
Glass	5.83^{b}	5.93^{b}	19.38^{a}	87.66^{cd}	1.09^{ab}

[A] Samples treated with 7% NaCl or 7% NaCl and 1.2% of phosphate salt
[B] %Pi determined by ^{31}P NMR after all phosphate species had hydrolyzed to monophosphate
[C] Values within a column with different superscripts are significantly different at $p < 0.05$ using Duncan's least significant difference.
[D] pH of meat following marination but prior to cooking

Treatment with NaCl only produced an increase in weight after marination (17.52%). With the exception of TSPP, phosphate-treated samples had significantly higher weight gain (18.97% to 19.45%). Higher yields after cooking were obtained with NaCl treatment (77.73%). Yet higher yields were obtained with TSPP (90.79%), TKPP (92.36%), STPP (89.58%), and glass phosphate (87.66%).

FID measurements

Figure 1 shows the free induction decay curve for chicken breast muscle treated with DSPP. For comparison, curves for unmarinated and dried DSPP-treated muscle (20% moisture) are also shown. In general, all treated samples showed FID curves with both fast and slow decaying components. The sample dried to 20% clearly shows the decay associated with solids. Protons associated with the meat solids decayed within 25 to 30 μs. Moisture contents were calculated using Eq. 5, with S_t at t = 30 μs, and are presented in Table 2. NMR-based values were compared with those from vacuum-oven measurements (data not shown.) Moistures (%M) based on FID calculations were linearly correlated with oven moistures, with $\%M_{FID} = 0.914*\%M_{oven} + 4.97$ (r = 0.95). The largest difference between FID and oven moisture was 1.87%. In general, moisture content was greater for treated in contrast to untreated samples, but no differences were observed among treated samples.

Spin-lattice relaxation

Effective longitudinal relaxation (T_1) times are shown in Table 2 and include values for uncooked, cooked, and cooked/compressed samples for each of the phosphate treatments. Also shown are T_1 measurements for the liquid expressed during compression of untreated and salt-treated samples. In general, spin-lattice relaxation data were well fit with a single exponential curve; standard errors for such fits were 0.002 or less. Bi-exponential fits (data not shown) showed 96% to 98% of a component with T_1 in the range 586 to 673 ms, and 2% to 4% with T_1 values between 76 to 84 ms. Single T_1 values for intact muscle ranged from 568 to 637 ms for uncooked samples, 330 to 403 ms for cooked samples, and 227 to 274 ms for compressed samples. These values are of the same magnitude as those found by others studying muscle systems. For example, Fung and Puon (1981) found T_1 values between 400 and 450 ms for glycerinated rabbit psoas muscle at 20 MHz; Chang and others (1976) show values between 600 and 680 ms during post-mortem aging of rat skeletal muscle. However, Guiheneuf and others (1997) found T_1 values on the order of 940 to 1080 ms for fresh and frozen/thawed pork. For both cooked and uncooked samples, values for untreated pieces had lower T_1 values than for those treated with salt and phosphate.

Many factors contribute to changes in ^1H relaxation in complicated systems such as muscle (Schmidt and Lai 1991). Binding or interactions of water at macromolecular surfaces enhances relaxation of aqueous protons. Cross-relaxation may occur as magne-

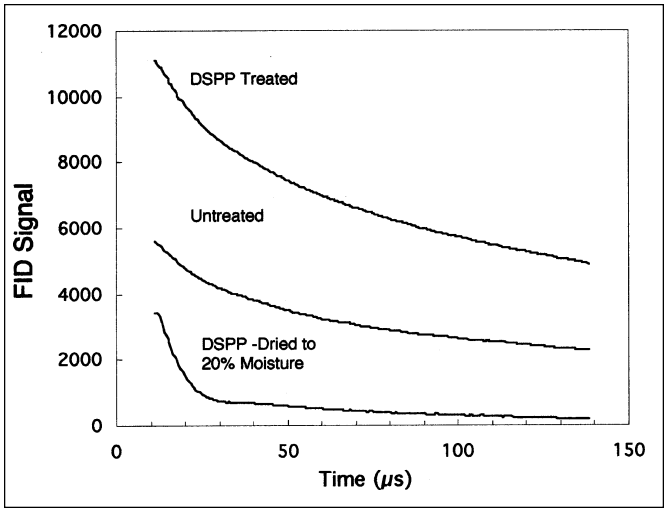

Fig. 1—Free induction decay for uncooked samples of chicken breast meat, meat treated with 7% salt/1.2% DSPP, and meat treated with 7% salt/1.2% DSPP and dried to 20% moisture

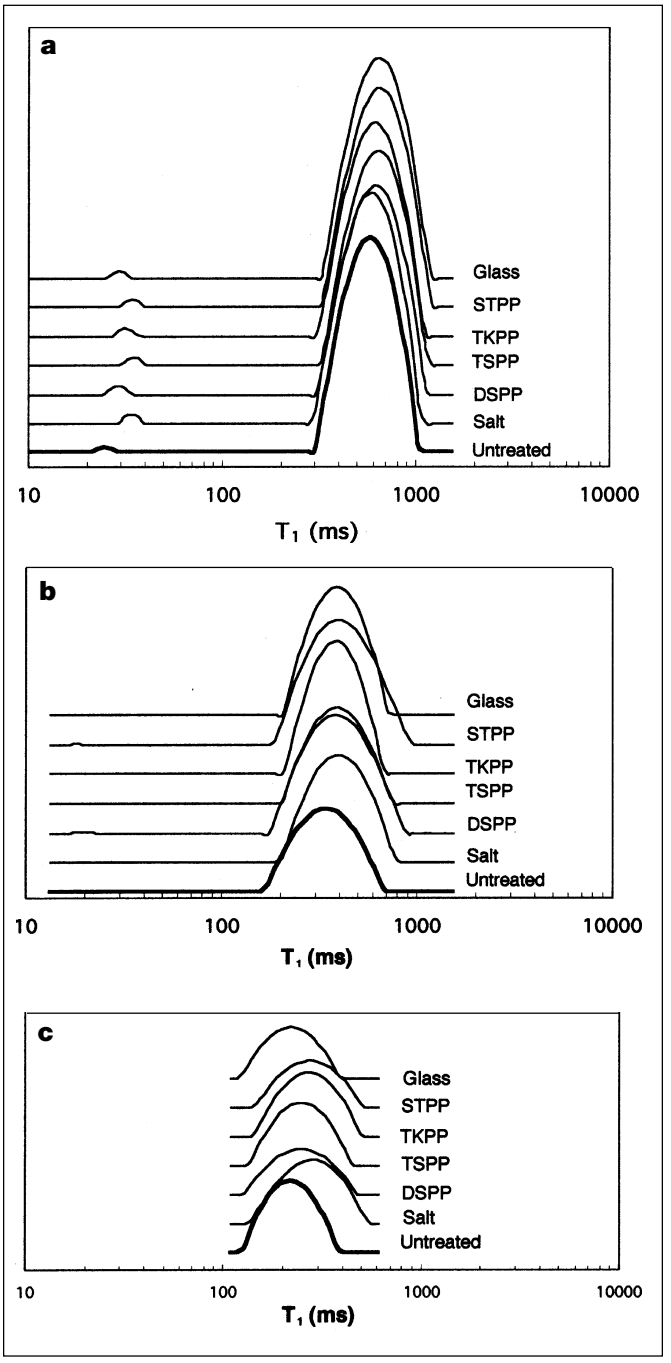

Fig. 2—Distribution of longitudinal (T_1) relaxation times for marinated and tumbled chicken breast. Samples were (a) uncooked, (b) cooked, and (c) cooked then compressed at 400 N. Treatments are salt (7% NaCl) or 7% NaCl plus 1.2% disodium pyrophosphate (DSPP), tetrasodium pyrophosphate (TSPP), tetrapotassium pyrophosphate (TKPP), sodium tripolyphosphate (STPP), or glass phosphate.

Table 2—Spin-lattice (T_1) relaxation times for chicken breast before cooking, after cooking, after cooking and compression at 400 N, and for expressed liquid[C]

Treatment[A]	Uncooked T_1 (ms) n = 6	Cooked) T_1 (ms) n = 6	Cooked/ compressed) T_1 (ms) n = 6	Expressed liquid T_1 (ms) n = 7	Moisture content[D] (%) n = 9	Expressible Moisture[E] (%) n = 7
Untreated	568[b] (6)	330[c]	227[f]	1289	76.88[c] (0.50)	40.3[g](7.1)
Salt	589[ab] (23)	378[d]	266[ef]	1276	78.97[ab] (0.59)	33.3[hi](2.5)
DSPP	642[a] (54)	381[d]	236[ef]	—	79.20[ab] (0.64)	37.0[ghi](2.8)
TSPP	612[ab]	382[d]	259[ef]	—	78.99[ab] (0.42)	38.2[gh](2.8)
TKPP	611[ab]	403[d]	274[e]	—	79.50[a] (0.91)	37.5[ghi](5.5)
STPP	637[ab]	396[d]	263[e]	—	78.80[b] (0.27)	32.1[i](2.6)
Glass	601[ab]	400[d]	251[ef]	—	78.79[b] (0.48)	40.1[g](4.6)
Correlation with[B]:						
Exp Moisture r =	–0.45	–0.34	–0.53			
% Moisture r =	0.68	0.89	0.71			

[A] Samples vacuum tumbled with 0.2L/kg marinades containing either 7% salt or 7% salt and 1.2% of phosphate salt
[B] Coefficients (r) are for correlations between expressible moisture (or moisture content) and T_1 values for uncooked, cooked, or cooked/compressed samples.
[C] Samples within a column with different superscripts are significantly different at $p < 0.05$, using Duncan's least significant **D**ifference.
[D] Moisture content as percent of total weight after marination but prior to cooking
[E] Weight of moisture expressed at 400 N as compared to weight of water in the original sample

tization is transferred between aqueous and macromolecular protons. In addition, relaxation may be enhanced by chemical exchange of protons between water molecules in different states or between water and other molecules with exchangeable protons. For heterogeneous systems with distinct aqueous environments, diffusion of water molecules between regions is also a factor (Lillford and others 1980). If diffusion times are long compared to NMR relaxation times, then distinct T_1 values associated with each region are observed. If diffusion times are relatively short, then an effective T_1 is observed that is a weighted average of each population. At intermediate diffusion times, relaxation processes are multi-exponential (Hills 1998). In systems with restricted spaces, as in gels or foods with extensive cellular structure, diffusion distances are relatively short. In such cases, all water molecules have sufficient time to diffuse to macromolecular surfaces and be affected by these interactions. For a heterogeneous distribution of such spaces, T_1 values are also distributed (Davies and Packer 1989).

Figure 2 shows plots of continuous distributions of T_1 values. For uncooked samples, a broad distribution was observed between approximately 300 and 1100 ms. Other researchers (Hazlewood and others 1974; Belton and others 1972; Chang and others 1976; Fung and Puon 1981; Renou and Monin 1985) have indicated that several distinct states of water exists in muscle tissue, such as "protein-bound" water, water in extracellular spaces, or water residing within myofibrils and the sarcoplasmic reticulum. Lillford and others (1980) and Fung and Puon (1981) suggest that the large fraction of water is associated with intracellular water. These latter researchers showed that within the cell, ^1H relaxation is most likely enhanced by hydrogen exchange between water and functional groups on proteins, including those on protein filaments. Lillford and others (1980) showed that in muscle, the observed T_i is roughly proportional to the pore size. In our results, the distribution of T_1 values may reflect the heterogeneity of enclosed structures in muscle.

The processing of samples in salt or various salt/phosphate combinations had only a small effect on the T_1 distributions. Salt caused shifting of the T_1 distributions to higher values, while salt and phosphates shifted the distributions to even higher values.

The effects of salt and phosphate on water states and associated NMR relaxation phenomena are complex. First, addition of phosphates often results in pH changes in the treated muscle (Table 1). Such changes may alter NMR relaxation, particularly for processes dominated by hydrogen exchange. Dependence of the proton exchange rate on pH has been observed for a variety of compounds containing labile protons (Rabenstein and Fan 1986). Measured values of pH in the samples were: untreated (6.0), salt (6.0), DSPP (5.7), TSPP (6.1), TKPP (6.2), STPP (6.1), glass (5.9). It is unlikely that such small changes would cause major changes in proton exchange rates. Also, there is no obvious correlation between the direction of pH change and changes in T_1.

A more likely reason for the observed increases is the effect that salt and phosphate has on the structural elements of the muscle. Both act synergistically to solubilize, and thus disrupt, protein myofilaments in the cell. As such, the diffusion distance between protein filaments is increased, and this would tend to increase observed T_1 values. In addition, the action of salt and phosphates is also known to cause swelling among the myofilaments (Offer and Trinick 1983), and this too would lead to higher values of T_1. In another study (data not shown) on chicken breast meat treated with salt and the same group of phosphates and analyzed by transmission electron microscopy and Warner-Bratzler shear values, we found that STPP-treated samples had substantial degradation of I-bands, Z-lines, and M-lines, while DSPP-treated samples had complete loss of H-zones and slight dispersal of I-bands. In addition, STPP and DSPP produced the lowest shear values of the group, which was presumed to be caused by the fact that less force was required to slice through a sample with greater structural degradation. Here, DSPP- and STPP-treated samples had the highest average T_1, but no claims of statistically significant difference could be made. Obviously, any changes were not substantial enough to produce large changes in T_1 values, or the variety of changes were compensatory in their effect on the average T_1 values.

T_1 values for cooked samples were substantially lower than those for uncooked samples (Table 2), with ranges from 330 to 403 ms. As with uncooked samples, T_1 values were higher in salt-treated samples, and higher still for salt/phosphate-treated samples. However, the type of phosphate had little effect on T_1 values. The decrease in T_1 values may be explained by changes in the muscle upon cooking. Cooking is known to cause shrinkage of the muscle and loss of moisture (Offer and Trinick 1983). In addition, heating causes gelling of solubilized protein, and this may further reduce average diffusion distances. Hills and others (1989) showed that protein aggregation reduces the water proton relaxation time, as dipole-dipole interactions are less efficiently averaged by rotational motion.

Compression of the 1 cm^3 cooked samples under a 400N load resulted in a further reduction in the observed T_1 values (Table 2), with T_1 values in the range of 227 to 263 ms. Such changes can be attributed to further reduction in cellular and extracellular spaces, as well as lower water/solids ratios as moisture is expressed.

Values for the moisture content of uncooked meat and the expressible moisture of cooked meat are shown in Table 2. In gen-

eral, processing with salt or salt/phosphate caused a decrease in expressible moisture, and therefore, an increase in water-holding capacity. However, only salt- and STPP-treated samples had significantly lower expressible moisture than untreated samples.

According to Lillford and others (1980) and Trout (1988), NMR relaxation values should be related to water-binding capacity in muscle foods, particularly as they measure water associated with pores of different size. Lillford and others (1980) presented spin-spin relaxation curves for cooked and uncooked beef muscle and fit the results with 3 or 4 exponential terms. They found differences in relative proportions of T_2 components among raw, cooked, and cooked/drained samples. However, there was no comparison of this data with common water-binding capacity measurements. Trout (1988) suggested that NMR is a good measure of water-binding capacity, as WBC is related to pore-size distributions. As shown in Table 2, moisture content was correlated ($r = 0.89$) with T_1 values for cooked meat and less so for cooked/compressed ($r = 0.71$) and uncooked ($r = 0.68$) meat. Expressible moisture had only slight negative correlation with T_1 values for the cooked/compressed ($r = -0.53$), uncooked ($r = -0.45$), and cooked ($r = -0.34$) meat.

Spin-spin relaxation

Plots of spin-spin relaxation rates ($R_2 = T_2^{-1}$) as a function of τ, the delay time between the initial 90° pulse and the subsequent 180° refocusing pulse, are shown in Fig. 3 for uncooked, cooked, and compressed samples that were treated with STPP. Results for expressed juice are also shown for comparison. Within a like category (such as cooked or uncooked), differences in T_2 among different phosphate treatments were not significant, except for DSPP samples, which had higher T_2 values. Such plots have proven useful in analyzing diffusive exchange between regions with different intrinsic relaxation processes (Hills 1998). For the juice, a clear dispersion in rates was found. The type of curve is typical for solutions of biopolymers such as polysaccharides (Hills and others 1991) or proteins (Hills and others 1989). In such cases, spin-spin relaxation rates are greatest at long τ values, as processes such as proton exchange with, and intrinsic relaxation away from the biopolymer, have time to contribute.

Raw samples showed little dispersion, which suggested a structure with restricted biopolymer mobility. For example, we have found that solutions of pectin show substantial dispersion but that such dispersion is lost once the system is gelled (Kerr and others 1998). Similarly, Hills and others (1989) showed protein aggregation and gel formation resulted in increased relaxation rates and less dispersion in rates. They attributed this to hindered biopolymer mobility, in which the protein-proton relaxation time was reduced because of hindered rotational averaging of the protein dipolar interactions.

Cooked samples showed lower T_2 values (increased rates) than for uncooked samples. We attribute this to denaturation and gelling of extracted protein as well as lower overall moisture content. Differences in rates among different treatments were also more noticeable than for uncooked samples. Of particular interest is the fact R_2 decreased slightly at long τ values and increased slightly at short τ values. This behavior is contrary to what is usually observed and would certainly not be fit by a 2-site diffusive exchange model (Carver and Richards 1972). Such experiments should be verified at higher field strengths where relaxation dispersion is more pronounced (Hills and others 1989).

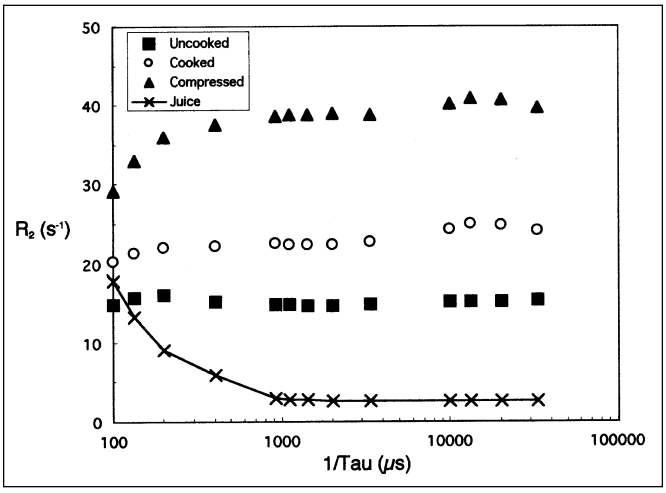

Fig. 3—Transverse relaxation rates ($R_2=1/T_2$) against the reciprocal of the 90% to 180% pulse spacing (τ) in the CPMG pulse sequence. Samples were (■) uncooked , (○) cooked, and (▲) cooked then compressed at 400 N. Rates for expressed juice (×) are also shown for comparison.

Conclusions

TOTAL MOISTURE CONTENT WAS READILY DISCERNED BY FID measurements and agreed well with vacuum-oven measurements. Treatment of raw chicken with salt and phosphates produced only small increases in measured T_1 or T_2 values. This was somewhat surprising, as such treatments are known to extract protein from myofibrils and enhance water-binding properties of the meat. For uncooked meat, this may reflect the fact that treated samples swell and have more moisture. T_1 times were distributed in 1 region (300 to 1000 ms), suggesting that water molecules interact with cell molecules within confined spaces. This notion is supported by the fact that transverse relaxation rates were effected little by variation of the inter-pulse spacing.

As NMR relaxometry has been used to measure water binding or mobility, it seemed a reasonable hypothesis that such measurements could be used to gauge water-holding capacity of muscle foods. Our results suggest, however, that the relationship is not straightforward. One would generally associate reduced relaxation times with greater water binding or decreased mobility. We found that, if anything, T_1 and T_2 values were higher for samples with greater water-holding capacity. A simple interpretation is that this was due to increased water in these samples.

Materials and Methods

Marination

Boneless, whole chicken breast muscle (Gold Kist Poultry, Athens, Ga., U.S.A.) was split and trimmed, then sear branded with different numbers for identification of individual pieces. The weight of each piece was recorded for yield studies. Samples were formulated using 5 kg prepared breast muscle and 1 L of marinade. The marinades were prepared with 10 °C deionized water and contained either 7% by weight of NaCl or 7% NaCl and 1.2% by weight of the phosphate salt [disodium pyrophosphate (DSPP), tetrasodium pyrophosphate (TSPP), tetrapotassium pyrophosphate (TKPP), sodium tripolyphosphate (STPP), or sodium phosphate glass ($Na_{n+2}P_xO4_{n-1}$, $n = 15 \pm 3$]. All chemicals were obtained from Sigma Chemical Company (St. Louis, Mo., U.S.A.) The chicken and marinade were processed in a pilot plant vacuum tumbler (U-Mec, Los Angeles, Calif., U.S.A.) for 20 min. The tumbler was 61 cm in

dia, 46 cm deep, and operated at a speed of 8 rpm. Tumbling was carried out under vacuum (< 9.89 kPa absolute pressure) at a temperature of 10 ± 2 °C. Marinated samples were stored at 0 °C for 24 h prior to further investigation.

The degree of marinade and phosphate pickup was assessed by measuring the weight gain following marination/tumbling, overall yield, and inorganic phosphate content of the cooked muscle. Weight gain was defined as:

$$\%\text{WtGain} = \frac{W_{mar} - W_{init}}{W_{init}} \times 100 \quad (1)$$

where W_{init} is the initial weight of a given chicken piece, and W_{mar} is the weight following marination and tumbling. Following cooking, overall yield was determined from:

$$\%\text{Yield} = \frac{W_{cook}}{W_{init}} \times 100 \quad (2)$$

Phosphate content of the treated samples was determined by ^{31}P NMR. Proton-decoupled ^{31}P NMR spectra were acquired on a Bruker AC-300 300 MHz NMR spectrometer. Prior to NMR analysis, samples were cut into 1.5 cm cubes, mixed with 10% (w/w) EDTA-Na_2, then chopped in a blender. Three grams of this mix were homogenized in an Omni-Mixer (Ivan Sorvall, Inc, Norwalk, Conn., U.S.A.) with 7 mL water (3 parts D_2O and 7 parts water). Samples were homogenized 3 times for 20 s at 20-s intervals. Samples (0.5 mL) were transferred to 5 mm NMR tubes, and a capillary tube containing 2.0337% methylenediphosphonic acid (MDP) was inserted into the tube as an external reference and primary standard (Matsunaga and others 1982). All data was collected at a ^{31}P resonance frequency of 121.5 MHz, sweep width of 82.3069 ppm, and 8K data points, giving a resolution of 2.441 Hz/point. The 90°**p**ulse width was 3.1 μs, the relaxation delay 3.0 s, and the data was averaged over 32 scans. Initial experiments showed that all phosphate forms eventually were hydrolyzed to monophosphate. Once a constant monophosphate level was reached (and other species had reached undetectable levels), the weight percent of monophosphate ($\%W_{Pi}$) in a 0.5 mL sample was calculated according to:

$$\%W_{Pi} = W_s \left(\frac{M_{Pi}}{M_s}\right)\left(\frac{N_s}{N_{Pi}}\right)\left(\frac{A_{Pi}}{A_s}\right)\left(\frac{100}{0.15}\right) \quad (3)$$

where W_s, N_s, and A_s are the weight, number of nuclei contributing to the peak, and peak area of the standard (2.0337% methylenediphosphonic acid). N_{Pi} and A_{Pi} are the number of nuclei and peak area associated with monophosphate. The factor 0.15 represents the amount of chicken (in g) present in the 0.5 mL sample, accounting for the dilution by D_2O/H_2O.

Cooking

The marinated breast meat was cooked in a single layer on a clam-shell type grill (ANETS' Model CFA-12, Northbrook, Ill., U.S.A.) until the center temperature at the thickest point had reached 72 °C. Both the top and bottom plates were heated to 176 °C prior to loading the meat, and the temperature maintained by the internal thermal control system. The temperatures of 2 pieces on the furthest diagonal position of the grill were continuously monitored through thermocouples placed in the samples and connected to a data acquisition system (OM270, Omega, Stamford, Conn., U.S.A.)

Expressible moisture

Pieces from the breast mid-section were further cut into 1-cm cubes for expressible moisture (EM) measurements. One piece of Whatman No. 1 filter paper (12.5 mm dia) was applied on top and bottom of the samples to absorb expressed moisture. The EM measurements were conducted with a Model 5500 Instron Universal Testing Machine (Instron Engineering Corp., Canton, Mass., U.S.A.) with a 10-cm dia flat disc attachment. The attachment was lowered at 100 mm/min until the load cell sensed 400 N force. The load was reached within 15 s, then held constant for a total testing time of 60 s. Typically, the samples were deformed approximately 85%. The weight of the sample was recorded to the nearest 0.1g before and after compression. EM was expressed as a percentage of the total moisture content prior to compression:

$$\%\text{EM} = \frac{W_1 - W_2}{W_m} \times 100 \quad (4)$$

where W_1 and W_2 are the sample weights before and after compression, and W_m is the weight of water in the original sample.

Total moisture

Moisture content was determined by weight difference before and after vacuum drying at 102 °C for 24 h (AOAC 1995).

pH measurements

The pH of each marinade was measured before use. Before and after marination, the pH of the chicken muscle was measured with an Orion Model 82-63 pH meter (Orion Research, Inc., Beverly, Mass., U.S.A.) with a Model 8163BN glass body spear tip probe.

Proton (^{1}H) NMR measurements

A MARAN-20 pulsed NMR spectrometer (Resonance Instruments Ltd., Oxon, U.K.), operating at 20 MHz, was used in this study. A cork borer was used to remove 1.6-cm dia samples from the breast muscle, which were then sliced to approximately 2.5 cm in length and such that fibers were oriented transverse to the length. Samples were placed in 18-mm dia NMR tubes that were then covered with parafilm to limit moisture loss. All measurements were made at 25 ± 0.1 °C.

FID measurements. Free induction decay measurements were made using a 90 ° pulse of 4.3 ms. After a 10 ms delay, the transverse NMR signal was collected every 0.5 ms for a total of 256 data points. A total of 8 scans were taken with a recycle delay (RD) 8.0 s between each scan. Moisture content of the sample was calculated using (Ruan and Chen 1998):

$$\%\text{Moisture} = \frac{S_t}{S_o} \times 100 \quad (5)$$

where S_o is the initial signal (at t = 10 μs), and S_t is the signal at time t, presumably shortly after the magnetization from protons in solids had fully decayed. Samples of meat were also dried in a vacuum oven at 102 °C and removed intermittently. This provided samples of various moisture levels down to 12% moisture, which were then measured using the FID procedure. This allowed us to determine a time at which the signal inten-

sity associated with solids had essentially decayed to 0, while that associated with water had changed little from its initial value.

Spin-lattice relaxation. Spin-lattice (T_1) relaxation experiments were accomplished using an inversion recovery sequence, with τ varying logarithmically from 1 to 5000 ms over 126 steps, an RD of 8.0 s, and signal averaging over 8 scans. Relaxation curves were analyzed using a multi-exponential fitting routine (EXP3, Resonance Instruments) that fits up to 3 exponential terms. That is:

$$\frac{M(\tau)}{Mo} = \sum_{i=1}^{3} P_i[1 - 2\exp(-\tau/T_{1,i})] \quad (6)$$

where M_o is the initial magnetization, P_i is a weighting factor, and $T_{1,i}$ represents each of the 3 possible time constants. In addition, data were analyzed using a distributed exponential routine (DXP, Resonance Instruments). Based on the "contin" routine of Provencher (1982), this routine calculates a distribution of T_1 terms that best describe the data.

Spin-spin relaxation. Spin-spin (T_2) relaxation curves were determined using a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. In initial experiments, the 90° to 180° pulse spacing (τ) was set to 1000 μs. Data were analyzed as described for spin-lattice measurements. In subsequent measurements, variable τ values between 30 and 20,000 μs were specified.

Statistical design and analysis

Seven sample treatments were tested including a "no treatment" control, one with 7% salt only, and five with 7% salt and 1.2% of each of the specified phosphate salts. Each sample batch was prepared from 5 kg of meat, containing approximately 30 to 35 individual pieces. Samples were withdrawn at random for analysis. The number of total replications N for a given analysis is presented in Tables 1 and 2. The cooking procedure was duplicated for each treatment, with 15 pieces cooked per replicate. SAS statistical software (SAS Institute, Inc., Cary, N.C., U.S.A.) was used to analyze data for differences between treatments. A linear ANOVA model was utilized to test for effects of treatments. The Duncan test was used to differentiate significant differences among treatments. Correlation coefficients between measurements were also calculated and compared. The level of significance was defined as $p < 0.05$.

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