ABSTRACT  The objective of this study was to evaluate effects of marinades containing varying calcium concentrations on the biochemical and texture characteristics of peri-rigor chicken breast fillets. Breast muscles from 200 broiler chickens were excised immediately post-mortem and marinated in 0, 50, 100, 150, or 200 mM CaCl₂. The treatments had no effect on meat pH either before or after cooking, but as calcium concentration increased, the normal post-mortem conversion of adenosine triphosphate (ATP) to inosine monophosphate (IMP) increased, according to the IMP:ATP ratios (R-values). Calcium treatment at all levels tested improved meat tenderness, but both marinade absorption and cooking losses increased as the calcium concentration in the marinades increased. It was concluded that although treating peri-rigor breast muscle with calcium might be useful in reducing or eliminating the conditioning period to assure tender chicken, methods must be developed for restoring the moisture binding properties that are damaged by the calcium.

(Key words: calcium, marination, tenderness, moisture binding, chicken meat)

INTRODUCTION

For many years, poultry was sold in the U.S. primarily as whole, ready-to-cook birds; but beginning in the 1950s, changes occurred in the marketplace that resulted in birds being sold as parts or deboned products. As the practice of cutting carcasses moved from the retail meat market to the processing plant, it became clear that a 6 to 8 h post-mortem aging period prior to cutting up was necessary to prevent toughening (de Fremery and Pool, 1963; Klose, et al., 1972; Lyon and Wilson, 1986). Because nearly 90% of the poultry is sold in the U.S. today as parts or as further processed products (W. P. Roenigk, National Broiler Council, 1155 15th St. N.W., Washington, DC 20005, personal communication), the practice of aging front halves or whole carcasses has become an important cost consideration. Numerous studies have been conducted to better understand the post-mortem toughening and tenderizing process and to develop methods for eliminating the aging practice for both poultry muscle and for muscle of other species (Lyon and Wilson, 1986; Lyon and Lyon, 1991; Sams and Janky, 1991; Papinaho and Fletcher, 1995; Papinaho et al., 1995). These studies have led to an improved understanding of the rigor process that begins shortly after the death of an animal. At that time, glycolytic activity results in the depletion of adenosine triphosphate (ATP) and accumulation of lactate in the sarcoplasm. Consequently, the sarcoplasmic reticulum loses its ability to control cytosolic calcium concentration, resulting in up to a 10-fold Ca²⁺ concentration increase (Goll et al., 1983). This increase activates myosin adenosine triphosphatase, resulting in contraction of the muscle (rigor mortis).

Because rigor develops much faster in avian than in mammalian muscle (Sams and Janky, 1991), broiler carcasses are usually in the early stages of rigor when they emerge from the chiller and the muscles are rigid, firm, and inextensible. However, within 6 to 8 h, the muscles become less rigid and regain some of their extensibility (resolution of rigor). Resolution remains an incompletely understood phenomenon, but evidence indicates that certain structures—most notably the Z-disks and elements of the cytoskeleton—in the myofibril undergo degradation (Sayer, 1970; Henderson et al., 1970; Penny, 1980; Nelson and Traub, 1983; Ouali et al., 1983; Ouali, 1984; Koohmaraie et al., 1988b; Whipple and Koohmaraie, 1991).

There are two main theories than explain the tenderization process. The first suggests that the increased ionic strength of the sarcoplasm due to the post-mortem influx of calcium ions solubilizes myofibrillar structural elements, resulting in their degradation (Ouali, 1990). The second theory supports the notion that endogenous calcium-activated proteinases (calpains) are activated by the influx of calcium ions and that they subsequently proteolyze some of the structural elements, leading to degradation of the myofibrillar matrix.
(Koohmaraie, 1992). There is no preponderance of evidence to support either view, but results indicate that proteolysis is, at least in part, responsible for post-mortem tenderization (Koohmaraie et al., 1987, 1988b; Koohmaraie, 1988, 1992; Wheeler et al., 1990; Whipple and Koohmaraie, 1991; Kendall et al., 1993).

Several attempts have been made to take advantage of the ability of the calpains to hasten tenderization of mammalian muscle tissue (Koohmaraie et al., 1988a, 1989, 1990). These attempts have generally involved infusion of calcium salts into the carcass via the circulatory system or injection of the salts into the muscle tissue. These processes reportedly lead to tenderization of some mammalian muscles within hours post-mortem compared to days post-mortem for untreated muscles (Koohmaraie et al., 1988a).

Even though extensive research has been conducted on effects of calcium treatment on mammalian muscles, little information is available on effects of such treatments on poultry muscle tissue. Young et al. (1991) reported that calcium chloride marination of chicken fillets that had been harvested immediately post-mortem produced fillets that were more tender than similar fillets marinated in NaCl, MgCl₂, or MnCl₂ solutions. However, prerigor fillets marinated in solutions containing divalent cations had depressed water-holding capacity as compared to the fillets marinated in solution containing only monovalent cations. Previous work suggested that the effects of calcium might be related to calcium effects on ATP metabolism and muscle pH. The objective of this study was to evaluate the effects of various calcium marinades on texture, moisture binding characteristics, pH, and ATP metabolism of chicken breast fillets harvested immediately post-mortem (peri-rigor).

**MATERIALS AND METHODS**

**Treatments**

Fifty commercially reared broilers were obtained from a local poultry processor, transported to the laboratory, and held overnight in order to minimize effects of catching and handling. The birds were stunned electrically (50 V alternating current for 10 s) and killed by exsanguination. They were not scalded or picked. After a 2-min bleeding period, the left Pectoralis major from each bird was immediately excised and weighed (wtᵢ). Batches of 10 muscles each were vacuum-tumbled for 15 min with 10% (wt/vol) of 0, 50, 100, 150, or 200 mM CaCl₂ plus sufficient NaCl so that all solutions were isoionic (0.6 M). The solutions were formulated thus to ensure that any effects were specific to calcium and not general ionic effects. Each muscle was immediately sampled for R-value analysis, weighed again (wtᵣ), and its pH recorded (pHᵣ). Each muscle was then cooked, weighed (wtᵢ), sealed in a plastic bag, cooled overnight in a 4 C refrigerator, and sampled for shear analysis. The experiment was repeated for a total of four replicates.

**Cooking Procedure**

Each muscle was vacuum-sealed in an individual cooking bag. Two of the muscles had meat thermometers inserted into their geometric centers. The bagged muscles were then immersed in a 90 C water bath and cooked until internal temperatures of those equipped with the thermometers reached 78 C (about 20 min).

**Moisture Binding Properties**

Marinade absorption and cooking loss were calculated as:

\[
\text{Absorption} = 100 \times \frac{(w_{t2} - w_{t1})}{w_{t1}}
\]

and

\[
\text{Cooking loss} = 100 \times \frac{(w_{t1} - w_{t2})}{w_{t1}}
\]

**pH Measurements**

The pH of marinated and cooked samples was measured with the aid of a pH meter equipped with a needle electrode. The probe was inserted into the geometric center of the thickest part of the muscle and pH recorded when a stable reading on the meter was observed for 10 s.

**R-Values**

The ratios of adenine:inosine nucleotides (R-values) in the muscle tissue were observed according to the procedure of Honikel et al. (1981). Briefly, the procedure was to extract the nucleotides in 1 M HClO₄ and then observe the ratio of light absorption at 250 and 260 nm. All samples for R-value analysis were stored in the 1 M HClO₄ until the absorption characteristics could be evaluated (no more that 6 h).

**Shear Values**

The cooked fillets were tempered in a 20 C water bath for 30 min prior to removing them from the plastic bags. A 1.27-cm wide template was used as a guide to cut each muscle to a standard size. The template was oriented from anterior to posterior because of the extreme contraction of some of the muscles. The force required to shear the samples was observed with an Instron Universal Testing Machine equipped with a Warner-Bratzler shear device.

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1Roschermatic TU 120 tumbler, Roscherwerk GMBH, Postfach 3566, Osnabruck, Germany.
2Orion Research Inc., Boston, MA 02129.
3Instron Corp., Canton, MA 02021.
**TABLE 1. Effect of calcium chloride marinades on moisture binding and biochemical and textural properties of peririgor chicken Pectoralis major**

<table>
<thead>
<tr>
<th>Treatment (mM Ca++)</th>
<th>Marinade absorption (%)</th>
<th>Cooking loss (%)</th>
<th>Marinated pH</th>
<th>Cooked pH</th>
<th>R-value</th>
<th>Shear value (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.64±b</td>
<td>14.9a</td>
<td>6.33</td>
<td>6.23</td>
<td>0.958±c</td>
<td>7.4a</td>
</tr>
<tr>
<td>50</td>
<td>5.74±a</td>
<td>17.3ab</td>
<td>6.31</td>
<td>6.32</td>
<td>0.979±c</td>
<td>5.4b</td>
</tr>
<tr>
<td>100</td>
<td>6.12±a</td>
<td>18.2b</td>
<td>6.35</td>
<td>6.31</td>
<td>0.986±c</td>
<td>5.4b</td>
</tr>
<tr>
<td>150</td>
<td>6.16±a</td>
<td>18.6b</td>
<td>6.26</td>
<td>6.29</td>
<td>1.001±b</td>
<td>5.1b</td>
</tr>
<tr>
<td>200</td>
<td>6.01±a</td>
<td>19.7c</td>
<td>6.29</td>
<td>6.30</td>
<td>1.025±a</td>
<td>5.5b</td>
</tr>
<tr>
<td>SEM</td>
<td>0.12</td>
<td>0.24</td>
<td>0.003</td>
<td>0.001</td>
<td>0.01</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*a*-Means within a column with no common superscript differ significantly (*P* < 0.05).

Full scale load was 100 kg and cross head speed was 50 cm/min. Duplicate observations were made on each sample, and the duplicate mean was recorded as the shear value (in kilograms) for the sample.

**Statistical Analysis**

The data were analyzed by analysis of variance using treatments (i.e., marinades) and replicates as main effects. As the treatment by replicate interaction was not significant, the data were pooled over replicates and treatments tested for statistical significance using the overall error mean square. Treatment means were separated by Duncan’s multiple range test at the 0.05 level of probability (Steel and Torrie, 1960).

**RESULTS AND DISCUSSION**

**Moisture Binding Properties**

As calcium content of the marinades increased, moisture absorption also increased (Table 1). However, muscles that received the highest calcium treatment and, thus absorbed the most moisture, also had the highest cooking losses. These results differ from those of Young and Lyon (1986), who found that moisture absorption by CaCl₂ plus NaCl-treated breast fillets was similar to that of fillets treated with NaCl alone, but that cooked yield of the fillets treated with CaCl₂ and NaCl was greater than that of controls treated with NaCl alone. However, the former study was conducted with commercially processed, fully aged muscles, whereas the present study was conducted with peri-rigor muscles. High cooking loss is economically important, so a method must be found to reduce cooking losses in order to take advantage of any beneficial effects of calcium treatment. In the previous study with aged meat (Young and Lyon, 1986), moisture absorption was greater and cooked yield was the same for muscles treated with calcium plus sodium tripolyphosphate (STPP) compared to those treated with STPP alone. However, because the previous study involved only postrigor muscle, application of STPP to the cooking loss problem will require further study.

**Muscle pH**

Calcium treatments had no significant effect on either marinated pH or cooked pH (Table 1). Post-mortem pH values of avian muscle declines very rapidly from near neutrality to about 5.6 to 5.8 within 6 to 8 h (Stewart et al., 1984). Because an increase in cytosolic calcium is a precipitating factor in the onset of rigor mortis, one would expect that the addition of calcium would hasten the process and lower the pH of calcium-treated muscles within 1 to 2 h post-mortem. It is possible the post-mortem pH decline was so precipitous that small effects caused by the treatments could not be detected with the methods that were employed. Differences in moisture binding properties by meat tissue are often ascribed to pH effects. However, in the present case, differences in moisture binding properties must be ascribed to other, as yet undefined, factors because pH was unaffected by treatment.

**R-value**

The R-value is a measure of the degree of conversion of adenosine to inosine nucleotides and can, therefore, be used to monitor development of rigor (Papa and Fletcher, 1988). They indicated that R-values of *P. major* muscles typically reach 0.95 to 0.97 within 15 to 30 min post-mortem and 1.2 to 1.3 within 2 to 4 h post-mortem. The present data (Table 1) indicate that the onset of rigor was significantly hastened by the calcium treatments. In fact, R-values for the highest levels of calcium were equivalent to 2 to 4 h post-mortem even though they were observed only 15 to 30 min post-mortem. However, ultimate R-values typically are 1.35 (Papa and Fletcher, 1988; Young et al., 1991), indicating that the muscles were in a peri-rigor state when cooked.

**Shear Values**

Calcium-treatment, regardless of concentration, significantly reduced shear values when compared to controls (Table 1). Thus, it appears that peri-rigor avian muscle responds to calcium in a manner similar to that of mammalian muscle by becoming more tender (Koo-
TABLE 2. Distribution of shear values of cooked control and calcium-treated chicken *Pectoralis major* muscles according to the sensory categories of Lyon and Lyon (1991)

<table>
<thead>
<tr>
<th>Sensory tenderness</th>
<th>Control (%)</th>
<th>Calcium-treated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very tender</td>
<td>2.5</td>
<td>9.5</td>
</tr>
<tr>
<td>(shear value &lt; 3.62 kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderately to slightly</td>
<td>35.0</td>
<td>72.8</td>
</tr>
<tr>
<td>tender (shear value 3.62 to 6.61 kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slightly tender to slightly tough</td>
<td>42.5</td>
<td>15.2</td>
</tr>
<tr>
<td>(shear value 6.62 to 9.60 kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slightly to moderately</td>
<td>20.0</td>
<td>1.9</td>
</tr>
<tr>
<td>tough (shear value 9.61 to 12.60 kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very tough</td>
<td>0.0</td>
<td>0.6</td>
</tr>
<tr>
<td>(shear value &gt; 12.60 kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

mariae, 1988, 1992; Koohmaraie et al., 1988a; Koohmaraie and Shackelford, 1991). Using the standards established by Lyon and Lyon (1991), mean shear values of control muscles (0 calcium) corresponded to a sensory score of “slightly tender to slightly tough”, whereas those of the calcium-treated muscles corresponded to “moderately to slightly tender”. However, of greater practical interest than the mean shear values are the extremes. Table 2 shows the percentages of muscles with shear values in each of the five tenderness groups described by Lyon and Lyon (1991). Twenty percent of the controls exhibited shear values greater than 9.61 (slightly to moderately tough) compared to 2.5% for the calcium-treated muscles. Shear values indicate that treating early harvested breast meat with calcium would reduce toughness and could improve consumer satisfaction. However, other quality attributes, such as yield and juiciness, must also be considered before technology can be developed to take full advantage of this potential.

REFERENCES


