



A simple, reliable and reproductive method to obtain experimental pale, soft and exudative (PSE) pork[☆]

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ABSTRACT

The inferior quality and economic risk of pale, soft and exudative (PSE) pork warrant continuing research. However, such research efforts are often hindered by the challenge to obtain reliable PSE muscle samples with similar quality characteristics. The objective of this study was to establish a reliable and convenient method to produce PSE-like pork. A PSE condition was induced by incubation of 30-min postmortem *Longissimus* muscle at 35 °C for 7 h followed by chilling to 4 °C. Compared to normal red, firm and non-exudative (RFN) pork (kept at 4 °C), PSE muscle had consistently lower pH_{2h} (5.46 vs. 5.74) and pH_{4h} (5.35 vs. 5.52), higher *L** (lightness) value (56.5 vs. 51.0), and reduced protein solubility and thermal stability (enthalpy and temperature) than RFN muscle ($P < 0.05$). The highly reproducible results indicate that incubation of muscle immediately postmortem at 35 °C offers a simple and consistent method to produce PSE pork.

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1. Introduction

Pale, soft and exudative (PSE) pork is a defective product resulting from both preslaughter and postmortem factors, for example, animal genetics, nutrition, season of the year, stress during animal transportation, and carcass processing and storage conditions (Barbut et al., 2008; Lesiów & Kijowski, 2003; Scheffler & Gerrard, 2007). Failure to rapidly chill the pork carcass immediately postmortem can lead to PSE conditions. For example, it has long been recognized that when the temperature of pork muscle remains high postexanguination, it would activate glycogen phosphorylase and also phosphofructokinase, leading to a fast accumulation of lactic acid and pH decline (Kastenschmidt, Hoekstra, & Briskey, 1968). There are continuous efforts to diminish the occurrence of this phenomenon and to find reliable quality indicators (pH, color parameter, i.e., lightness at 24 h postmortem, electrical conductivity, etc.) to separate PSE pork and then process such meat to obtain final products with improved properties and consumer acceptability.

Both objective and subjective methods have been used to separate pork into different quality groups, i.e., RFN (red, firm and non-exudative), PSE, and DFD (dark, firm and dry) (Kazemi, Ngadi, & Gariepy, 2011; O'Neill, Lynch, Troy, Buckley, & Kerry, 2003; van de Perre, Ceustermans, Leyten, & Geers, 2010). Subjective methods rely on the examiner's experience, while objective methods are typically based on the measurements of pH, lightness (*L**), and drip

loss of fresh muscle. The indicator value for PSE can differ depending on particular plant conditions. In the literature, many researchers classify pork meat on the basis of drip loss and the *L** value, e.g., drip loss > 6% and *L** > 50 for PSE, and drip loss < 6% and *L** < 50 for RFN (Joo, Kauffman, Kim, & Park, 1999; Ryu, Choi, & Kim, 2005; van Laack & Kauffman, 1999). Others separate pork by combining drip loss, *L**, and pH, e.g., drip loss > 5% and *L** > 50 (or *L** > 55) and pH_{1h} < 5.6 for PSE (Channon, Payne, & Warner, 2002; Šimek, Grolichová, Steinhäuserová, & Steinhäuser, 2004; Warner, Kauffman, & Greaser, 1997; Wilson & van Laack, 1999). PSE meat is also identified when pH taken 45 min after slaughter is less than 5.7–6.0, and normal RFN meat is identified when pH_{24h} is between 5.5 and 5.8 (O'Neill et al., 2003) or from 5.8 to 6.2 (Mota-Rojas et al., 2006).

The value standardization for aforementioned indicators is difficult, and the problem intensifies when one compares the pH/*L** values obtained by different researchers. For example, pork loins were classified into PSE and RFN when the respective pH/*L** values were 5.32/55.9 and 5.64/45.1 (van Laack & Kauffman, 1999); 5.2/61.9 and 5.6/54.6 (Lien et al., 2002); 5.47/54.9 and 5.69/48.1 (Nam, Ahn, Du, & Jo, 2001); and 5.60/51.5 and 5.96/44.8 (Kuo & Chu, 2003). These discrepancies demonstrate that meat researchers who study PSE meat are challenged by not being able to obtain PSE muscle samples with similar quality characteristics, let alone the effort to develop ingredient and processing technologies for utilizing PSE pork. Hence, research is needed to address how to model and create PSE-like pork muscle through postmortem manipulation so as to generate such samples for fundamental studies.

The influence of chilling temperature on the occurrence of PSE meat has been studied by a number of researchers. In general, an extended exposure of hot carcasses to high temperatures (35 to 40 °C) predisposes pork (Brewer, Zhu, Bidner, Meisinger, & McKeith, 2001; Fernandez,

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Forslid, & Tornberg, 1994; McCaw, Ellis, Brewer, & McKeith, 1997), turkey (Molette, Réminon, & Babilé, 2003) and chicken (Zhu, Ruusunen, Gusella, Zhou, & Puolanne, 2011) to the development of PSE-like conditions. The present study attempted to establish a convenient tempering method to produce PSE-like pork. The PSE condition was validated by monitoring postmortem glycolysis (pH reduction), changes in meat color parameters with different colorimetric measurements, protein denaturation profiles, and protein solubility.

2. Materials and methods

2.1. Materials and experimental design

A total of 15 crossbred pigs [Yorkshire×Duroc, (Yorkshire×Landrace)×Duroc] from a known halothane gene negative breed line were raised for each of two separate experiments at the University of Kentucky Swine Farm on a typical commercial corn–soybean meal diet (14.0% protein, 3.34 Mcal/kg metabolizable energy). Pigs were slaughtered at the University of Kentucky Meat Plant, a USDA-inspected facility. The average hot carcass weights were 89.2 ± 5.0 and 76.6 ± 3.3 kg for the first and the second experiment groups, respectively. From eight randomly selected pork carcasses immediately after carcass splitting (within approx. 30 min postexanguination), loins (*Longissimus lumborum*) were excised. Loins were used because they are prone to PSE incidences (van de Perre et al., 2010). The loins were divided into two equal portions (4 for each experiment replication) and each loin was cut in the middle perpendicularly to the long axis. The average temperature of the pork loins, measured by a thermocouple 30 min postmortem, was 36.3 ± 1.3 °C. A thin slice (approx. 2 mm) was excised from the middle of the loin, kept on ice, and tested by pH at 30 min postmortem (pH_{30min}). The two halves from each loin were dipped in 10 mM NaZ₃ (an antimicrobial) and placed in two separate plastic bags (Ziploc bags, Gal size). One bag was stored at 4 °C for 24 h to generate RFN and the other was submerged in a 35 °C water bath for 7 h followed by chilling and storage at 4 °C for a total of 24 h postmortem to induce PSE.

The pH of RFN and PSE muscle samples was measured at 0.5, 2, 4, 6, 12 and 24 h postmortem. Duplicate pork chops were obtained from the 24 h postmortem loins for the measurement of color parameters. After the color measurement, thin layers (1–2 g) of muscle from PSE and RFN were sliced, placed in small conic centrifuge tubes (50 mL capacity) and kept on ice for thermal stability measurement the following day (48 h postmortem). Thereafter, chops and remaining meat from both PSE and RFN groups in separate plastic bags were vacuum packaged, placed in a –80 °C freezer, and used for protein solubility determination.

2.2. pH and color determination

The pH of raw meat was determined using a combination electrode connected to pH meter AB 15 (Accumet, Fisher Scientific, Fair Lawn, NJ, USA) calibrated with pH 4.0 and 7.0 standard buffers. An aliquot of 5 g muscle samples was mixed with 15 mL iodoacetate (5 mM) solution and homogenized 30 s at speed 3 (7,500 rpm) with a PT 10-35 Polytron blender (Kinematica, AG, Littau/Lucerne, Switzerland).

Color measurements were taken on RFN and PSE pork loins 24 h postmortem using Minolta Chroma Meter (Model CR-310) and Hunterlab colorimeter (LabScan XE, Hunter Associates Laboratory, Reston, VA, USA) using illuminant C or D₆₅ within the instrument. The Minolta Chroma Meter was calibrated with a white plate according to the manufacturer's instructions. Color parameters, i.e., lightness (L^*), redness (a^*), and yellowness (b^*), were measured at three different locations on each pork chop, and average values were reported. The Hunter LabScan with a 0.64 cm port was calibrated with a black and a white tile according to manufacturer's instructions. The color measurements on each pork chop were taken at two different locations and the

average values were reported. Hue angle [$h = \tan^{-1} (b^*/a^*)$] and saturation index [$C = (a^{*2} + b^{*2})^{1/2}$] were also calculated.

2.3. Myofibrillar protein preparation and solubility measurement

Frozen meat samples were thawed at 4 °C overnight. After the removal of visible fat and connective tissue, meat was ground through a 3 mm orifice plate and subjected to myofibrillar protein (MP) isolation in a 4 °C walk-in cooler. Briefly, ground meat was washed two times with 4 vol (v/w) of 50 mM NaH₂PO₄ buffer (pH 7.0) containing 0.1 M NaCl and 5 mM EDTA. Each time the mixture was homogenized with a Waring blender at high-speed for 60 s followed by centrifugation at 2,000 g for 15 min. The final pellets were washed two times with 4 vol (v/w) of 0.1 M NaCl solution as described above. For the 0.1 M NaCl washing, protein homogenates were adjusted to pH 6.0 with 1 M HCl before centrifugation. The protein concentration of the final MP pellet was determined by the Biuret method (Gornall, Bardawill, & David, 1949).

To measure protein solubility, MP isolates prepared from both RFN and PSE meat were suspended by gently stirring in a pre-determined volume of 50 mM NaH₂PO₄ buffer (pH 6.0) containing 0.6 M NaCl (final concentration) to a 5 mg/mL protein concentration. An 8-mL aliquot of each MP suspension (5 mg/mL protein) was placed in a 15-mL centrifuge tube and centrifuged at 5,000 g for 15 min at 2 °C. The protein concentration of the supernatant was determined by the Biuret method. Protein solubility (%) was expressed as protein concentration of the supernatant divided by the protein concentration of the original suspension times 100.

2.4. Thermal stability of muscle proteins

Thermal stability or degree of denaturation of proteins in PSE versus RFN loin samples at 48 h postmortem was measured using the Model 2920 modulated DSC (TA Instruments, Inc., New Castle, DE, USA). A 15–20 mg meat sample was carefully placed and sealed in a polymer-coated aluminum hermetic pan. A sealed empty pan was used as reference. A constant heating rate of 10 °C/min was applied to scan samples from 10 to 95 °C. The enthalpy change (ΔH) associated with protein denaturation was estimated by measuring the area above the DSC transition curve. For an individual endotherm, a straight baseline was constructed from the start to the end of the peak to measure the ΔH ; for combined endotherms, a straight line was constructed from the start of the first peak to the end of the last peak to measure the ΔH . Also, the temperature at the peak of each endotherm, i.e., the temperature at the maximum heat flow (T_{max}) for each muscle sample was recorded and analyzed using the universal analysis 2000 Thermal Advantages software supplied by the DSC manufacturer.

2.5. Statistical analysis

Two identical but independent experiments were conducted on two different days. In each experiment, four loins from four different pork carcasses were used as replicates. Results collected from the total eight individual loins were analyzed using the Statistix 8.0 program (Analytical Software, Tallahassee, FL). One-way analysis of variance was done to determine significant differences (P at 0.05) between RFN and PSE muscle samples. Differences between means were identified by Duncan's all-pair wise comparisons test.

3. Results and discussion

3.1. pH

Holding the warm *Longissimus* muscle at the near-body temperature (35 °C) immediately postmortem caused rapid pH decline when compared with muscle that was kept in the chilling room (4 °C) (Fig. 1).

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