Duplication of the pale, soft, and exudative condition starting with normal postmortem pork

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ABSTRACT: The objective of the current study was to create an in vitro model that duplicated the development of PSE pork. Postrigor pork chops with various pH values and normal color were vacuum-packaged, and heated at approximately 42°C for various times (0, 15, 30, 60, 120, or 240 min), or heated to temperatures that occur early postmortem (34, 37, 39, or 42°C for 60 or 120 min) in a water bath. Chops were cooled and allowed to bloom, after which changes in Minolta and Hunter color values were assessed, and purge loss was determined. Warming postrigor pork with normal color and pH to early postmortem body temperature for various times successfully duplicated the characteristics of PSE pork. After 60 to 120 min at 42°C or above, chops with pH < 5.8 lightened until L values were similar to those typical of PSE pork (Minolta L = 61.0). Change in chop color depended on length of time the samples were warmed, as well as on pH. Below 34°C, temperature had no (P ≥ 0.28) effect on color (Minolta L, a, b, and Hunter L*, a*, b*); however, at higher temperatures, color change depended on pH and warming time. A comparison of the time and temperature relationships for changes in lightness and purge suggested that the mechanisms of the two processes are not identical. The similarities in the dynamic range of color change, change in absolute color values, and time frame for changes in vitro and in vivo suggest similarity of the processes creating PSE in a carcass and in the in vitro model.

Key Words: Denaturation, In Vitro Model, pH, Postmortem, Pale, Soft, and Exudative Pork, Temperature


Introduction

Pale, soft, and exudative pork is a quality problem that occurs in 10 to 16% of US market pigs (Kauffman et al., 1992; Cannon et al., 1995). Pork with the PSE condition is undesirable to packers because of increased drip/purge loss, which represents value lost as “shrink,” and the light color makes PSE pork less appealing to consumers (Brewer and McKeith, 1999).

The PSE condition results from rapid postmortem metabolism of glycogen to lactic acid before muscle temperatures have cooled (Asghar and Pearson, 1980). The resulting combination of low pH and high temperature results in partial denaturation of muscle proteins, which increases light reflectance and decreases waterholding capacity (Bendall, 1973; Brewer, 2004).

It is not entirely clear which proteins denature during PSE development. Changes have been reported in both soluble sarcoplasmic proteins, such as myoglobin and lactate dehydrogenase, and in contractile proteins (Fischer et al., 1977; Swatland, 1993; Zhu et al., 2001). Denaturation refers to a variety of changes in proteins caused by pH, heat, oxidation, and other conditions. Proteins may partially denature, with changes occurring in one region but not in the whole protein. Therefore, in a complex array of proteins such as muscle, several pathways of protein denaturation may exist. For example, myoglobin can exist in at least three distinct denatured states, independent of oxygenation, at the same time (Alonso et al., 1991; Goto and Fink, 1994), indicating the potential complexity of protein denaturation in muscle pigments alone.

An in vitro model for replicating the development of the PSE condition would facilitate research into the mechanism(s) of genotype-independent PSE pork. Therefore, the objective of the current study was, using normal posttrigor pork as a starting material, to duplicate in vitro some of the postmortem conditions that result in PSE.

Materials and Methods

Experimental Design

The experiment was set up in two phases. The purpose of Phase I was to determine the effects of pH and
duration of exposure to temperature approximating initial postmortem muscle on color and purge. Boneless pork chops were warmed to approximately 42°C, and then held for various times (15, 30, 60, 120, or 240 min) with one control chop held on ice at approximately 2°C for 240 min. The purpose of Phase II was to determine the effects of pH and various muscle temperatures using times established in Phase I (30 and 60 min) on color and purge. Chops were held for 30 or 60 min at four temperatures (34, 37, 39, and 42°C), with one control chop held on ice at approximately 2°C.

Instrumental Color Determination

Color was determined using a Hunter Laboratory MiniScan XE colorimeter (Hunter Associates, Reston, VA) and a Minolta Chroma Meter CR-300 colorimeter (Minolta, Ramsey, NJ). Spectral reflectance was determined over the 400 to 700 nm range. Commission Internationale de l’Eclairage (CIE, 1978) L*, a*, and b* values were calculated from the Hunter spectral curve, and L, a, and b values were calculated from the Minolta spectral curve. As a measure of the divergence from the true red axis of the color space, hue angle was calculated as the arctangent of b/a (Minolta) or b*/a* (Hunter; Hunter and Harold, 1987).

The Hunter and Minolta colorimeters were calibrated before each use using the manufacturer’s color tile standards in a vacuum bag for the first two measurements, and with the tile standards only for the bloomed readings. The Hunter colorimeter was calibrated with a white and a black tile using illuminant D65 and the 10° standard observer. The Minolta colorimeter was calibrated with four standard tiles (white, orange, brown, and dark pink) using illuminant D65 and the 2° standard observer.

pH Determination

One gram (fresh tissue) of sample was placed into 20 mL of distilled water and held on ice until homogenization. Samples were homogenized at high speed on a Polytron homogenizer (Brinkmann, Westbury, NY) with a 2.5-cm stator, and pH was determined with an Orion 720A pH meter equipped with an Orion 8172 Ross “fast-flow” combination electrode (Thermo Electron, Waltham, MA). The pH meter was calibrated with pH 4.0, 5.0, and 7.0 buffers. Initial pH values (before heat treatment = ultimate pH) were determined on the day that pork loin sections were fabricated into chops. Final pH values (after heat treatment) were determined on the day chops were warmed, cooled, and subsequently removed from vacuum packaging.

Preparation of Samples

Longissimus muscle sections were removed from 18 randomly selected pork carcasses between 1 and 3 d after slaughter at the University of Illinois Champaign-Urbana Meat Science Laboratory. A 15- to 30-cm-long boneless LM section was removed from the carcass and used immediately, or vacuum-packaged (533 Pa; Cryovac Sealed Air Corp, Duncan, SC) and held at about 4°C for up to 4 d before sample preparation. Six to nine samples were cut from each loin, producing a total of 125 boneless chops. At least 3 cm were discarded from both ends of each LM section to account for purge effects because of vacuum-packaging; 1.2-cm-thick chops were sequentially cut and labeled A through F (Phase I) or A through I (Phase II), with A-labeled chops the most anterior and F- or I-labeled chops the most posterior. Labels were used for random treatment assignment. An approximately 1-g core was removed from each chop near the lateral midpoint for pH determination; the chop was weighed (initial weight), and vacuum-sealed at 533.3 Pa in Cryovac bags. Each chop was packaged immediately after cutting to avoid potential oxygenation, which minimized possible variation from this source.

Maintaining aseptic conditions in the preparation of the samples was required to minimize microbial contamination that might alter color or fluid loss because the chops were to be held near body temperature, ideal for microbial growth. Immediately before sample preparation, all knives, core samplers, and cutting boards were washed and rinsed with 70% ethanol, followed by sterile distilled water. In addition, vinyl gloves, worn during preparation, were rinsed with ethanol and distilled water before use. The scale platform was covered, and all vacuum-packaging bags were removed directly from the box in which they were shipped and visually inspected for cleanliness.

Experimental Procedure

After vacuum packaging, chops were allowed to stabilize overnight on ice in a cooler at approximately 2°C, so that essentially all the oxygen bound to the myoglobin would be consumed. This minimized initial color variation among individual chops from the same loin and more closely simulated postmortem in vivo conditions. After overnight stabilization, instrumental color was determined on each chop through the vacuum bag. Vacuum-packaged chops were then warmed to postmortem carcass temperatures by placing chops into a 42°C stirred water bath for 15, 30, 60, 120, or 240 min (Phase I). Samples were added at staggered intervals, so that all were removed at the same time (i.e., 240-min chops were placed in the water baths first, then 120-min chops, and so on) and to minimize the thermal load on the water bath. In Phase II, chops were held for 30 or 60 min at four temperatures (34, 37, 39, and 42°C). Control samples were not warmed but were held on ice in the cooler at approximately 2°C. At the end of the warming period, all samples were removed from the water bath and immediately placed into an ice water bath for 10 min to rapidly decrease temperature.
Figure 1. Effect of warming time on L value and purge of pork LM chops cut from a single loin heated for the indicated time at 42°C.

Results

The warming temperature used in Phase I (42°C) was chosen as a temperature that might be reasonably expected in muscles during the postmortem period (Rosenvold et al., 2002; Schafer et al., 2002). Loss of natural metabolic cooling mechanisms results in an increase in interior muscle temperature to above normal body temperature during the first 60 min postmortem. Warming postrigor pork, with normal color and pH, to early postmortem body temperature (42°C) for various times successfully duplicated the characteristics of PSE pork. The photograph of a series of pork chops from a single pig warmed to 42°C for various times illustrates the changes in lightness and purge typical of the in vitro PSE model (Figure 1). The samples from this pig changed from a normal color (L = 48) to nearly PSE in 30 min (L = 58) and continued to lighten with time.

Starting with a mean L value of 51.5, chops warmed to 42°C lightened to the PSE color range. Roughly half the color change occurred during the first 30 min, with progressively smaller changes thereafter (Table 1). After 60 to 120 min at 42°C, the color of in vitro model chops almost matched the lightness standard typical of PSE. Minolta L values of the National Pork Board (2000) color standards are 43 to 49 for normal pork and ≥61 for PSE pork.

Change in chop color clearly depended on pH, as well as the length of time the samples were warmed (Table 1). Chops with an initial pH < 5.8 consistently became paler (P < 0.05) with warming, indicated by an increasing L or L* value, whereas those with higher initial pH values exhibited little change. Results were nearly identical for Minolta and Hunter lightness values (Table 1). In samples with pH < 5.8, Minolta b and Hunter b* values also increased (P < 0.05) with time at 42°C, although the change was smaller than that observed in L* values on a numerical basis. Hunter a* values decreased slightly in samples with lower initial pH, whereas Minolta a values did not. Hue angle combines redness and yellowness to provide an indication of perceived color or hue. Below pH 5.8, samples became less...
true red ($P < 0.05$), whereas at pH > 5.8, chops remained the same color for the 240-min warming time.

Purge into the vacuum-bag increased ($P < 0.05$) from 3 to 12% over the 4-h period (Table 2), indicating that the model replicated development of both the color and exudation typical of PSE pork. Chops with a pH < 5.8 exhibited large purge losses during warming, whereas chops with a pH > 5.8 had small losses (Table 2). However, purge increased in a linear manner over the entire 4-h warming period, which was in contrast to color, where most of the change occurred within the first hour.

Because holding at 42°C resulted in nearly maximal changes in L* value within 60 min, our next step was to examine the influence of other warming temperatures. In general, warming chops to 34°C had little effect on color values (Table 3); however, when the temperature was increased to 37°C or above (approximately postmortem carcass temperature), color began to change, and lightness (Minolta L and Hunter L*) values began to increase ($P < 0.05$). Minolta a and b values, and hue angle changed similarly, with relatively smaller changes occurring below body temperature, and increasing changes above 37°C. Changes in Hunter a* and b* values were less consistent but changed the most at 42°C ($P < 0.05$). The effect of temperature on purge loss was small at 39°C or less, but it became greater at 42°C.

Results from Phase I (effect of time at 42°C) and Phase II (effect of temperature for 60 min) were combined to further examine the in vitro model in more detail. Temperature, time at a particular temperature, and hydrogen ion concentration ([H+] or pH) affected ($P < 0.05$) both measures of lightness (L and L*). Two-way (temperature × time) and the three-way interactions (temperature × time × [H+]) were significant ($P < 0.05$) for lightness values (Table 4).

Compared with lightness values, time and temperature effects on a or a* and b or b* values were small enough that they could not be separated into individual linear components (Table 4). The time × temperature interaction term represented energy transfer to the muscle sample, and an effect of time independent of temperature would have no practical meaning in this experiment. Likewise, purge was affected by temperature, time, and pH, with significant two- (temperature × time; $P < 0.001$) and three-way interactions (temperature × time × [H+]; $P < 0.05$).

Using the combined results, contour plots were created from predicted values relating pH and temperature to color and purge at various times. Figure 2 illustrates changes in Minolta L value after 30 and 60 min at various pH values and temperatures. At lower temperatures, the contour lines show the expected relationship between pH and color independent of denaturation, with L value increasing as pH decreases. At temperatures below 34°C, there was little change in L value with temperature at any particular pH; however, above 34°C, the L value rose sharply with increased tempera-

Table 1. Reflectance values of bloomed, warmed (42°C) LM chops from carcasses with different ultimate (24 h) pH values

<table>
<thead>
<tr>
<th>pH &lt;5.8</th>
<th>pH &gt;5.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warming time, min</td>
<td>Minolta L</td>
</tr>
<tr>
<td>0</td>
<td>51.5 ± 3.0</td>
</tr>
<tr>
<td>15</td>
<td>52.9 ± 2.4</td>
</tr>
<tr>
<td>30</td>
<td>56.4 ± 2.4</td>
</tr>
<tr>
<td>60</td>
<td>59.3 ± 2.8</td>
</tr>
<tr>
<td>120</td>
<td>61.4 ± 1.5</td>
</tr>
<tr>
<td>240</td>
<td>62.8 ± 0.4</td>
</tr>
</tbody>
</table>

aValues are means ± SD. Purge, % = ([initial weight – final weight]/ initial weight) × 100.

bNumber of carcasses used to prepare chops.

table 2 Purge (%) of vacuum-packaged chops warmed to 42°C for various times

<table>
<thead>
<tr>
<th>Warming time, min</th>
<th>pH &lt;5.8</th>
<th>pH &gt;5.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purge, %a</td>
<td>No.</td>
<td>Purge, %a</td>
</tr>
<tr>
<td>0</td>
<td>3.9 ± 1.1</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>5.0 ± 1.7</td>
<td>6</td>
</tr>
<tr>
<td>30</td>
<td>5.6 ± 1.9</td>
<td>14</td>
</tr>
<tr>
<td>60</td>
<td>6.6 ± 2.3</td>
<td>16</td>
</tr>
<tr>
<td>120</td>
<td>8.8 ± 3.0</td>
<td>7</td>
</tr>
<tr>
<td>240</td>
<td>11.1 ± 2.7</td>
<td>5</td>
</tr>
</tbody>
</table>

aValues are means ± SD. Purge, % = ([initial weight – final weight]/ initial weight) × 100.

bNumber of carcasses used to prepare chops.
Table 3. Reflectance values and purge for chops warmed for 60 min at various temperatures

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>Minolta L</th>
<th>Minolta a</th>
<th>Minolta b</th>
<th>Hunter L</th>
<th>Hunter a</th>
<th>Hunter b</th>
<th>Purge, %</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>52.0 ± 3.3</td>
<td>10.3 ± 0.9</td>
<td>4.6 ± 1.6</td>
<td>56.1 ± 2.9</td>
<td>10.5 ± 1.3</td>
<td>17.6 ± 1.3</td>
<td>3.9 ± 1.4</td>
<td>9</td>
</tr>
<tr>
<td>34</td>
<td>52.7 ± 3.0</td>
<td>10.7 ± 0.9</td>
<td>4.8 ± 1.4</td>
<td>56.7 ± 2.9</td>
<td>10.6 ± 1.7</td>
<td>17.5 ± 1.1</td>
<td>5.0 ± 3.7</td>
<td>9</td>
</tr>
<tr>
<td>37</td>
<td>54.1 ± 2.4</td>
<td>10.9 ± 1.4</td>
<td>5.2 ± 1.3</td>
<td>57.8 ± 2.6</td>
<td>10.8 ± 2.3</td>
<td>17.8 ± 0.9</td>
<td>5.8 ± 4.3</td>
<td>9</td>
</tr>
<tr>
<td>39</td>
<td>55.2 ± 3.9</td>
<td>11.2 ± 1.5</td>
<td>5.4 ± 0.7</td>
<td>58.5 ± 4.0</td>
<td>9.9 ± 1.1</td>
<td>17.4 ± 0.5</td>
<td>6.0 ± 2.8</td>
<td>4</td>
</tr>
<tr>
<td>42</td>
<td>59.6 ± 3.2</td>
<td>10.2 ± 1.0</td>
<td>6.1 ± 1.6</td>
<td>63.1 ± 3.1</td>
<td>9.2 ± 1.2</td>
<td>18.0 ± 0.7</td>
<td>6.3 ± 2.9</td>
<td>9</td>
</tr>
</tbody>
</table>

*Values are means ± SD.

*aInitial value of the specific color attribute at time = 0 was included as a covariate in each model.

Table 4. Probabilities of independent variable effects on pork LM quality attributes

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Hunter L*</th>
<th>Hunter a*</th>
<th>Hunter b*</th>
<th>Minolta L</th>
<th>Minolta a</th>
<th>Minolta b</th>
<th>Purge, %</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>[H+]c</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>&lt;0.001</td>
<td>—</td>
<td>—</td>
<td>&lt;0.001</td>
<td>—</td>
<td>—</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>—</td>
<td>&lt;0.001</td>
<td>—</td>
<td>—</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Temperature × time</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Temperature × time × [H+]c</td>
<td>≤0.01</td>
<td>—</td>
<td>&lt;0.01</td>
<td>≤0.05</td>
<td>—</td>
<td>—</td>
<td>≤0.05</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.99</td>
<td>0.98</td>
<td>0.97</td>
<td>0.99</td>
<td>0.97</td>
<td>0.94</td>
<td>0.90</td>
<td></td>
</tr>
</tbody>
</table>

*Initial value of the specific color attribute at time = 0 was included as a covariate in each model.

**Figure 4** shows the effects of pH and time on Minolta L value and purge when samples were held at or above 39°C. When samples had a pH between 5.6 and 5.8, lightness changes over time seemed to be primarily a function of pH, as indicated by a shallow slope. Below 5.6, however, the length of time at a given temperature became the major contributor to change in lightness, as indicated by a change from linear contours to curved contours with steeper slopes. This relationship mimics the development of PSE in carcasses with low pH when temperature remains high. The time dependency for purge changes was similar to changes in L value except that inflection in the time and temperature contours occurred at higher pH values and was more sharply curved. Comparison of the contour plots for Minolta L value and purge suggests that the mechanisms of the two processes are not identical.

Chops with initial L values > 60 from carcasses that had developed PSE in vivo following slaughter were also evaluated. These chops exhibited little to no color or purge change when warmed to 42°C (results not shown). Similarly, chops with normal color that were warmed, cooled, and then rewarmed for an additional 2 to 4 h experienced no further lightening. Together, these observations suggest an equivalent process of protein denaturation for PSE development in vivo and in the in vitro PSE model.
Discussion

Other investigators have described model systems to evaluate muscle color changes with pH or temperature (Swatland, 1995); however, we are unaware of other models that comprehensively replicate the physical development of the PSE condition. The in vitro model we described herein effectively replicates in situ postmor-
Figure 3. Effect of warming temperature and ultimate (24-h) pH on purge loss from pork LM chops held at the indicated temperatures for 30 or 60 min. The pH axis is plotted on an inverse log scale, corresponding to a linear scale for [H⁺].

tem carcass conditions that result in PSE in swine LM. This is characterized by changes in lightness (L and L* values) and by increased purge losses. Moreover, samples from PSE carcasses did not respond further to temperature increases in the in vitro model.

Characteristics of the in vitro PSE model emphasize several important points about the development of PSE. First, the in vitro model suggests that different processes may be involved in the two primary changes characteristic of PSE. The differences are apparent in the shape and transitions for pH and time contour plots of lightness and purge. Two likely explanations for these differences exist. First, there may be two proteins (or two groups of proteins) being denatured, with one protein (or group) accounting for the changes in waterholding capacity and the other accounting for color...
Figure 4. Effect of ultimate (24-h) pH and warming time at ≥39°C on Minolta L value and purge loss of pork LM chops.
changes. An alternative explanation is that denaturation of different regions within an individual protein (or group of proteins) occurs at different combinations of pH and temperature independently. The contour plots also illustrate how PSE characteristics are superimposed on the known pH dependency of meat color and water-holding characteristics (Brewer et al., 2001). At lower temperatures, both lightness and purge are a function of pH, with little temperature effect reflecting generally reversible changes in protein structure. At higher temperatures, however, there is a transition to a strong temperature dependency, indicating irreversible protein denaturation.

A second aspect of the in vitro model is that it highlights the potential for pigs to develop PSE independent of genotype. This is particularly notable because, in the National Genetic Evaluation Program, roughly two-thirds of the pigs that produced PSE carcasses did not carry the halothane gene (Christian, 1995). The clear relationship of paleness and exudation to low pH exposure at or near normal body temperature, regardless of genetic background, demonstrates that anything that abnormally accelerates postmortem metabolism has the potential to create the PSE condition.

In vivo postmortem experiments have shown that lightness and drip loss are highly correlated to pH and temperature (Goldspink and McLoughlin, 1964; Schafer et al., 2002). However, with in vivo postmortem experiments, because temperature and pH are highly independent, clear separation of independent effects is difficult. Electrical stimulation can be used to alter postmortem metabolism resulting in accelerated pH decline at high carcass temperatures (Hammelman et al., 2003; Rees et al., 2003). This decreased pH at temperatures seen during normal postmortem metabolism may then be related back to quality changes; however, because heat production results from metabolism, pH and temperature cannot be evaluated independently. In addition, because of the thermal mass of a carcass, potential manipulation or control of temperature decline is limited. Even with showering or cooling at -30°C, several hours are required to achieve a significant temperature difference in intact loins (Maribo et al., 1998; Ohene-Adjei et al., 2002). The model system we have described allows for independent control of pH and temperature.

Clearly, pigs differ in susceptibility to PSE regardless of genotype. Variation in fiber type accounts for some of this variation because of myosin isoform-specific differences in denaturation characteristics (Eggert et al., 2002). The in vitro model described here could be used to assess intrinsic resistance to PSE development. A particular advantage of this method is that it is essentially independent of time postmortem, thereby simplifying practical implementation.

**Literature Cited**


