The use of sodium bicarbonate for marination of broiler breast meat

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ABSTRACT This study aimed to evaluate marination performances and the effect on meat quality traits of sodium bicarbonate, used alone or in combination with sodium chloride, when compared with sodium trypolyphosphate by using advanced analytical tools, including low-field nuclear magnetic resonance and differential scanning calorimetry. In total, 140 samples (cylindrical shape of 1 × 4 cm size) were obtained from a batch of 24-h postmortem broiler breast meat (Ross 708, females, 47 d old). Six of the groups were used for subsequent marination treatments, whereas the last group was kept as a nonmarinated control. Samples were subjected to vacuum tumbling in a special equipped laboratory rotary evaporator with a 12% (wt/wt) water:meat ratio using 6 marinade solutions: 7.7% (wt/wt) NaCl (S); 2.3% (wt/wt) $Na_4O_7P_2$ (P); 2.3% (wt/wt) $NaHCO_3$ (B); S and P; S and B; S, P, and B. Samples marinated with bicarbonate alone or in combination (B, SB, and SPB) significantly increased ($P < 0.05$) the meat pH by approximately 0.7 units compared with that of the control, whereas phosphate alone or in combination with salt increased ($P < 0.05$) the pH by 0.2 units. The combination containing all of the ingredients (SPB) produced the highest marinade performances; however, SB was able to guarantee a better marinade uptake and water retention ability with respect to that of SP. According to low-field nuclear magnetic resonance, the combined use of B and P with S determined a remarkable increase in proportion of entrapped water into the myofibrillar spaces, while the extramyofibrillar water fraction was not modified. Moreover, water gain following marination does not correspond to an increase in the freezable water amount, as detected by differential scanning calorimetry. In conclusion, B is a very promising marinating agent, and it can be exploited to develop processed poultry products with no added phosphates to match the request to avoid the nutritional drawbacks recently indicated with the use of phosphates.

Key words: poultry meat, marination, sodium bicarbonate, water status, differential scanning calorimetry

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INTRODUCTION

Marination is a commonly used method for adding value to different types of meats, which involves injection or tumbling to disperse in a muscle a solution of water, salt, and other ingredients. To add flavor and to increase the shelf life of meat products, spices and extracts with antimicrobial and antioxidant properties are also added in marinades (Alvarado and McKee, 2007).

Injecting or adding nonmeat ingredients to enhance the water-holding capacity (**WHC**) of poultry meat products has been extensively investigated in literature (Smith, 2010). Water is often overlooked as a functional ingredient in processed meats. Although water is a major component of raw meat, when additional water is added as part of the curing process, the water becomes a nonmeat ingredient as well as a meat component (Sebranek, 2009). Generally speaking, the WHC of meat is minimal when the pH is close to the isoelectric point of myofibrillar proteins (about 5.2–5.3 in poultry meat). On either side, the ionic strength could be steadily increased by adjusting the pH, thus leading to an increased WHC of meat products (Barbut, 2002).

According to Offer and Trinick (1983), increased moisture-retention ability by marination is due to muscle fiber expansion (swelling) caused by electrostatic repulsion that allows more water to be immobilized in the myofibril lattices. Several additives have been demonstrated to improve the quality of meat products, the most common being sodium chloride and phosphates (Alvarado and McKee, 2007).

A sodium chloride concentration of 4.6 to 5.8% is known to produce maximum swelling of myofibrils and a simultaneous high water uptake. Sodium chloride plays a key role in the solubilization of myofibrillar proteins for subsequent denaturation and aggregation to improve water retention and acceptable rigidity and elasticity of the meat gels (Barbut, 2002). Mechanisms about sodium chloride improving the WHC of meat have been reviewed by Offer and Knight (1988) and Ruusunen and Puolanne (2005). Also, the addition of

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phosphate salts, particularly pyrophosphate and tripolyphosphate, increases the water-binding capacity of meat. A phosphate concentration of about 0.3% or higher is believed to act on muscle proteins by increasing the pH, ionic strength, and specifically by complexing protein-bound Mg and Ca, which results in increased solubilization of myosin and actin (actomyosin dissociation and depolymerisation of thick and thin filaments; Xiong, 2004). A strong synergistic effect between sodium chloride and phosphates in poultry meat was noted (Xiong et al., 2000). Marinades consisting of salt and polyphosphates are used to improve the texture and yield of muscle food products (Young and Lyon, 1997; Xiong and Kupski, 1999a,b; Smith and Young, 2007). Although phosphates have been shown to improve meat quality, several countries have banned their use in raw meat production (Sebranek, 2009).

Some ingredients have been put forward to replace phosphates in meat products. A few studies focused on using bicarbonate to minimize the problem of pale, soft, and exudative in pork (Kauffman et al., 1998; Van Laack et al., 1998; Wynveen et al., 2001) and poultry (Woelfel and Sams, 2001; Alvarado and Sams, 2003). More recent studies found that sodium bicarbonate was able to reduce shear force and improve the yield of enhanced pork and poultry meat (Sheard and Tali, 2010; Sen et al., 2005; Petracci et al., 2009b). The greater effect of bicarbonates may be due to a higher buffering capacity and ionic strength than phosphates (Wynveen et al., 2001). In contrast to the more commonly used enhancement ingredients, the basic mechanisms responsible for the enhancement properties of sodium bicarbonate are far from understood in detail.

Although previous studies have provided information about changes in meat upon marination with sodium chloride, polyphosphate, and bicarbonate, scarce information has been obtained at present about marination effects on the interaction of water with the biopolymers inside the single-compartment meat (namely intra- and extramyofibrillar spaces). As a consequence, it can be very useful to investigate water distribution and mobility by applying advanced techniques, such as low-field nuclear magnetic resonance (**LF-NMR**) and differential scanning calorimetry (**DSC**).

The registration of proton transverse relaxation time ($\mathbf{T_2}$) weighted signals, obtained by means of LR-NMR, has been successfully applied to study water distribution and water properties in meat. Studies conducted on pork (Bertram et al., 2002), turkey (Bianchi et al., 2004), and rabbit meat (Petracci et al., 2009a) have shown that weighted T_2 signals allow one to separately observe water tightly associated to macromolecular constituents of meat located inside and outside of the myofibrils. The characteristics of these 2 latter water pools have been found to be strictly related to WHC and other meat-quality traits. Recently proton T_2 has been found to strongly correlate with salt-induced swelling in pork (Bertram et al., 2008). Such swelling is consistent with the influence of pH and ionic strength on proton NMR T_2 relaxation characteristics of extracted myofibrils.

From a calorimetric point of view, bound water has been traditionally determined by DSC as the amount of unfreezable water within a sample after being cooled at low temperature (e.g., −70°C; Simatos et al., 1975). In principle, when the temperature of the cooled samples is increased at a constant rate in the calorimeter, the fusion of ice is detected as an endothermic peak, with the area proportional to the amount of ice, being identified. Unfreezable water represents the difference between the total water content and the amount of water detected by the fusion endotherm (Cornillon, 2000). Differential scanning calorimetry has been used to monitor the gross phase changes of water in polymeric networks (Capitani et al., 2003) and in food systems, such as meat (Venturi et al., 2007).

This study was aimed at evaluating marination performances and the effect on meat quality traits of sodium bicarbonate, used alone or in combination with sodium chloride, when compared with sodium trypolyphosphate. Water distribution and mobility changes induced by marination treatments have been investigated by LF-NMR and DSC.

MATERIALS AND METHODS

Experimental Procedures

A batch of 35 broiler breast fillets was obtained 24 h postmortem from a flock of birds (Ross 708, females, 47 d old, 2.54 kg) grown and slaughtered under commercial conditions. From each Pectoralis major muscle, 4 samples of cylindrical shape with the dimensions of 1-cm height and 4-cm diameter were cut, resulting in a total of 140 samples weighing about 15 g, and were divided into 7 homogeneous groups (20 samples/group) according to their pH and color (L*, a*, and b*) and tagged for identification. Six of the groups were used for subsequent marination treatments, whereas the last group was kept as a nonmarinated control. Samples were subjected to vacuum tumbling with a 12% (wt/wt) water:meat ratio using 6 marinade solutions: 7.7% (wt/wt) NaCl (**S**); 2.3% (wt/wt) $Na_4O_7P_2$ (**P**); 2.3% (wt/wt) $NaHCO_3$ (**B**); 7.7% (wt/wt) NaCl and 2.3% (wt/wt) $Na_4O_7P_2$ (**SP**); 7.7% (wt/wt) NaCl and 2.3% (wt/wt) $NaHCO_3$ (**SB**); and 7.7% (wt/wt) NaCl, 2.3% (wt/wt) $Na_4O_7P_2$, and 2.3% (wt/wt) $NaHCO_3$ (**SPB**). The target final concentration (g/100 g of meat) of NaCl in marinated samples was approximately 1% for the S treatment, and 0.3% sodium trypolyphophates and sodium bicarbonate for the P and B treatments, respectively, which are within the common range used in chicken products. To simulate the commercial process, tumbling was conducted using a laboratory rotary evaporator (Heidolph, Germany) connected to a vacuum pump controlled by a specially designed device able to strictly control pressure throughout the treatment. The samples and marinade were placed in a 500-mL

evaporator flask and tumbled for 40 min under vacuum (3 kPa) at a temperature of 2 ± 1°C. Before and after being tumbled, samples were weighed to determine the marinade uptake and placed in covered plastic boxes on raised wire racks in a 2 to 4°C cooler. After 24 h, samples were again weighed to determine the drip loss, and color (L*, a*, and b*) was also measured. From each group, 10 samples were used to determine pH, expressible moisture, and LR-NMR relaxation properties on uncooked meat, whereas the remaining 10 samples were individually vacuum-packed under 5 kPa, cooked in an 80°C water bath for 12 min until the internal temperature reached 80°C, and assessed for cooking loss, pH, color, total moisture, and LR-NMR relaxation properties. Moreover, the water activity ($\mathbf{A_w}$) and freezable water (**FW**) by DSC were assessed on 3 raw samples per group after marination and after cooking.

Analytical Methods

Color Measurement. The CIE (1976) system color profile of lightness (L*), redness (a*), and yellowness (b*) was measured by a reflectance colorimeter (CR-400, Minolta, Milano, Italy) using illuminant source C. The colorimeter was calibrated throughout the study using a standard white ceramic tile. The color was measured in single on the center of the samples before tumbling, after tumbling, and after cooking.

pH Measurement. The pH was determined using a modification of the iodoacetate method that was initially described by Jeacocke (1977). Approximately 2.5 g of meat sample before tumbling, after tumbling, and after cooking were used, minced by hand, homogenized in 25 mL of a 5 m*M* iodoacetate solution with 150 m*M* potassium chloride for 30 s, and the pH of the homogenate was determined using a pH meter calibrated at pH 4.0 and 7.0.

Weight Changes During Marination and Cooking. The weights of the individual samples were recorded before tumbling (wt_1), after tumbling (wt_2), after 24 h of storage (wt_3), and after cooking (wt_4). The following calculations were made:

$$\text{Marinade uptake (\%)} = [(wt_2) - (wt_1)/(wt_1)] \times 100,$$

$$\text{Drip loss (\%)} = [(wt_2) - (wt_3)/(wt_2)] \times 100,$$

$$\text{Cooking loss (\%)} = [(wt_3) - (wt_4)/(wt_3)] \times 100, \text{ and}$$

$$\text{Yield (\%)} = (wt_4/wt_1) \times 100.$$

Expressible Moisture. Expressible moisture was measured with a TA.HDi Heavy Duty texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK) by a method described by Parks et al. (2000). Samples were cut into 1-cm cubes, and 2 sheets of 12.5-cm Whatman #1 filter papers were positioned on the top and bottom of the sample to absorb expressed moisture. A 12.5-cm diameter flat disc attachment was lowered onto the sample at a rate of 100 mm/min. A maximum load of 400 N was applied to the sample for 15 s. Samples typically reached a deformation of 88%. The sample weight before and after compression was recorded, and the expressible moisture was expressed as a percentage of the net weight difference from the initial weight.

A_w. The A_w was measured at a constant temperature (25 ± 1°C) by a water activity meter mod Aqualab (Decagon Devices Inc., Pullman, WA) that bases its measure on the chilled-mirror dewpoint technique. For each marination treatment, the A_w was detected on 3 samples before tumbling, after tumbling, and after cooking.

FW. The amount of FW was evaluated by a Pyris 6 DSC (Perkin Elmer Corp., Wellesley, MA) on 3 samples per group after tumbling and after cooking. The DSC was equipped with a low-temperature cooling unit Intacooler II (Perkin Elmer Corp.). Temperature calibration was performed with ion-exchanged distilled water (melting point 0.0°C), indium (melting point 156.60°C), and zinc (melting point 419.47°C). Heat flow was calibrated using the heat of fusion of indium ($\Delta h = 28.71$ J/g). For the calibration, the same heating rate used for sample measurements was applied, and a dry nitrogen gas flux of 20 mL/min was used. Each sample (about 20 mg) was weighed in a 50-μL aluminum pan with a small spatula, hermetically sealed, and then loaded onto the DSC instrument at room temperature, using an empty pan of the same type as a reference. Samples were then cooled at 5°C/min to −60°C, held for 1 h, and then scanned at 5°C/min to 20°C (Brake and Fennema, 1999). The FW was determined as follows:

$$FW = \frac{\Delta H_m}{\Delta H_w},$$

where ΔH_w (325 J/g) is the latent heat of melting per gram of pure water at 0°C (Roos, 1986), and ΔH_m (J/g) is the measured latent heat of melting of water per gram of sample obtained by the integration of the melting endothermic peak. The FW amount was expressed as grams per gram of fresh sample weight.

NMR Relaxation Measurements. The proton transverse relaxation (T_2) decays in breast meat after marination and after cooking were recorded at the operating frequency of 20 MHz with a Bruker (Milan, Italy) Minispec PC/20 spectrometer using a standard Carr-Purcell-Meiboon-Gill pulse sequence (Meiboom and Gill, 1958). A sample of about 600 mg of meat was placed inside a 10-mm (outer diameter) NMR tube, thus forming a small cylinder where the height did not exceed the active region of the radio frequency coil. Each measurement was comprised of 30,000 points with a τ-spacing (time between subsequent 180° pulses) of 80 μs and a relaxation delay of 3.5 s. All of the measurements were performed at a constant temperature of 24°C. The Carr-Purcell-Meiboon-Gill decays were

Table 1. The pH of marinades and the pH and color of broiler breast meat before and after marination and after cooking[1]

Item	C	S	P	B	SP	SB	SPB	SEM	*P*-value
Marinade solution pH	—	6.99	9.04	8.35	7.61	7.84	7.70	—	—
Before marination[2]									
pH	5.81	5.81	5.81	5.81	5.81	5.81	5.81	0.01	NS
Lightness (L*)	55.9	55.9	56.1	56.3	56.3	55.6	55.9	0.24	NS
Redness (a*)	1.77	1.86	1.99	2.18	1.98	2.05	1.86	0.07	NS
Yellowness (b*)	2.32	2.50	2.38	2.46	2.08	2.11	2.53	0.09	NS
After marination									
pH[3]	5.81^{c}	5.83^{c}	6.05^{b}	6.50^{a}	6.02^{b}	6.50^{a}	6.57^{a}	0.04	***
Lightness (L*)[2]	55.9^{a}	50.6^{c}	57.3^{a}	52.9^{b}	49.7^{cd}	48.3^{d}	48.2^{d}	0.36	***
Redness (a*)[2]	1.77^{a}	0.83^{d}	1.26^{bc}	1.82^{a}	1.23^{cd}	1.40^{abc}	1.68^{ab}	0.06	***
Yellowness (b*)[2]	2.32^{bc}	3.51^{a}	3.25^{a}	1.91^{c}	2.96^{ab}	1.64^{c}	-0.17^{d}	0.14	***
After cooking[3]									
pH	6.05^{d}	6.03^{d}	6.17^{c}	6.40^{b}	6.16^{c}	6.50^{a}	6.56^{a}	0.03	***
Lightness (L*)	85.7^{a}	85.1^{ab}	85.1^{ab}	84.7^{ab}	83.8^{bc}	82.8^{c}	81.4^{d}	0.23	***
Redness (a*)	2.09	1.52	2.18	2.16	2.09	1.99	1.70	0.07	NS
Yellowness (b*)	9.34^{b}	8.53^{c}	9.68^{ab}	10.41^{a}	8.29^{c}	8.13^{c}	8.14^{c}	0.14	***

[a–d]Means within a row followed by different superscript letters differ significantly ($P \leq 0.05$).

[1]C = control (nonmarinated); S = salt; P = phosphate; B = bicarbonate; SP = salt and phosphate; SB = salt and bicarbonate; SPB = salt, phosphate, and bicarbonate.

[2]For the data, n = 20.

[3]For the data, n = 10.

***$P \leq 0.001$.

normalized to the corresponding sample weight and transformed into relaxograms (i.e., continuous distributions of relaxation times) through the program UPEN (Borgia et al., 1998). Each relaxogram was interpreted in agreement with previous studies on pork (Bertram et al., 2002) and turkey (Bianchi et al., 2004).

Total Moisture. The moisture content of the cooked meat samples was determined by the procedures of AOAC (1990). Ground samples of about 5 g were dried in a conventional oven at 100 to 102°C for 16 h.

Statistical Analysis

Data were analyzed by one-way ANOVA testing the type of marination treatment (C, S, P, B, SP, SB, and SBP) as the main effect. When the effect was significant, the means were separated using Duncan's multiple range test. The calculations were performed on SAS software (SAS Institute, 1988).

RESULTS

Marinade solution pH, as well as meat pH and color, after tumbling and after cooking are shown in Table 1. The pH and color before marination were similar among groups, and this consistency was important because extreme meat pH and color have been shown to affect marination absorption and cooking yield (Qiao et al., 2002; Barbut et al., 2005). As expected, the pH of the marinade solution with S was close to neutrality, whereas P and B alone, or in combination, increased the pH (from 7.61 to 9.04). As a consequence, meat pH was increased by the alkaline marinades. Samples marinated with B alone or in combination (SB and SPB) significantly increased ($P < 0.05$) meat pH by approximately 0.7 units compared with that of the control, whereas P alone or in combination with salt (SP) increased ($P < 0.05$) the pH by 0.2 units. The pH after cooking evidenced a similar trend, even if absolute differences with respect to the control group were of a lower extent.

As for meat color, after marination, samples had a darker color when compared with that of the controls, with the exception of the P samples. Samples marinated with combinations of salts (SP, SB, and SPB) exhibited the darkest color. With regard to cooked meat color, marinated samples with ingredient combinations exhibited a darker color than that of the controls, whereas lightness from S, P, and B groups did not differ from that of the control. There appears to be some effect of marination treatments on redness and yellowness, but these effects are not always consistent or necessarily dramatic (may be of relatively little practical importance).

Marinade uptake, drip loss, expressible moisture, cooking loss, yield, and total moisture after cooking exhibited significant differences among treatments ($P < 0.001$; Table 2).

The percentage of marinade uptake observed in this study was lower than typical levels for industry, most likely because of the use of a laboratory rotary evaporator for tumbling. The most pronounced effect was seen in marinade combinations of B with S without or with P (SB and SPB) showing the highest marinade uptake values (10.2 and 11.6%, respectively). The SPB samples also exhibited the lowest drip loss (0.74%). The use of P or B alone resulted in the lowest marinade uptake, whereas samples marinated in S without or with P (S and SP) resulted in intermediate marinade uptakes. Groups P and B also exhibited a worse ability to retain liquid, as assessed by expressible moisture

Table 2. Marination and cooking performances of marinated broiler breast meat[1]

Item (%)	C	S	P	B	SP	SB	SPB	SEM	*P*-value
Marinade uptake[2]	—	7.1^{b}	5.5^{c}	5.3^{c}	8.3^{b}	10.2^{a}	11.6^{a}	0.31	***
Drip loss[2]	0.99^{b}	1.30^{a}	1.23^{ab}	1.33^{a}	1.31^{a}	1.23^{ab}	0.74^{c}	0.04	***
Expressible moisture[3]	15.2^{a}	12.3^{b}	16.5^{a}	15.3^{a}	11.5^{b}	9.3^{c}	11.9^{bc}	0.44	***
Cooking loss[3]	21.8^{b}	19.5^{c}	25.4^{a}	20.8^{bc}	15.0^{d}	10.3^{e}	9.2^{e}	0.70	***
Total moisture[3]	67.9^{d}	70.4^{bc}	69.9^{c}	71.3^{b}	71.3^{b}	72.8^{a}	73.1^{a}	0.22	***
Yield[3]	77.5^{f}	85.2^{d}	77.8^{f}	82.0^{e}	90.6^{c}	98.2^{b}	101.5^{a}	1.13	***

$^{a–f}$Means within a row followed by different superscript letters differ significantly ($P \leq 0.05$).

[1]C = control (nonmarinated); S = salt; P = phosphate; B = bicarbonate; SP = salt and phosphate; SB = salt and bicarbonate; SPB = salt, phosphate, and bicarbonate.

[2]For the data, n = 20/group.

[3]For the data, n = 10/group.

***$P \leq 0.001$.

when compared with samples marinated in S alone or in combinations with B or P (SP, SB, and SPB).

Cooking loss covered a large range, from 9.2 to 25.4%. Samples marinated in only P exhibited the highest losses. The lowest losses occurred when S and B irrespective of the presence of P (SB and SPB) were used, whereas the SP combination showed a reduced ability to retain liquid during cooking. The SPB and SB samples after cooking exhibited higher total moisture as a result of higher marinade uptake and lower cooking losses. Yields are also shown in Table 2. All marinated samples had a significantly higher yield than that of the control ($P < 0.05$), except for P alone, which did not differ significantly from the control. The combination containing all of the ingredients (SPB) produced the highest yield; however, the use of S and B (SB) obtained a very similar result.

Figure 1 shows a typical T_2 relaxogram of both raw and cooked meat obtained during the present investigation through LR-NMR. The proton pool with lower relaxation time, representing roughly 4% of the relaxogram's signal, was assigned according to Bertram et al. (2002) to water tightly associated to proteins and macromolecular constituents of meat (bound water, $T_2 < 20$ ms); the main population, with a T_2 between 20 and 60 ms, was assigned to myofibrillar water or water entrapped in the contractile protein reticulum. Finally, the population with a higher relaxation time was assigned to extramyofibrillar water or water physically located outside of the protein network ($T_2 > 60$ ms). According to the two-site chemical exchange model described by Hills (1998), a significant contribution to the 3 proton pools is given also by the biopolymers' protons chemically exchanging with the water located in the different sites. For this reason, from this section on, the 3 proton pools will be simply referred to as bound water, myofibrillar protons, and extramyofibrillar protons. Table 3 reports their T_2 and absolute values (% intensities). If no difference was observed in both the extramyofibrillar protons' intensity and T_2, water gain following marination with S (alone or in combination with the other studied compounds) generally increased the area of the peaks assigned to myofibrillar protons. Such an increase was accompanied by a movement toward a higher T_2. A comparison between marinated and cooked samples revealed that the major effect of cooking was the increase of the bound-water proton pool signal, which made the total signal higher in the cooked meat than in the marinated counterparts before cooking. This apparent contradiction was explained by considering that a part of the bound-water proton pool could not be observed through Carr-Purcell-Meiboon-Gill in the marinated samples because of the too-low T_2, whereas the same protons could be seen in the cooked meat (Venturi et al., 2007). Due to this artifact, the bound-water proton pool will no longer be considered in the remaining part of the present discussion. Finally, among marinated cooked samples, only SB and SPB groups exhibited higher T_2 than that of the control.

Meat A_w and FW after marination and after cooking, are shown in Table 4. The A_w of a fresh meat sample was 0.990, in agreement with previous data on raw meat (Chirife and Fontan, 1982), whereas the FW

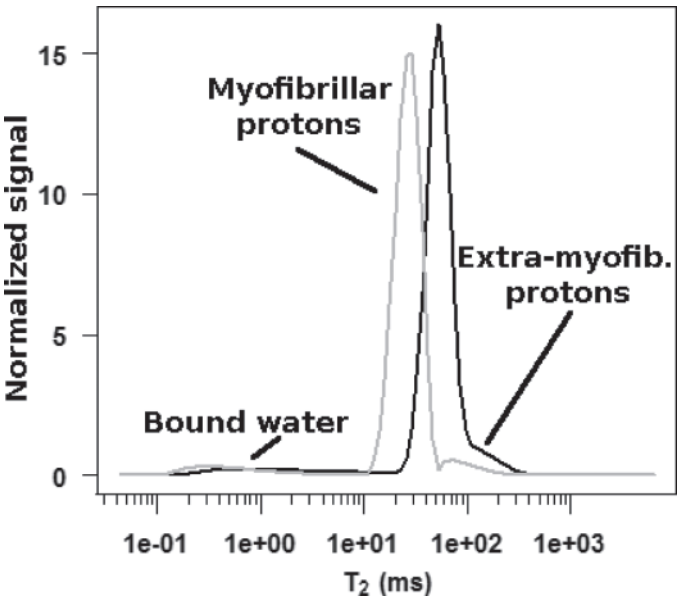

Figure 1. Two typical transverse relaxation time spectra (T_2) obtained on the raw (black line) and cooked (gray line) meat samples. To allow for a direct comparison among the treatments, the intensities are scaled so that the total area equals 100.

Table 3. Nuclear magnetic properties of broiler breast meat after marination and after cooking (n = 10/group)[1,2]

Item	Property	C	S	P	B	SP	SB	SPB	SEM	*P*-value
After marination										
Extramyofibrillar water	intensity (%)	3.7	4.0	3.7	3.2	4.4	3.9	3.7	0.09	NS
	T_2 (ms)	140.6	171.0	143.0	136.6	135.7	136.8	139.5	3.22	NS
Myofibrillar water	Intensity (%)	91.7^{bc}	93.2^{ab}	91.5^{c}	91.3^{c}	92.8^{abc}	93.3^{a}	94.4^{a}	0.24	***
	T_2 (ms)	42.3^{bc}	49.5^{a}	43.1^{bc}	41.9^{c}	49.9^{a}	45.8^{b}	50.0^{a}	0.66	***
Bound water	Intensity (%)	4.6^{a}	3.1^{b}	4.6^{a}	5.4^{a}	2.9^{b}	2.7^{b}	2.5^{b}	0.23	***
	T_2 (ms)	0.6	0.7	0.6	0.7	0.6	0.6	0.6	0.26	NS
After cooking										
Extramyofibrillar water	Intensity (%)	2.6	1.9	2.6	2.4	2.0	1.8	1.6	0.10	NS
	T_2 (ms)	83.7	107.6	105.1	116.4	112.4	105.4	122.8	4.44	NS
Myofibrillar water	Intensity (%)	91.7	91.6	93.4	93.1	93.1	93.1	93.9	0.33	NS
	T_2 (ms)	25.2^{c}	29.3^{bc}	24.9^{c}	28.7^{bc}	30.7^{bc}	33.3^{ab}	37.2^{a}	0.97	**
Bound water	Intensity (%)	5.4	6.7	4.7	5.5	5.6	5.5	5.5	0.30	NS
	T_2 (ms)	0.5	0.5	0.5	0.4	0.4	0.4	0.7	0.03	NS

[a–c]Means within a row followed by different superscript letters differ significantly ($P \leq 0.05$).

[1]To allow for a direct comparison among the treatments, the intensities are scaled so that the control samples' total area equals 100.

[2]C = control (nonmarinated); S = salt; P = phosphate; B = bicarbonate; SP = salt and phosphate; SB = salt and bicarbonate; SPB = salt, phosphate, and bicarbonate.

**$P \leq 0.01$; and ***$P \leq 0.001$.

was 0.612 g/g of fresh sample weight, which means that in raw meat, about 90% of the total water had enough mobility to freeze. Compared with the nonmarinated control samples, only samples treated with S or in combination with B and P (SP, SB, and SPB) evidenced significantly lower A_w values. Actually, S, SB, and SPB samples showed values of 0.985, 0.984, and 0.985, respectively, whereas the A_w of the SP sample was 0.981. No differences were observed in terms of FW. Nonmarinated cooked control samples evidenced a slight reduction in A_w, but only sample P showed a significantly different, higher A_w value compared with that of the control and SP, SB, and SPB treatments. The cooking process did not produce any significant differences in the FW amount.

DISCUSSION

As expected, the use of the alkaline marinades increased meat pH. However, when used at the same level (0.3%), B showed a greater ability to increase meat pH with respect to P (0.7 vs. 0.3 pH units, respectively). These results are in agreement with those reported by other authors for P and B (Alvarado and Sams, 2003; Sen et al., 2005). The different effects of P and B may be due to differences in buffering capacity and ionic strength. Moreover, it was also confirmed that S did not interfere with the alkaline effect of P and B. Differences in raw meat remained after cooking even if absolute differences with respect to the control group were of a lower extent.

The darkening effect of marination with combinations of salts (SP, SB, and SPB) in both raw and cooked meat is in agreement with previous findings (Alvarado and Sams, 2003; Sen et al., 2005), whereas Young and Lyon (1997) found no effect of salt and phosphate marinade on meat lightness. It is well-know that raw meat with high pH appears darker because its surface scatters less light than meat with low ultimate pH (Swatland, 2008). Differences in color related to pH were also found in cooked meat, in agreement with Trout (1989), who noticed that a high pH reduced heat denaturation of myoglobin during cooking, thus leading to increased darkness. Previously, Young and Lyon (1997) found that phosphates tend to decrease the redness of cooked breast meat. Because meat color is very important both for the selection of deboned and skinless raw meat as well as being critical for the final evaluation of many

Table 4. Water activity (A_w) and freezable water content (FW) of broiler breast meat after marination and after cooking (n = 3/group)[1]

Item	C	S	P	B	SP	SB	SPB	SEM	*P*-value
After marination									
A_w	0.990^{a}	0.985^{b}	0.989^{a}	0.989^{a}	0.981^{b}	0.984^{b}	0.985^{b}	0.001	***
FW (g·g fw^{-1})[2]	0.612	0.632	0.630	0.648	0.622	0.630	0.612	0.008	NS
After cooking									
A_w	0.986^{bc}	0.987^{ab}	0.990^{a}	0.983^{bc}	0.984^{bc}	0.982^{c}	0.983^{bc}	0.001	*
FW (g·g fw^{-1})	0.502	0.579	0.525	0.527	0.518	0.550	0.558	0.028	NS

[a–c]Means within a row followed by different superscript letters differ significantly ($P \leq 0.05$).

[1]C = control (nonmarinated); S = salt; P = phosphate; B = bicarbonate; SP = salt and phosphate; SB = salt and bicarbonate; SPB = salt, phosphate, and bicarbonate.

[2]fw = fresh sample weight.

*$P \leq 0.05$; and ***$P \leq 0.001$.

cooked products, the overall effect of B on product appearance should be properly modulated.

The results confirmed a significant improvement of S (1%) over the control in terms of water-holding ability and yield (Barbut, 2002). The use of 0.3% P did not determine an improvement of yield with respect to the nonmarinated controls due to the scarce ability to hold liquid during cooking. This result agrees with Xiong et al. (2000) who stated that phosphates have only a relatively small effect on ionic strength when used alone, whereas a strong synergistic effect was noted when used together sodium chloride. In contrast, the use of the same concentration of B allowed a higher yield to be obtained when compared with the control and P groups but lower than samples marinated with S. The performances of B, higher than those of P, can be related to its higher alkaline effect. Likewise, the combining of 2 or more ingredients resulted in a higher yield than that of each ingredient used alone. The combination of P and S acted synergistically in reducing liquid losses during cooking. This was in agreement with previous studies showing that through the addition of salt (around 1%) together with phosphates to a meat product, proteins can immobilize high levels of added water (Young and Lyon, 1997; Xiong and Kupski, 1999a). Moreover, Xiong and Kupski (1999b) indicated that salt was able to reduce phosphate functionality when high concentrations were employed. The combination of S and B evidenced a higher ability to improve water uptake during tumbling and to minimize cooking losses. As introduced before, this effect must be mainly related to its alkalinisation effect that moved the meat pH away from the isoelectric point of myofibrillar proteins and increased the net negative charge. This leads to muscle fiber expansion (swelling) caused by electrostatic repulsion that allows more water to be immobilized in the myofibrillar lattice (Offer and Knight, 1988). Otherwise, the effect of P is mainly due to their capabilities to complex protein-bound Mg and Ca, which results in the increased solubilization of myosin and actin (actomyosin dissociation and depolymerisation of thick and thin filaments; Xiong, 2004).

Modifications on water distribution and mobility can be more deeply explained by considering LR-NMR outcomes. Indeed, the data obtained through LR-NMR can be rationalized according to the two-site chemical exchange model proposed by Hills (1998). At 20 MHz, the T_2 shorter than that of pure water (≈1500 ms) mainly reflects the proton exchange between water and biopolymers (with a typical T_2 of milliseconds). The T_2 of the protons pertaining to a certain compartment containing water can thus decrease when 1) the biopolymers:water ratio increases, and 2) the pH drifts away from the isoelectric point of the biopolymers, thus increasing the number of exchangeable protons.

When the S marinade solution was used, it determined a significant water gain without affecting the pH, thus increasing the myofibrillar water peak and shifting it toward higher T_2 values, which corresponds to a higher mobility of the water within the meat structure due to higher water content caused by marinating, as discussed by Bertram et al. (2008). However, when bicarbonate was involved, the water increase was accompanied by a pH shift from the isoelectric point, leading to an increased number of exchangeable protein sites. Finally, when the cooking treatment lowered the water content of a compartment, the corresponding peak lowered and shifted toward lower T_2 (Figure 1). Results obtained in the present study showed that water entering the meat structure along marination mainly reached the myofibrillar network, as can be seen from both myofibrillar water peak intensity and T_2. In no cases did cooking dry this compartment to the original level. Sodium chloride, P, and B showed a similar performance in this respect, whereas the combined uses led to higher myofibrillar water in the cooked product.

Overall, the parameters related to water mobility through the DSC technique showed no significant modifications for all of the considered marinating treatments. According to recent findings (Pearce et al., 2011), water in meat is structurally arranged in layers around polar molecules and between layers of cellular materials. About 5% of the water contained in muscle tissue exists as true hydration water that is bound to proteins by macromelecular of multimolecular adsorption. This water is not free; it has an ice-like structure (liquid crystal), is unfreezable, is unaffected by charges on the muscle protein (pH), and is unavailable to participate in reactions. In this study, FW outcomes evidenced that in the control samples, the unfreezable water content was about 10% of the total amount and its changes caused by marination were not significant. As recently reviewed by Pearce et al. (2011), NMR transverse relaxometry permit us to better understand water distribution kinetics in the different parts of meat microstructures. The bound-water amount obtained by the evaluation of the T_2 peak intensity for fresh meat was about 4% of the total signal, showing a substantial agreement with previous findings on the amount of true hydration water in fresh meat.

The state and mobility of water evidenced by LR-NMR was observed with difficulty through the A_w. Indeed, the effect of marination on A_w was significant only for raw samples treated with S, alone or in combination with the other marinade ingredients. These treatments (S, SP, SB, and SPB) showed lower values (ranging between 0.981 and 0.985) of A_w compared with that of the control. They also showed significantly lower values of expressible moisture. According to Barbut (2002), expressible moisture is mainly represented by the water in the extracellular spaces, which is likely the water fraction more correlated with A_w. According to these findings, it seems that the total moisture increase of enhanced samples, mainly dependent on myofibril swelling, does not correspond to an increase in A_w and FW, even promoting their decrease in some cases. The concomitant increases of solute concentrations with humectant action, protein solubilization

(that increase their capacity to bind water), as well as electrostatic interactions between actin and myosin and marinade ions (Xiong, 2004) can be the main causes for the detected A_w decrease.

In conclusion, this study showed that the use of P and B together with S resulted in higher marinade performances and cook yields than when the ingredients were used alone. However, the use of sodium bicarbonate in conjunction with S can allow a better marinade uptake to be obtained and water retention ability even superior to that of using P. According to LR-NMR, the combined use of B or P with S determined a remarkable increase in the proportion of entrapped water into the myofibrillar spaces, while the extramyofibrillar water fraction was not modified. Moreover, water gain following marination does not correspond to an increase in the FW amount, as detected by DSC. Based on these results, B is a very promising marinating agent, and it can be exploited to develop processed poultry products with no added phosphates to match the request to avoid the nutritional drawbacks recently indicated with the use of phosphates. However, pH increasing and darkening effects related to B use should be further investigated to properly modulate possible negative outcomes on product shelf life and appearance, respectively; also, the effect on sensory acceptability needs to be quantified.

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