Meat Science 115 (2016) 34–40

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Disorder of endoplasmic reticulum calcium channel components is associated with the increased apoptotic potential in pale, soft, exudative pork

Bing Guo [a], Wangang Zhang [a], Ron K. Tume [b], Nicholas J. Hudson [c], Feng Huang [d], Yan Yin [a], Guanghong Zhou [a,*]

[a] Key Laboratory of Meat Processing and Quality Control, Synergetic Innovation Centre of Food Safety and Nutrition, College of Food Science and Technology, Nanjing Agriculture University, Nanjing 210095, China
[b] CSIRO Food and Nutrition Flagship, 39 Kessels Road, Queensland 4108, Australia
[c] CSIRO Agriculture Flagship, 306 Carmody Road, Queensland 4067, Australia
[d] Institute of Agro-Products Processing Science and Technology, Chinese Academy of Agricultural Science, Beijing 100193, China

ARTICLE INFO

Article history:
Received 2 April 2015
Received in revised form 24 December 2015
Accepted 9 January 2016
Available online 11 January 2016

Keywords:
Calcium imbalance
Mitochondrial
Apoptosis
PSE meat

ABSTRACT

Eight pale, soft and exudative (PSE) and eight reddish-pink, firm and non-exudative (RFN) porcine longissimus muscle samples were selected based on pH and L* at 1 h postmortem (PM), and drip loss at 24 h PM, and used to evaluate the cellular calcium and apoptosis status. We found that SERCA1 was decreased, while IP3R was decreased in PSE meat ($P < 0.05$), indicative of the overloaded sarcoplasmic calcium status. In PSE meat, the pro-apoptotic factor BAX was increased while the anti-apoptotic factor Bcl-2 was decreased ($P < 0.05$). The significantly increased activity of caspase 3 and the expression of its cleavage fragment suggested higher apoptotic potential in PSE meat compared with RFN meat ($P < 0.05$). Moreover, the significantly higher expression level of cytochrome C ($P < 0.05$) suggests the important role of mitochondria during apoptosis appearance in PSE meat. Taken together, our data inferred that the calcium channel disorder present in PSE meat was associated with the increased apoptotic potential.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The occurrence of PSE (pale, soft and exudative) meat in commercial pork and chicken carcasses causes large economic losses to the industries (Barbut, 1997; Barbut et al., 2008). Although this meat quality problem has been extensively investigated, with much of the research focusing on the relationship between the genetic background of the pigs and the incidence of PSE meat, the underlying molecular mechanism still needs further investigation (Barbut et al., 2008).

The presence of high sarcoplasmic calcium concentrations shortly after slaughter in PSE meat has been well documented (Greaser, Cassens, Briskey, & Hoekstra, 1969). The components of calcium channels located in the membrane of sarcoplasmic reticulum (SR), are lined with sarcoendoplasmic reticulum calcium-ATPase 1 (SERCA1), ryanodine receptor (RyR) and inositol 1, 4, 5-trisphosphate receptor (IP3R) and have been demonstrated to be correlated with the calcium concentration variance in sarcoplasm of many tissues, including skeletal muscle (Berchtold, Brinkmeier, & Muntener, 2000). For instance, genetic mutation of RyR and SERCAs is highly correlated with the appearance of PSE meat in both pig and chicken (Chai, Xiong, Zhang, Shang, et al., 2010; Chai, Xiong, Zhang, Zheng, et al., 2010; Sporer, Zhou, Linz, Booren, & Strasburg, 2012; Toyoshima, Nakasako, Nomura, & Ogawa, 2000; Weaver, Dixon, & Schaefer, 2000). Also, the expression variance of both SERCA and IP3R can induce the skeletal muscle calcium disorders and PSE meat appearance (Chai, Xiong, Zhang, Shang, et al., 2010; Chai, Xiong, Zhang, Zheng, et al., 2010; Li et al., 2009). In skeletal muscle, disorder of these factors inevitably leads to elevated sarcoplasmic calcium concentrations and these are usually initiated by stressful situations in live animals or by dexamethasone (DEX) in cell model systems (Chai, Xiong, Zhang, Shang, et al., 2010; Chai, Xiong, Zhang, Zheng, et al., 2010; Greaser et al., 1969).

Overloaded sarcoplasmic concentrations of calcium (>200 μM) are responsible for the activation of muscle metabolism and acceleration of lactate production in postmortem (PM) muscle (Carafoli, 2002). These high calcium concentrations have also been implicated as initiators of apoptosis via some signaling pathways in skeletal muscle (Demaurex & Distelhorst, 2003). As a pluripotent organelle, mitochondria (MT) plays multiple roles in calcium homeostasis, apoptosis and physiology in PM skeletal muscle (Contreras, Drago, Zampese, & Pozzan, 2010; Hudson, 2012; Umaki, Mitsui, Endo, Akaike, & Matsumoto, 2002). Under high calcium environment, the integrity of MT would be destroyed, leading to the release of cytochrome C and other pro-apoptotic factors, which finally triggers apoptosis (Berchtold et al., 2000).

* Corresponding author.
E-mail address: gh.zhou@njau.edu.cn (G. Zhou).

http://dx.doi.org/10.1016/j.meatsci.2016.01.003
0309-1740/© 2016 Elsevier Ltd. All rights reserved.

From the perspective of calcium concentration, there is a considerable similarity in the intracellular environments of PSE meat and skeletal muscles from patients with muscular atrophy partially due to apoptosis (Dirks-Naylor & Griffiths, 2009). Further, the functional and morphological disorders of MT were also found in PSE meat (Dutson, Pearson, Merkel, & Spink, 1974; Malila et al., 2013), which were considered to induce or represent the appearance of apoptosis in skeletal muscle (Cao et al., 2010; Umaki et al., 2002). Therefore, in the current study we aimed to explain the calcium status observed in the sarcoplasm in both PSE and RFN (reddish-pink, firm and non-exudative) meat based on the protein expression levels of two calcium channel components (SERCA1 and IP3R). We also investigated the protein expression levels or enzyme activities of the functional apoptotic factors to determine the differences in apoptosis status between porcine PSE and RFN meat.

2. Materials and methods

All animal procedures were reviewed and approved by the Animal Care and Use Committee at the Chinese Academy of Agricultural Sciences.

2.1. Animals and meat samples

A total of 120 castrated Erhualian × Landrace × Yorkshire crossbred pigs weighing between 100 and 120 kg live weight were slaughtered in a commercial abattoir (Jiangsu Sushi Food Group, Huai'an, China) following electrical stunning (110 V, 4 s). Pigs from different production farms were kept in separate compartments during transportation to the abattoir, and held in lairage with water showering for 12 h before slaughter. After the pigs were slaughtered (40 to 60 min), one longissimus muscle (LM) (from the 12th to the last rib) was removed from each of 40 carcasses which were randomly selected from the full set of 120 pigs. The samples were then classified into PSE or normal meat (RFN) based on muscle L* value and pH at 1 h PM, and drip loss at 24 h PM (PSE: L* 1 h > 50, drip loss 24 h > 10%, pH 1 h < 6.0; RFN: L* < 50, drip loss 24 h < 10%, pH 1 h > 6.0). Based on the above criteria, 8 PSE loin samples and 8 RFN loin samples were selected. At 1 h PM, the selected LM samples were immediately put into a 4 °C chiller for the meat quality measurements (Teledoor GmbH, Meller, GER; air velocity: 1 m/s). Simultaneously, a small piece of meat weighing approximately 2 g was removed from each LM samples and stored in liquid nitrogen for both the protein expression and enzyme activity measurements.

2.2. Meat quality measurements

The pH of collected LM samples was measured at 1 and 24 h PM using a portable pH meter (HI9025, HANNA, Co. Italy). The color of the surface meat was measured in triplicate on a freshly cut surface using a portable colorimeter (Minolta Cameras CR 400, Japan) (CIE LAB coordinates, light source D 65, 8-mm diameter) after allowing 10 min for blooming at 4 °C. Three small pieces were removed from each LM samples and then cut into $1 \times 2 \times 2$ cm^3 pieces and weighed. Then they were hung in paper cups at 4 °C for 24 h and re-weighed. The percentage of weight loss, which was determined by the initial weight and the 24 h weight, was recorded as a drip loss. Drip loss was determined in triplicate for each LM sample and the results were obtained to determine an average value.

2.3. SDS-PAGE and Western blotting

We used the meat samples collected 1 h PM and stored in the liquid nitrogen for the SDS-PAGE process. Half a gram of meat was lysed in 3.6 mL lysis buffer (10 mM sodium phosphate buffer, 0.2% SDS [wt/vol], pH 7.0) and the solution was centrifuged at 10,000 ×*g* at 4 °C for 10 min. The supernatant was collected and its concentration was measured using a BCA Protein Assay kit (Thermo Fisher Scientific, Inc., Rockford, IL). The final concentration was adjusted to 10 μg/μL with lysis buffer. One volume of each diluted sample was combined with 0.95 volume of tracking dye solution which was prepared and 0.05 volume of β-mercaptoethanol with a final concentration of 5 μg/μL. The final protein samples were heated at 50 °C for 20 min and then stored at −80 °C.

Gel samples were thawed and subjected to electrophoresis on polyacrylamide separating gels (acrylamide: N,N′-bis-methylene acrylamide = 37.5:1 [wt/wt], 10% [wt/vol] SDS, 0.5% [vol/vol] N′N′N′N′-tetramethylethylenediamine (TEMED), 0.05% [wt/vol] ammonium persulfate (APS), and 500 mM Tris–HCl, pH 8.8) for the determination of caspase 3 (12% acrylamide), SERCA1 and IP3R (7.5% acrylamide), BAX, Bcl-2 and cytochrome C (10% acrylamide), respectively. A 4% polyacrylamide gel (acrylamide: N,N′-bis-methylene acrylamide = 37.5:1 [wt/wt], 10% [wt/vol] SDS, 0.125% [vol/vol] TEMED, 0.075% [wt/vol] APS, and 125 mM Tris–HCl, pH 6.8) was used for the stacking gel.

2.3.1. Running conditions

Gels (10 cm wide × 12 cm tall × 1.5 mm thick) used for analysis of SERCA1, IP3R, BAX, Bcl-2, cytochrome C and caspase 3 were run on a Mini-PROTEAN Tetra cell (Bio-Rad Laboratories, Hercules, CA). The running buffer contained 25 mM Tris, 192 mM glycine, and 0.1% [wt/vol] SDS. Gels were loaded with 120, 100, 80, 80, 100 and 120 μg per lane of total protein for SERCA1, IP3R, BAX, Bcl-2, cytochrome C and caspase 3 respectively, and run at a constant voltage of 60 V for 45 min, and then a constant voltage of 100 V for 85 min.

2.3.2. Transfer conditions

Gels were transferred to polyvinylidene difluoride membranes (PVDF; NEN Life Science Products Inc., Boston, MA) at a constant voltage of 100 V for 70 min for SERCA1 and IP3R, and at a constant voltage setting of 90 V for 45 min for BAX, Bcl-2, caspase 3 and cytochrome C using a Wet Transfer Cell (Bio-Rad Laboratories, Hercules, CA), respectively. The transfer buffer consisted of 25 mM Tris–HCl (pH 8.3), 192 mM glycine, and 10% [vol/vol] methanol. The temperature of the transfer buffer was maintained at 0 °C for all proteins.

2.3.3. Immuno-blotting

We selected the primary antibodies based on the following principle: the amino acid sequence of the antigens must have more than 95% similarity with the corresponding protein to be tested in our study. The blast process was carried out by using “BLAST” function in NCBI. Primary antibodies used included polyclonal rabbit anti-BAX (SC-6236; Santa Cruz, USA; antigen is the C-terminal 21 amino acid of mouse origin BAX; diluted 1:1500), monoclonal mouse anti-Bcl-2 (SC-509; Santa Cruz, USA; antigen is a synthetic peptide corresponding to amino acids 41–54 of human Bcl-2; diluted 1:1000), monoclonal mouse anti-cytochrome C (SC-13560; Santa Cruz, USA; antigen is the full length denatured cytochrome C of human origin; diluted 1:800), monoclonal rabbit anti-caspase 3 (ab90437; Abcam, UK; antigen is the recombinant full length protein of human caspase 3; diluted 1:500), monoclonal mouse anti- SERCA1 (ab109899; antigen is the recombinant fragment corresponding to amino acids 522–613 of human SERCA1; Abcam, UK; diluted 1:4000) and polyclonal goat anti-IP3R (SC-6093; Santa Cruz, USA; antigen is epitope mapping at the C-terminus of IP3R of mouse origin; diluted 1:2000). Secondary antibodies included goat anti-rabbit horseradish peroxidase (HRP) (AP132P; Millipore, USA; diluted 1:5000 for BAX and caspase 3), sheep anti-mouse (HRP) (ab97100; Abcam, UK; diluted 1:4000 for Bcl-2, for cytochrome C and SERCA1), and rabbit anti-goat horseradish peroxidase (HRP) (ab97023; Abcam, UK; diluted 1:4000 for IP3R). BAX, Bcl-2, cytochrome C, SERCA1 and IP3R were indicated at the 25, 29, 12, 110 and 270 kDa bands, respectively. The zymogen and cleaved fragment of caspase 3 were indicated at the 32 and 18 kDa respectively. The electro-blotted membranes were blocked at room temperature in blocking buffer (TTBS: 0.1% [vol/vol] Tween 20, 20 mM Tris, pH 7.5,

Download English Version:

https://daneshyari.com/en/article/2449558

Download Persian Version:

https://daneshyari.com/article/2449558

Daneshyari.com