THE EFFECT OF ORGANIC ACID MARINATION ON TENDERNESS, COOKING LOSS AND BOUND WATER CONTENT OF BEEF

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ABSTRACT

Longissimus *muscle obtained from beef carcasses was used in this research. Initially, 0.5%, 1.0% and 1.5% lactic and citric acid solutions were prepared. Meat was marinated in these solutions (1:4 w/v) in polyethylene bags at 4C for 72h. Bound water, pH, weight gain, cooking loss and Warner Bratzler shear (WBS) were evaluated. Differential Scanning Calorimetry (DSC) was used to determine the bound water content in meat samples. The latent heat of melting* (ΔH_m) *and bound water were found to be functions of moisture content of marinated meat. There was a significant decrease in pH due to marination. Compared to lactic acid, the samples marinated with citric acid held less water. The WBS values in control samples were higher than in marinated samples. Cooking loss was lower in samples marinated with lactic acid compared to citric acid marinated samples.*

INTRODUCTION

Meat tenderness is one of the main attributes of meat quality. Currently, marination is widely used by consumers to improve meat tenderness and flavor (Whipple and Koohmaraie 1993). Being a mechanical property, tenderness is related to final pH, postmortem temperature, sarcomere length, connective tissue content and enzymatic proteolysis of myofibrillar proteins (particularly troponin and desmin) (Yu and Lee 1986; Perez *et al.* 1998). Desirable meat tenderness is obtained when carcasses are chilled slowly, and then allowed to hang, which, for beef, may be two weeks or more, at chill temperatures. Although condition-

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ing improves beef quality, the cost of holding carcasses for an extended period at chill temperatures can be very high.

Processors are seeking ways of reducing the conditioning time without forfeiting quality. Artificial tenderization by acid marination, the soaking of meat in acid solution, is a commonly used culinary technique. The mechanism of muscle tissue tenderization in weak solutions of organic acids is still not thoroughly understood. A limited number of studies have been conducted on muscle tissue. Marination affects tenderness in 3 ways, potentially: (1) pH-induced swelling of muscle fibers and/or connective tissue; (2) accelerated or additional proteolytic weakening of muscle structure, and (3) increased solubilization of collagen upon cooking (Offer and Trinick 1983; Offer and Knight 1988; Ertbjerg *et al.* 1999). Numerous studies have shown that the operational component involved in marination affecting pH and tenderness is the concentration of the acid. Howat *et al.* (1983) reported that meat tenderness was not increased by marination in weak acid (pH 2.56). However, Wenham and Locker (1976) and Gault (1985, 1991) showed that tenderness of meats marinated with acid solutions (pH 2.58 to 3.17) increased while Seuss and Martin (1993) showed that meat became somewhat more tender as acid concentration increased at pH values between 1.8 and 3.0. The tenderization observed at pH values below 5.0 was believed to be caused mainly by the effects of acidic pH on the water-holding capacity of muscle proteins. Oreskovich *et al.* (1992) marinated meat with 0.1M phosphate buffers ranging in pH from 3.25 to 10.15, 0.1M sodium chloride, 0.4M phosphoric acid or 0.7M acetic acid. They reported that low muscle pH after marination had positive effects on texture and resulted in increased water-binding capacity, moisture content and decreased cooking losses. Many physical properties of meat (including color, texture, firmness of raw meat, juiciness and tenderness of cooked meat) are partially dependent on water holding capacity (Judge *et al.* 1989). It is generally accepted that high-quality meat should contain a high percentage of bound water. But, until recently, there has been little or no information available in the literature regarding the effects of marination on the free and bound water content of beef.

The present study was carried out to determine the influence of varying concentrations of lactic and citric acids on the weight gain, cooking loss, WBS and bound water content of beef.

MATERIAL AND METHODS

Materials

Meat samples were obtained from the *Longissimus dorsi* muscle of four year old (279.6 ± 7.3 kg) Eastern Anatolian Red beef carcasses from a major

slaughter-house (Et ve Balık Kurumu Kombinası, Erzurum, Turkey). After slaughter, carcasses were chilled for 24 h in a cooling room (4C). Following chilling, all trimmable fat and connective tissue were removed.

Marination of Meats with Lactic and Citric Acid

Steaks were cut (2.5-cm-thick) from the *Longissimus dorsi* muscle between the 12th rib and the 5th lumbar vertebrae of carcasses. Each steak was weighed and placed in a polyethylene plastic bag (15 cm × 30 cm, Sedat Tahir Ltd., Ankara, Turkey). Six marination solutions were prepared (lactic acid [Merck]: 0.5% [pH 2.73], 1.0% [pH 2.50], and 1.5% [pH 2.40]; citric acid [Sigma]: 0.5% [pH 2.67], 1.0% [pH 2.52], and 1.5% [pH 2.40]) and chilled to 4C. Marinating solution was added (1:4 w/v; meat: liquid) into the plastic bags. Samples were marinated for 72 h at 4C.

pH and Moisture Analysis

The pH was evaluated on a 10 g ground sample homogenate in 100 mL of distilled water before and after marination and after cooking. Moisture percentages were determined on the raw materials, cooked and uncooked steaks from each treatment. Moisture determinations were performed on ground 10 g samples after drying for 24 h in a 105C oven.

Weight Gain and Cooking Loss

Meat was removed from marination bags, blotted lightly with paper towels to remove excess surface moisture, and weighed. Marination solution weight gain was calculated as:

$$\% \text{ weight gain} = \left(\frac{\text{Initial wt} - \text{Blotted, marinated wt}}{\text{Initial wt}}\right) \times 100$$

Blotted samples were cooked in cooking bags (Cook Roasting Bags, 25 cm × 38 cm, Sedat Tahir Ltd., Ankara, Turkey) for 30 min at 200C in an oven (Dold, Fellbach-Stuttgart, Germany) to an internal temperature of 70C. Temperature was monitored using constantan-copper thermocouple wires inserted into the geometric center of the steak and attached to a Tri-Sense multipoint recorder (Tri-Sense Kit, Model 37000-90, Cole-Parmer Instrument Co., Chicago, Ill.). Cooked steaks were cooled to room temperature, blotted dry, and reweighed. Cooking loss was calculated as:

$$\% \text{ cooking loss} = \left(\frac{\text{Blotted, marinated wt - Chilled, Blotted, cooked wt}}{\text{Blotted, marinated wt}}\right) \times 100$$

Warner Bratzler Shear Force

Tenderness was determined with a Warner-Bratzler shear (Model 5KH29GK88, GR Electric Manufacturing, Manhattan, Kansas) on cooked samples, and is expressed as kg required to shear a 2.54 cm sample.

Bound Water by Differential Scanning Calorimetry

The freezable water content of meat samples was determined using a DSC-50 (Shimadzu Corp., Kyoto, Japan). Approximately 10 mg samples were weighed into aluminum hermetic cells (Shimadzu 201-53090) and sealed with a crimper. A sealed empty cell was used as a reference. Samples were preheated to 30C, then cooled to -80C and heated to 40C using a temperature change rate of 5C/min. Liquid nitrogen was used to cool cells at a flow rate of 30 mL/min (99.9% N_2) to avoid condensation. For temperature calibration, Indium (Mettler standard, mp: 156.6C), and water (mp: 0C deionized, distilled) were used. The heat flow was calibrated using the heat of fusion of Indium (28.45 J/g). The most common method for calculation of unfreezable water is through use of latent heat of melting (ΔH_m) (Ross 1978; Roos 1986). The melting curve was integrated to determine the latent heat of melting (ΔH_m). ΔH_m was divided by the value by that of water (333 J/g). The unfreezable water content was calculated as:

$$\% \text{ Unfreezable water content} = \left(\text{Total water content} - \frac{\Delta H_m \text{ of sample}}{\Delta H_m \text{ of water}}\right) \times 100$$

Statistical Analysis

This experiment was conducted as a completely randomized block design using three replicates. Analysis of variance of all data was conducted using the General Linear Model (GLM) procedure (SAS 1999). Means which were significantly different were separated using Duncan's multiple range.

RESULTS AND DISCUSSION

pH and Moisture

Moisture content and pH values of the meat are shown in Table 1. Values in control samples were close to the values of fresh red meat. Significant differences were found for pH and moisture contents at the end of the

marination and marination/cooking procedures (Table 1). Acid type and concentration had significant effects on pH values and moisture content (Table 2). Acid type × acid concentration (Fig. 1) and marination treatment × acid concentration (Fig. 2) interactions had significant effects on pH. The pH is an important criteria for changes brought about by soaking in food acids. Compared to citric acid, samples marinated with lactic acid had lower pH values (Fig. 1). Gault (1991) and Seuss and Martin (1993) made similar observations during investigations with various organic acids. To reach a pH of 4.0, only 15 g tartrate and 18 g lactate were needed per kg meat, whereas 28 g citrate and 48 g acetate were necessary. One reason for this difference in behavior is the different pH values of the individual acids. This may result in stronger acidification of the solutions and subsequently, of the muscles themselves, if it is assumed that a higher concentration of hydrogen ions is an important factor.

TABLE 1.
MOISTURE CONTENT AND pH VALUES OF MARINATED BEEF

			pH	Moisture (%)
		Concentration (%)		
After marination	Lactic acid	0	5.72 [a] ± 0.06	74.13 [d] ± 0.07
		0.5	3.98 [b] ± 0.09	85.90 [c] ± 0.07
		1.0	3.67 [c] ± 0.07	87.45 [b] ± 0.04
		1.5	3.49 [d] ± 0.05	88.81 [a] ± 0.05
	Citric acid	0	5.72 [a] ± 0.06	74.13 [d] ± 0.07
		0.5	4.28 [b] ± 0.08	82.35 [c] ± 0.05
		1.0	3.83 [c] ± 0.04	84.42 [b] ± 0.04
		1.5	3.70 [d] ± 0.06	85.07 [a] ± 0.07
After marination/cooking	Lactic acid	0	5.93 [a] ± 0.07	62.80 [d] ± 0.04
		0.5	4.24 [b] ± 0.07	79.17 [c] ±0.05
		1.0	3.83 [c] ± 0.06	83.92 [b] ± 0.07
		1.5	3.68 [d] ± 0.07	85.68 [a] ± 0.09
	Citric acid	0	5.93 [a] ± 0.07	62.80 [d] ± 0.04
		0.5	4.42 [b] ±0.05	68.61 [c] ±0.05
		1.0	3.90 [c] ±0.06	79.40 [b] ±0.06
		1.5	3.72 [d] ±0.07	81.80 [a] ±0.04

±: Standard deviation of three replicates

[a-d] Values in a column with the same superscript are not significantly different ($P<0.05$)

TABLE 2.
EFFECTS OF ACID, TREATMENT AND CONCENTRATION ON pH AND MOISTURE CONTENT

	Total moisture (%)	pH
Acid		
Lactic acid	79.03 [a] ± 5.33	4.72 [b] ± 0.56
Citric acid	76.58 [b] ± 4.36	4.80 [a] ± .052
Treatment		
Control	75.12 [c] ± 0.13	5.67 [a] ± 0.05
Marination	82.78 [a] ± 1.53	4.17 [c] ± 0.10
Marination/ Cooking	75.51 [b] ± 2.82	4.45 [b] ± 0.04
Concentration (%)		
Control	70.68 [d] ± 0.21	5.60 [a] ± 0.06
0.5	77.71 [c] ± 0.23	4.71 [b] ± 0.10
1.0	80.88 [b] ± 0.15	4.43 [c] ± 0.12
1.5	81.93 [a] ± 0.17	4.32 [d] ± 0.20

±: Standard deviation for three replicates
[a-d] Values in a column with the same superscript are not significantly different ($P<0.05$)

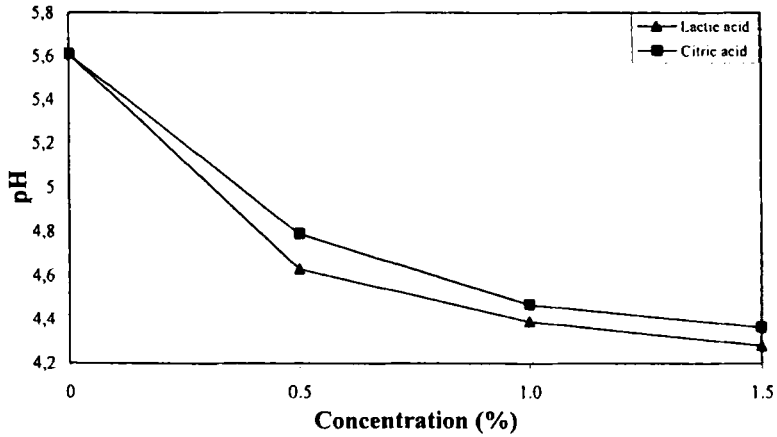

FIG. 1. ACID TYPE × CONCENTRATION INTERACTION EFFECTS ON pH VALUES

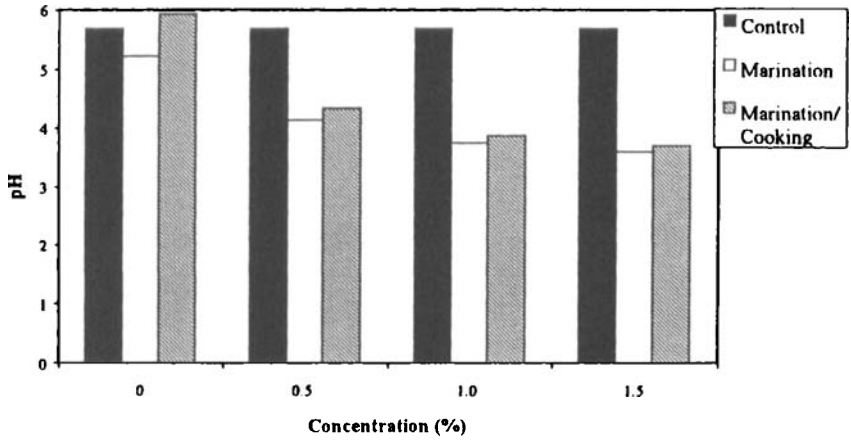

FIG. 2. THE EFFECT OF TREATMENT × CONCENTRATION INTERACTION ON pH VALUES

Even though pKa_1 of citric acid (3.06) is lower than that of lactic acid (3.86), citric acid can act like a buffer when it binds to the meat surface because it is a weak organic acid with three pKa values (pKa_1: 3.06, pKa_2: 4.74, pKa_3: 5.40) and has other available carboxylic groups. Citric acid cannot induce dramatic decreases in meat pH because of its buffering behavior and because of meat buffering capacity. Since lactic acid has one pKa value, there is no more buffering effect from a second or third pKa as in the case of citric acid. Thus, lactic acid can dramatically decrease meat pH. After cooking, the pH of meat increased. The increase in pH value of cooked meat is probably caused by a reduction in available carboxylic groups on proteins and also through the liberation of calcium and magnesium ions from proteins, as proposed by Medynski *et al.* (2000).

Weight Gain and Cooking Loss

Weight gain and cooking losses are shown in Table 3. The type and concentration of acid had significant effects on weight gain and cooking loss (Table 4). Acid type × acid concentration interaction affected weight gain and cooking losses ($P < 0.05$, Fig. 3). As can be seen in Table 3, the lower pH induced a higher moisture uptake. The water binding of proteins is better below (or above) the isoelectric point (approximately pH 5.2). Therefore, meat marinated in lactic acid absorbed much more moisture during marination; the total loss was also less (Table 4). Marination should make the meat remain juicy. Seuss and Martin (1993) pointed out that the low degree of hydration of

the myofibrillar proteins at their isoelectric point is responsible for a higher moisture loss during cooking. The lowest cooking loss was observed in samples marinated in the highest concentration of lactic and citric acids. This can be attributed to the strong effect of lower pH on proteins, as indicated by results reported by Rao *et al.* (1989) and Rao and Gault (1989). These groups reported that the influence of acids on the tissue depended on the type of fibers in the meat as well as on final acidification. Low acidification favors collagen swelling which surrounds bundles of muscle fibers (perimysium) as well as single fibers (endomysium).

TABLE 3.
WEIGHT GAIN, COOKING LOSSES, AND WBS VALUES IN BEEF MARINATED WITH ACIDS

	Concentration (%)	WBS (kg)	Weight gain (%)	Cooking loss (%)
Lactic acid	0	$15.31^{a} \pm 1.12$	$2.20^{d} \pm 0.60$	$40.08^{a} \pm 5.22$
	0.5	$9.75^{ab} \pm 3.52$	$50.69^{c} \pm 6.51$	$36.54^{a} \pm 2.86$
	1.0	$4.08^{bc} \pm 1.09$	$78.79^{b} \pm 8.47$	$24.45^{b} \pm 3.23$
	1.5	$3.51^{c} \pm 1.89$	$98.78^{a} \pm 11.56$	$18.06^{c} \pm 4.76$
Citric acid	0	$15.31^{a} \pm 1.12$	$2.20^{a} \pm 0.60$	$40.08^{b} \pm 5.22$
	0.5	$12.51^{b} \pm 0.58$	$27.68^{c} \pm 2.20$	$43.88^{a} \pm 2.38$
	1.0	$4.30^{d} \pm 0.63$	$55.03^{b} \pm 8.46$	$32.16^{c} \pm 3.28$
	1.5	$7.21^{c} \pm 1.35$	$67.02^{a} \pm 7.33$	$28.14^{d} \pm 3.85$

±: Standard deviation for three replicates
a-d Values in a column with the same superscript are not significantly different ($P<0.05$)

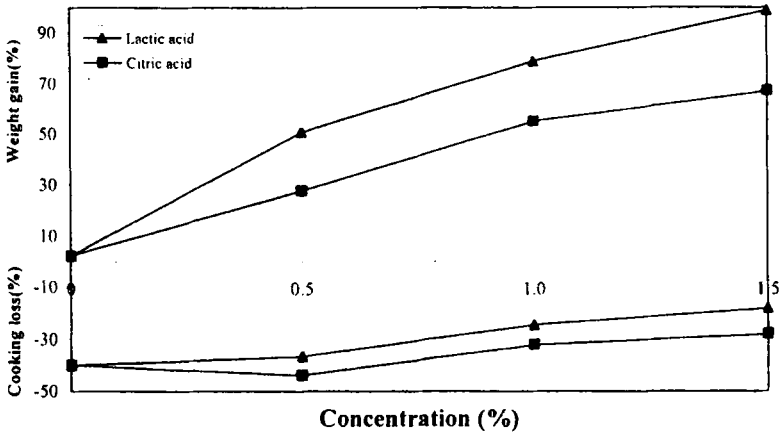

FIG. 3. ACID TYPE × CONCENTRATION INTERACTION EFFECTS ON WEIGHT GAIN AND COOKING LOSS VALUES

TABLE 4.
ACID TYPE AND CONCENTRATION EFFECTS ON WBS, WEIGHT GAIN AND COOKING LOSS OF MARINATED BEEF

	WBS (kg)	Weight gain (%)	Cooking loss (%)
Acid			
Lactic acid	8.16 [a] ± 1.90	57.61 [a] ± 6.78	29.78 [b] ± 4.01
Citric acid	9.83 [a] ± 0.92	37.98 [b] ± 4.62	36.06 [a] ± 3.67
Concentration (%)			
0	15.31 [a] ± 1.12	2.20 [d] ± 0.60	40.08 [a] ± 5.21
0.5	11.13 [b] ± 2.05	39.18 [c] ± 4.30	40.21 [a] ± 2.62
1.0	4.19 [c] ± 0.86	66.91 [b] ± 8.46	28.30 [b] ± 3.25
1.5	5.36 [c] ± 1.61	82.90 [a] ± 9.45	23.09 [c] ± 4.30

±: Standard deviation for three replicates
[a-d] Values in a column with the same superscript are not significantly different ($P<0.05$)

Using the electrostatic or osmotic theory of muscle swelling may explain these relationships. Addition of acid to the muscle results in the protonation of carboxyl groups. This idea is common to both theories. According to the electrostatic theory, the addition of acid below the isoelectric point of meat proteins gradually protonates negatively charged carboxyl groups, which should lead to the breaking of some electrostatic bonds with the adjacent protein chains. The increase in the net positive charge is thought to result in repulsion between protein groups of similar charge, thereby creating space for immobilization of added water.

The osmotic theory proposes another explanation. The anions of the acids form ionized salts with available cationic groups on the protein molecules resulting in an unequal distribution of free ions between the proteins and the surrounding acid solution. A consequence is a difference in osmotic pressure. The flow of water into the protein structure results in a reduction of this difference and the system swells (Medynski *et al.* 2000).

Warner Bratzler Shear Force

Acid marinated muscles had lower WBS values (more tender) than untreated muscles (Table 3). The WBS values were affected by acid type and concentration (Table 4). Acid type × acid concentration interaction had a significant effect on WBS values (Fig. 4). Lactic acid marination resulted in the most tender muscle. Measurement of the WBS on heat-treated meat that had been marinated for 72 h showed that it became somewhat more tender as acid concentration of the marinade increased (Fig. 4). Several authors have reported improved sensory tenderness in association with a decrease in shear force values

in beef due to marination with food acids (Wenham and Locker 1976; Gault 1985, 1991; Seuss and Martin 1993). This effect may be due to some solubilization of the collagenous tissue. Stanton and Light (1990) and Eilers *et al.* (1994) reported that degradation of connective tissue increased with lactic acid injection. Aktaş and Kaya (2001) reported that α and β components of the intramuscular connective tissue marinated with lactic and citric acids were clearly seen on SDS-PAGE, especially when lactic acid was used. The increased tenderness is not attributed solely to change in pH value; the texture of meat is also influenced by the amount of immobilized water in it. Further lowering of meat pH value caused increased tenderness, which may be associated with the enhanced water holding capacity.

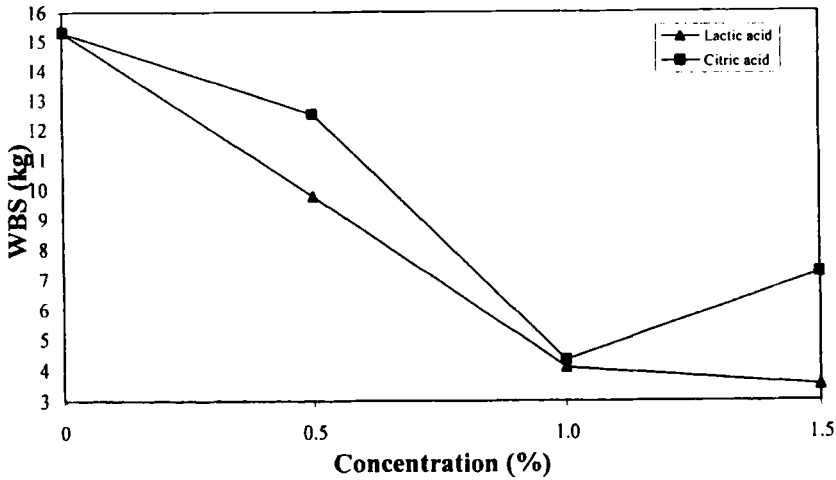

FIG. 4. ACID TYPE × CONCENTRATION INTERACTION EFFECTS ON WBS VALUES

Bound Water by Differential Scanning Calorimetry

The ΔH_m and unfreezable water content of samples are shown in Table 5. The highest total water content was 88.81%, and the lowest was 62.80% (Table 1). An increase in the total water content of a sample resulted in an increase in the freezable (free) water content and a decrease in the bound (unfrozen) water content (Table 5). Similar results have been reported by Riedel (1957), Chen (1985), and Pham (1987) for lean beef and for cod fish. The fact that an increase in total water content is accompanied by an increase in freezable water content and a decrease in bound water content may be due to a decrease in the ratio of the water binding components of the samples. Pham (1987) postulated

that a decrease in solid matter content of a sample results in a decrease in the water binding protein molecules, which in turn causes a decrease in the bound water content of a given food material. The ΔH_m determined from the peak area under the curve was 78.29-267.81 J/g. These results (Table 5) indicate that the heat of fusion depends chiefly on the dry matter fraction; it decreased as the dry matter fraction increased, in products of the same type. Most of the energy changes were related to changes in the state of water. This can be explained by the assumption that water in meat uncovers binding sites that might not be accessible at lower moisture content.

TABLE 5.
UNFROZEN WATER AND LATENT HEAT (ΔH_m) VALUES OF MARINATED AND MARINATED/COOKED BEEF SAMPLES

		After marination		After marination/cooking	
	Concentration (%)	Unfrozen water (%)	Latent heat (ΔH_m: J/g)	Unfrozen water (%)	Latent heat (ΔH_m: J/g)
Lactic acid	0	27.05 ^{a} ± 4.39	157.60 ^{b} ± 3.61	39.29 ^{a} ± 0.66	78.29 ^{b} ± 2.21
	0.5	11.43 ^{b} ± 0.55	247.99 ^{a} ± 1.83	11.51 ^{b} ± 7.92	217.71 ^{a} ± .6.32
	1.0	7.02 ^{b} ± 4.45	267.81 ^{a} ± 4.77	16.59 ^{b} ± 3.73	224.22 ^{a} ± 2.40
	1.5	9.14 ^{b} ± 4.53	265.32 ^{a} ± 5.09	15.95 ^{b} ± 0.89	209.38 ^{a} ± 2.98
Citric acid	0	27.05 ^{a} ± 4.39	157.60 ^{b} ± 3.61	39.29 ^{a} ± 0.66	78.29 ^{b} ± 2.21
	0.5	11.72 ^{b} ± 5.38	235.20 ^{a} ± 7.92	9.53 ^{c} ± 7.04	197.27 ^{a} ± 3.00
	1.0	10.91 ^{b} ± 4.42	244.79 ^{a} ± 4.70	21.15 ^{b} ± 0.79	193.62 ^{a} ± 2.69
	1.5	7.85 ^{b} ± 4.46	257.14 ^{a} ± 4.85	19.97 ^{b} ± 5.89	205.89 ^{a} ± 5.63

±: Standard deviation for three replicates
$^{a-d}$ Values in a column with the same superscript are not significantly different ($P<0.05$)

Acid type and concentration, and marination treatment had significant effects on ΔH_m and bound water (Table 6). After marination with both acids, moisture uptake increased. However, there was a decrease in bound water content due to marination with both acids as compared to the control (Table 6). The cause of the bound water decrease is thought to be denaturation of the protein constituents during marination. At pH values below the isoelectric point, the protein carries a positive electric charge and water molecules may interact with these charges, thus contributing to solubilization. Moreover, protein chains carrying the same electrical charge have a tendency to repel each other and to dissociate or unfold (Fennema 1985). This unfolding of the molecules may have caused an increase in the ionic strength by strong electrostatic repulsion by ionized groups inside the molecule and the separation of water from the surface of the myofibrillar proteins, or dehydration of the myofibrillar proteins. This

may produce desirable juiciness (free moisture) provided the free water is not expelled from the tissue.

TABLE 6.
ACID TYPE AND CONCENTRATION, AND TREATMENT EFFECTS ON LATENT HEAT (ΔH_m) AND BOUND WATER OF BEEF

	Latent heat (ΔH_m : J/g)	Bound water (%)
Acid		
Lactic acid	$202.84^{a} \pm 10.44$	$18.13^{a} \pm 7.45$
Citric acid	$192.73^{b} \pm 9.89$	$18.17^{a} \pm 7.85$
Treatment		
Control	$185.74^{b} \pm 2.12$	$19.34^{a} \pm 6.64$
Marination	$229.18^{a} \pm 4.79$	$14.02^{b} \pm 4.10$
Marination/ Cooking	$178.43^{b} \pm 6.21$	$21.91^{a} \pm 3.97$
Concentration (%)		
Control	$140.54^{b} \pm 3.23$	$28.56^{a} \pm 4.07$
0.5	$211.61^{a} \pm 9.48$	$14.14^{b} \pm 5.87$
1.0	$216.98^{a} \pm 5.40$	$15.72^{b} \pm 4.62$
1.5	$221.99^{a} \pm 6.72$	$15.26^{b} \pm 4.95$

±: Standard deviation for three replicates
[a-d] Values in a column with the same superscript are not significantly different ($P<0.05$)

CONCLUSIONS

Low muscle pH induced by marination resulted in increased binding of water. The percentage of bound water decreased when muscle pH was lower than that of normal muscle. The higher pH muscle had lower WBS values and was more tender than control samples. These results suggest that substantial changes in meat tenderness can be achieved by altering the pH of the meat. The tenderization mechanism is likely to involve pH-induced swelling of the muscle structure.

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