The Effects of Different Chilling Methods on Meat Quality and Calpain Activity of Pork Muscle

Longissimus Dorsi

Yang Xu, Ji-Chao Huang, Ming Huang, Bao-Cai Xu, and Guang-Hong Zhou

Abstract: The objective of this study was to investigate the effects of conventional chilling (0 to 4 °C), rapid chilling (RC, –20 °C for 30 min, followed by 0 to 4 °C), and short-duration chilling (0 to 4 °C for 30 min, followed by 25 °C) on meat quality and calpain activity of pork muscle longissimus dorsi (LD). The muscle quality characteristics pH, color, cooking loss, pressing loss and tenderness, and calpain activities were measured 0-, 3-, 12-, and 24-h postmortem. Results show that the RC resulted in a faster temperature decline of the muscle, and prevented the meat pH and Commission Internationale de l’Eclairage L* value from declining during postmortem aging. RC also reduced meat cooking loss and pressing loss compared with the other two chilling methods. However, the chilling methods did not significantly affect meat shear force. During the first 24-h postmortem, there was not a noticeable change in the activity of m-calpain. But μ-calpain activity decreased regardless of chilling method. In the rapidly chilled carcasses, μ-calpain activity remained the same 3- and 12-h postmortem. However, in the short-duration chilled and conventionally chilled carcasses, the activity was visibly reduced. At 24-h postmortem, no clear zones on the gel were observed in all three treatments.

Keywords: calpain activity, carcasses, chilling methods, meat quality, pork

Practical Application: Conventional and RC methods are commonly used for pork in commercial practice nowadays. Compared with conventional chilling, the effect of RC on quality parameters of pork varies. In recent years, short-duration chilling (SC) is widely used in many Chinese pig slaughtering facilities. However, few researchers have studied the effect of SD on pork quality. Therefore, the present study investigated the effect of different chilling methods on functionalities or quality of chilled pork meat.

Introduction

Temperature of animals right after slaughter is usually about 37 to 39 °C. If it is not lowered promptly, microorganisms on carcasses will grow rapidly, resulting in meat shelf life and safety concerns (Honikel 1999). In addition, it has been shown that the rate of carcass heat transfer, temperature reduction, and pH decline also directly affect meat functionalities, such as evaporative moisture loss, water-holding capacity (WHC), tenderness, and color (Savell and others 2005).

The chilling step during meat processing is essential to lower the temperature of postmortem carcasses. It reduces the activity of microorganisms and histaminases in the meat, affects meat quality, and extends shelf life. How meat is chilled commercially depends on animal type, size, throughput, and so on.

Chilling methods that are used during meat processing include spray-chilling, delay chilling, conventional chilling (CC), rapid chilling (RC), and short-duration chilling (SC). Among them conventional, short-duration, and RC methods are commonly used for pork in commercial practice nowadays.

CC requires temperature of 0 to 4 °C for 24 h, RC requires temperature of –20 °C to –40 °C for 1 to 3 h (Huff-Lonergan and Page 2001), and they both aim at decreasing carcasses temperature as soon as possible, inhibiting microorganisms growth, and slowing down the degradation of muscle protein. Compared with CC, it has been shown RC can affect chilled pork quality (McFarlane and Unruh 1996; Springer and others 2003). For example, RC systems quickly reduced meat temperature and improved pork quality by lessening the incidence of pale, soft, and exudative (PSE) muscle, reduced the level of microbial population on pig carcasses, and extended shelf life (Milligan and others 1998). Furthermore, RC also consistently resulted in reduced evaporative moisture losses (Long and Tarrant 1990; Jones and others 1993) and chilling times (Dransfield and others 1991). All this has many economic benefits for the pork industry.

Savell and others (2005) concluded that the rate of thermal transfer relevant to the rate of temperature and pH decline influenced meat quality. Because the method of chilling directly influences the muscle’s temperature-pH-time relationship, the chilling process can significantly influence meat quality, particularly meat tenderness.

However, Janz and others (2001) observed that RC resulted in cold shrink. Some published reports show that the effect of RC on quality parameters of pork varies with pig genotypes and stress levels before chilling. Kerth and others (2001) observed a reduction in PSE incidence in loin and ham of stress-sensitive pigs after...
RC but not in stress-resistant pigs. Hambrecht and others (2004) concluded that the RC was unable to prevent the appearance of inferior pork quality caused by high prelaughter stress. Similarly, some other studies suggested that RC had no impact on WHC at all (van der Wal and others 1995; D’Souza and others 1998) and its effect on tenderness was uncertain (Weakley and others 1986; Long and Tarrant 1990).

SC requires temperature of 0 to 4 °C for 30 min and followed by storage at 25 °C. In recent years, SC is widely used in many Chinese pig slaughtering facilities. However, few researchers have studied the effect of SC on pork quality. Therefore, the objective of this study was to compare the effect of different chilling methods, RC, SC, and CC, on functionalities or quality and calpain activities of chilled pork meat.

Materials and Methods

Animals and slaughter procedures

Eighteen commercial crossbred pigs (the same genotype and age) that were fed and handled at the same farm were transported together for 30 min from farm to abattoir and slaughtered in the same facility. They were slaughtered as outlined in the guide for the care and use of experimental animals (Animal Experimental Special Committee of Nanjing Agricultural Univ.). After transport, pigs were allowed to rest for 2 h in the abattoir. Stunning was performed electrically (220 V, 2 A, 8 s) in a V-restrainer with a pair of stunning tongs. Immediately after stunning, the animals were shackled by one hind leg exsanguinated and scalded (5 min, 62 °C) vertically. Carcasses were individually placed in a dehairer, and residual hair was removed using flame and knife. The total slaughtering and carcass dressing time was 20 min.

Experimental treatment

All pigs were handled the same with the exception of chilling. They were randomly assigned to three treatment groups (n = 6): SC, in which carcasses were chilled at 0 to 4 °C for 30 min, and followed by storage at 25 °C; conventionally chilling (CC), in which carcasses were chilled at 0 to 4 °C; and rapidly chilling (RC), in which carcasses were chilled at –20 °C for 30 min, and followed by storage at 0 to 4 °C.

Meat quality measurements

Temperature and pH. Carcass temperature was measured using a digital thermometer (TESTO735, Testo Co., Lenzkirch, Germany). Two grams of muscle sample, devoid of connective tissue and large fat deposits, were hand-minced with a surgical scalpel and then homogenized on an ice bath in 16 mL of ultrapure water. Carcass pH was measured with a pH meter (FE-20, Mettler-Toledo Instruments Co., Ltd., Zurich, Switzerland) equipped with a probe-type glass electrode. The electrode was calibrated before each measurement using standard phosphate buffers (pH values of calibration buffers were 7.02 and 4.00 at 20 °C).

Meat color. Samples for color measurements were taken from the central part of *longissimus dorsi* (LD). The minimum thickness of samples was 2.5 cm. The samples were allowed to oxygenate for 10 min before Commission Internationale de l’Eclairage (CIE) *L*° (lightness), *a*° (red-green spectral axis), and *b*° (yellow-blue spectral axis) were measured using a Minolta Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan).

Water-holding capacity. WHC of muscle was determined using cooking loss and pressing loss method.

The LD was subsampled by cutting 5 × 3 × 2 cm cubes devoid of fat and connective tissue. Each cube, which was weighed (∏11), and individually placed inside polyethylene bags, was cooked in a water bath at 80 °C until an internal temperature of 70 °C was reached. During cooking, the internal temperature was tracked by the portable needle-tipped thermometer. The cooked samples were then cooled at 4 °C for 16 h, removed from the bags, patted dry with filter paper, and reweighed (∏2). The cooking loss was calculated according to the following equation:

\[
\text{Cooking loss} = \frac{\text{∏1} - \text{∏2}}{\text{∏1}} \times 100\%.
\]

The filter-paper press method described by Farouk and others (2004) was used to measure pressing loss. Cored LD samples, 2.5 cm in dia and 1.0 cm in thickness, were collected and weighed (∏3). Subsequently, the core was pressed by a force meter at 35-kg force for 5 min. Samples were reweighed (∏4) and pressing loss was calculated according to the following equation:

\[
\text{Pressing loss} = \frac{\text{∏3} - \text{∏4}}{\text{∏3}} \times 100\%.
\]

Tenderness

After measurement of cooking losses, the same steaks were used for determination of shear force at 0-, 12-, 24-, 48-, and 72-h postmortem. About 6 to 8 1.27-cm-dia cylindrical cores parallel to the muscle fiber orientation were removed from each steak. A single, peak shear force measurement was obtained for each core using a Warner-Bratzler meat shear machine (C-LM3B; Northeast Agricultural Univ., Harbin, China) and an average shear force was calculated and recorded for each steak.

Calpain activity

At 0-, 3-, 12-, and 24-h postmortem, 2 g of muscle samples were collected from the LD of each carcase, frozen in liquid nitrogen for subsequent measurement of calpain activity.

Sarcoplasmic protein extraction

The initial sarcoplasmic protein extraction procedure was conducted according to the method of Shackelford and others (1994), with modifications as noted in the following description. Finely minced, 2-g tissue samples from the LD were homogenized in 3 vol (wt/vol) of an extraction buffer containing 10-mM EDTA, 0.1% (vol/vol) β-mercaptoethanol (MCE), and 100-mM Tris-HCl, pH 8.3. The homogenate was centrifuged at 25000 × g for 30 min, and the supernatant was filtered through glass-wool. Protein content of each sample was determined following the procedure outlined by Bradford (1976). Filtered supernatant samples were collected for determination of relative calpain activity using casein zymography.

Casein zymography

Relative differences in calpain activity were determined in samples at each time point using casein zymography. The casein zymography procedure described by Raser and others (1995) was used with slight modifications. One volume of supernatant (from the extracted tissue sample) was combined with 1 vol of tracking dye solution (20% [vol/vol] glycerol, 0.75% [vol/vol] MCE, 0.02% [wt/vol] bromophenol blue, and 150-mM Tris-HCl, pH 6.8). Samples were loaded immediately onto nonde naturing 12.5% polyacrylamide gel electrophoresis (PAGE) casein gels (separating gel = acrylamide : N,N′-bis-methylene acrylamide = 75:1 [wt/wt], 0.05% [vol/vol] N,N′-tetramethylethylenediamine [TEMED], 0.05% [wt/vol] ammonium persulfate.
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[AP], casein [2.1 mg/ml], and 1.5M Tris-HCl, pH 8.8; stacking gel = acrylamide; N,N’-bis-methylene acrylamide = 75:1 [wt/wt], 0.125% [vol/vol] TEMED, 0.075% [wt/vol] AP, and 0.5M Tris-HCl, pH 6.8. Gels were run on mini slab electrophoresis units (Bio-Rad Laboratories, Hercules, Calif., U.S.A.).

Gels were prerun at 100 V for 15 min in a running buffer containing 192-mM glycine, 1-mM EDTA, 0.5% [vol/vol] MCE and 29-mM Tris, pH 8.3. Gels were then run at a constant voltage (100 V) for approximately 22 h. After electrophoresis was completed, the gels were removed, and then incubated in 3 changes (20 min each) of incubation buffer (4-mM CaCl$_2$, 0.05% [vol/vol] MCE and 50 mM Tris, pH 7.8). Gels were then incubated overnight in incubation buffer. The following day, gels were stained in a solution containing 0.1% [wt/vol] Coomassie brilliant blue R-250, 50% [vol/vol] methanol, and 10% [vol/vol] glacial acetic acid for approximately 1 h. Gels were destained using an excess of destain (10% [vol/vol] methanol and 10% [vol/vol] glacial acetic acid). Calpain activity was indicated by clear zones in the stained gels.

Statistical analysis

All statistical analysis of the differences between each treatment was carried out using a one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS version 13.0, SPSS, Inc., Chicago, Ill., U.S.A.). Each animal was considered as an experimental unit for data analyses. Differences in the mean values within the same treatments were compared by the Fisher’s protected least significant difference test ($P < 0.05$). A natural logarithm transformation was executed to satisfy the ANOVA homogeneity of variance assumption. All data are expressed as mean ± SD.

Results and Discussion

Carcass temperature decline

Changes of carcass temperature in each of the three treatment groups at 0-, 3-, 12-, and 24-h postmortem are shown in Figure 1. Muscle temperature of LD from SC carcasses was higher ($P < 0.05$) than those from RC and CC carcasses during chilling. The mean temperatures at 3 and 12 h for RC carcasses were lower ($P < 0.05$) than that from CC carcasses, which implied that RC results in a faster decline in carcass temperature compared with CC. The differences in mean temperature between RC and CC carcasses at 3 h were the greatest. The difference gradually decreased until ultimately reaching just below 7 °C at 24-h postmortem.

Since the difference in temperatures between carcasses and environment was greater during RC, the faster chilling rate was noticed in agreement with the result published in previous studies (Drumm and others 1992; Mallikarjunan and Mittal 1996).

Carcass pH decline

Changes of LD muscle pH within 3 treatment groups during postmortem period are presented in Figure 2. Muscle pH values from SC carcasses were lower ($P < 0.05$) than those from RC and CC carcasses during postmortem aging. The rate of pH decline between 3 and 12 h for the RC group was slower ($P < 0.05$) than those for the CC and SC groups. However, at 24 h there was no difference between the RC and CC groups ($P > 0.05$). After a relatively fast fall within the 1st 12-h postmortem, the mean pH values for all the carcasses underwent a slow decline until they reached the ultimate pH at 24-h postmortem.

Following slaughter, oxygen supplying to muscle tissue is disrupted. Aerobic oxidation of intramuscular glycogen is terminated. Muscle glycogen is degraded into lactic acid through glycolysis, and it lowered the pH of meat. Bertram and others (2001) concluded that temperature during postmortem aging affected the buffering capacity of muscle, which is an important factor to explain the difference between the effects of RC and CC on pH values. Pike and others (1993) observed that a lower chilling temperature slowed pH decline. Lawrie (1998) stated that the rate of biochemical reactions in the muscle was positively correlated with carcass temperature. Colder chilling temperature reduced the activity of glycolytic enzymes and decreased the consumption of adenosine-triphosphate, so it would reduce the rate of pH decline.

Water-holding capacity

Pressing loss. The effects of different chilling treatments on pressing loss of LD are showed in Figure 3. Considerable differences in pressing losses were observed between the chilling methods. Overall, the highest pressing loss was observed in meat from the SC group, followed by meat from the CC group. The lowest fluid loss was obtained in meat from the RC group. At 3-h postmortem, no differences ($P > 0.05$) were detected among the 3 treatments. Compared with SC and CC, the pressing loss of LD from the RC groups was significantly lower ($P < 0.05$) at 12-h and 24-h postmortem.

These results demonstrate that RC was advantageous to improve WHC of LD. Tomović and others (2008) state the freezing temperature in the RC that causes an ice layer to form on the surface of the carcass and thereafter reduces the rate of surface water evaporation. However, the lower WHC in SC group is possible...
due to higher temperature and lower pH causing protein damage and thus losing water-binding capacity.

Cooking loss. Figure 4 shows that cooking losses of LD irrespective of chilling treatment tended to increase with aging. At 3-h postmortem, the average cooking losses of the RC, CC, and SC were 13.0%, 12.8%, and 14.2%, respectively. There were no significant differences (P ≥ 0.08). However, at 12-h postmortem, an increase in cooking loss was observed in the SC group (P ≤ 0.02) when compared to those in the RC and CC groups (24.1%, 15.17%, and 18.14%, respectively), but no significant difference was observed between RC and CC (P = 0.10). At 24-h postmortem, there was no significant difference (P = 0.09) for cooking loss between RC and CC (21.57% and 23.54%, respectively). These results indicate that RC does not adversely affect cooking losses of pork compared with the CC.

Studies have shown that differences in cooking losses are largely dependent on ultimate pH of meat (Honikel 1999). The pH24h values of the RC group were not significantly different from the CC group. But pH of the SC group was lower than pH of either RC or CC group. This further demonstrates that there is a relationship between pH and cooking loss in pork muscle.

When WHC was considered by combining pressing losses and cooking losses, the mean value of SC muscle was the highest and RC the lowest. The preslaughter condition for all pigs in our experiments was the same. Therefore, it is possible that the quick decline of temperature made the RC group remain comparatively higher pH value and thus the higher protein functionality and water-binding capacity (Joo and others 1999).

Carcass tenderness. Shear force values measured over a 3-d aging period are presented in Figure 5. Shear force values in SC group were lower (P ≤ 0.03) but no difference was observed between RC and CC (P ≥ 0.16) at 12 h (7.47 kg, 7.05 kg, and 6.25 kg, respectively) and 24 h (6.14 kg, 5.84 kg, and 4.89 kg, respectively) postmortem. However, at 48-h postmortem, shear force values in the SC group were lower (P = 0.02) than those in the RC group (5.18 kg and 6.01 kg, respectively). Shear force values of 12-h aged LD were greater than those with longer aging periods. Higher shear force values of meat from early postmortem aging stage could be due to the postmortem contraction of the myofibrillar proteins. Following aging for 2 and 3 d, shear force values of the cooked pork decreased (meat became more tender), presumably as a result of breakdown of muscle myofibrillar structures caused by proteolytic enzyme activity and/or ice crystal formation. However, after 72 h, the mean shear force values of meat from RC and CC carcasses were higher (P = 0.001) than those from SC carcasses (4.71 kg, 4.49 kg, and 3.50 kg, respectively).

Relatively high shear force values with RC samples further support previous published results (Gigiel and James 1984; McGeehin and others 2002; Hannula and Puolanne 2004), in which RC was considered to be one of the most important causes of tough meat. Our discovery is also in accordance with the conclusion drawn by Howard and Lawrie (1956) who found that the rate of pH decline was inversely related to meat tenderness. On the contrary, Jones and others (1993) found no significant difference in the shear force value of semimembranosus (SM) muscle applying higher chilling rate (3 h at −4 ºC). Dransfield and others (1991) also found no significant increase in toughness of SM muscles in RC system (−15 ºC for 3 h, followed by 0 to −4 ºC). According to Locker and Haygard (1963), lowering the muscle temperature below 10 ºC within 10-h postmortem will cause cold-induced shortening. The results also indicate that application of RC for pork processing will take the risk of producing tough meat caused by cold shortening.

The work of Koohmaraie and others (1987) showed that at slaughter, all meat with the same pre-slaughter treatments had the same tenderness level, and that differences in tenderness were created in the 1st 24-h postmortem. The rate and extent of postmortem muscle pH and temperature decline can significantly affect meat tenderness (Rees and others 2003).

Meat color. The effects of chilling methods on meat color are presented in Table 1. The *L* values increased consistently from 45-min to 24-h postmortem in all pork muscles. According
to the average values for lightness, the color of all investigated LD muscles represented normal quality meat (reddish-pink color: \( L^* = 43 \) to 50 (Joo and others 1999), except for the meat from the SC carcasses. The highest numerical average \( L^* \) value (lightest color) was found in SC muscles (\( L^* = 51.19 \)), while R.C. and CC muscles at 24-h postmortem were visibly darker (\( L^* = 43.54 \) and \( L^* = 45.92 \), respectively). Joell and others (2004) found no effect of RC on the color (\( L^* \) value) of pork SM; however, our result shows that RC improved (\( P < 0.05 \)) LD muscle color. The lower \( L^* \) value of the rapidly chilled meat is typically associated with a high ultimate pH value (Lawrie 1998). Furthermore, no significant difference was found among 3 treatments (\( P > 0.05 \)) for \( a^* \) value. CIE \( a^* \) values were almost identical in all 3 groups of LD muscles at any given measuring time. The highest average \( b^* \) value was found in meat from the SC group (\( P < 0.05 \)), while no significant difference existed between RC and CC groups (\( P > 0.05 \)).

\( L^* \) value is correlated with WHC of pork LD muscle (Warriss and Brown 1987; van Laack and others 1994; Joo and others 1995). During the process of rigor mortis, some muscle proteins are denatured, which increased free water and juice loss, and further changed the surface reflection properties of meat. Our results show again that change in \( L^* \) values is associated with WHC in LD during postmortem aging.

Breuer and McKeith (1999) reported that color is an important factor in predicting consumer self-reported purchase intent of chilled, fresh pork. Zhu and Brewer (1999) reported that instrumental color characteristics (\( L^*, a^*, b^* \)) were highly correlated with visual redness of fresh meat. Therefore, consumers were visually perceptive to the instrumental color differences.

**Calpain activities.** A representative nondenaturing 12.5% PAGE casin gel result is shown in Figure 6. A comparison of calpain activity is made between the R.C., CC, and SC treatments based on the light intensity and size of the clear zones. During the 1st 24-h postmortem, there was not a great change in the activity of \( m \)-calpain regardless of chilling treatment. However, at 3- and 12-h postmortem, \( \mu \)-calpain of the RC group remained the same (similar intensity and size of clear zones indicate the similar \( m \)-calpain activities in the muscle), but intensity of \( \mu \)-calpain activity zone was reduced in both CC and SC groups. By 24-h postmortem, the absence of clear zones indicated that \( \mu \)-calpain completely disappeared in meat no matter what chilling method was used.

The differences in \( \mu \)-calpain activity correlated with the differences in pH decline noted among the 3 chilling treatments. For example, SC group had the fastest drop in pH and lost its \( \mu \)-calpain zone mostly among the 3 treatments. The increased rate of autolysis was significant because autolysis of \( \mu \)-calpain reduced the Ca\(^{2+} \) requirement for calpain activity from approximately 3 to 50 \( \mu \)M (pre-autolysis) to 0.5 to 2.0 \( \mu \)M, bringing the Ca\(^{2+} \) requirement closer to the physiological range (Zimmerman and Schlaepfer 1991).

**Tables**

**Table 1—Effects of various chilling methods (CC—conventional chilling, RC—rapid chilling, and SC—short-duration chilling) on meat color of Longissimus dorsi at 0-, 3-, 12-, and 24-h postmortem (means ± SD, \( n = 6 \)).**

<table>
<thead>
<tr>
<th>Time</th>
<th>Method</th>
<th>0 h</th>
<th>3 h</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>RC</td>
<td>SC</td>
<td>CC</td>
<td>RC</td>
</tr>
<tr>
<td>( L^* )</td>
<td>37.91 ± 1.62</td>
<td>38.42 ± 0.38</td>
<td>37.82 ± 0.69</td>
<td>41.48 ± 1.79</td>
<td>42.19 ± 0.41</td>
</tr>
<tr>
<td>( a^* )</td>
<td>3.34 ± 0.63</td>
<td>3.42 ± 0.33</td>
<td>3.44 ± 0.25</td>
<td>3.76 ± 0.16</td>
<td>4.76 ± 0.71</td>
</tr>
<tr>
<td>( b^* )</td>
<td>2.43 ± 0.14</td>
<td>2.38 ± 0.51</td>
<td>1.78 ± 0.25</td>
<td>2.82 ± 0.64</td>
<td>2.77 ± 0.67</td>
</tr>
</tbody>
</table>

**Figure 6—Casein zymography gels depicting \( \mu \)-calpain and \( m \)-calpain activities in sarcoplasmic extracts of the Longissimus dorsi at 0-, 3-, 12-, and 24-h postmortem for each of the chilling methods used. Lanes from left to right are 0 h control (A), 3 h-CC (B), 12 h-CC (C), 24 h-CC (D), 3 h-RC (E), 12 h-RC (F), 24 h-RC (G), 0 h control (H), 3 h-SC (I), 12 h-SC (J), 24 h-SC (K). Each lane was loaded with 40 \( \mu \)g of protein. The uppermost clear zone indicates \( \mu \)-calpain activity and the bottom clear zone indicates \( m \)-calpain activity.
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