Meat Science 145 (2018) 455–460

Contents lists available at ScienceDirect

Meat Science

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

Investigation of the effects of high pressure processing on the process of rigor in pork

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ARTICLE INFO

Keywords:
High pressure processing
Meat
Rigor
Myofibrillar fragmentation index
Enzyme activity
Texture

ABSTRACT

This study was conducted to determine whether the application of high pressure processing (HPP) could prevent the process of rigor in fresh pork and to identify the physical and chemical changes occurring in the meat that are responsible for this effect. Pressure (0–400 MPa) and time (10 min) were applied to the pork loin and enzyme samples. The effects of treatment on shear force, myofibrillar fragmentation, enzyme activity and meat texture were measured. The results showed that, compared with untreated samples, HPP treatment inhibited the occurrence of rigor in pork. The myofibrillar fragmentation indices of HPP treated samples were higher than those of control samples and increased linearly with increasing pressure. Application of HPP prevented the process of rigor at 0–300 MPa, reduced calpain activity and markedly decreased calpastatin activity, resulting in an increase of total calpains. Above 300 MPa, the mechanical action of pressure led to continuous prevention of rigor.

1. Introduction

Alongside a general improvement in quality of life in developed countries, consumption of meat and meat products is increasing. Of all the sensory characteristics of meat, tenderness is considered by consumers to be the most important (Miller, Carr, Ramsey, Crockett, & Hoover, 2001). This has led manufacturers to update or improve their existing meat processing technologies in order to retain the tenderness of meat and meat products during processing. One novel technology that has emerged as a result is high pressure processing (HPP), a cold pasteurization technique that can improve the safety and quality of prepacked foods by inactivating microorganisms that may be present (bacteria, viruses, yeasts, moulds), changing the food microstructure but with only minimal changes to nutrient content and taste (Balasubramaniam & Farkas, 2008). The technology is mainly used to improve food safety and to increase the shelf-life of meat products (Garriga & Aymerich, 2009; Sun & Holley, 2010). However, its effects on the tenderization of meat are also of interest and have been the subject of several previous studies (Cao, Xia, Zhou, & Xu, 2012; Hurtado, Montero, & Borderías, 2001). Especially be applied for the pre-rigor meat (Kennick, Elgasim, Holmes, & Meyer, 1980; MacFarlane, 1973; Morton, Lee, Pearson, & Bickerstaffe, 2018), while moderate pressure treatment of pre-rigor meat seems to have potential since the meat will be tender and look normal. Regarding post-rigor meat the benefits are less clear cut and loss in perceived color and possibly flavour quality due to the elevated temperatures required (Ma & Ledward, 2013).Values of meat tenderness obtained from samples that have been subjected to HPP are known to be generally higher than unprocessed samples (Kennick et al., 1980; Riffero & Holmes, 1983). However, few studies have explored the effects of HPP on the natural postmortem metabolic processes that occur in pre-rigor meat *via* the process of rigor, which results in the gradual toughening of the meat and reduces tenderness. Koohmaraie, Kennick, Elgasim, and Anglemier (1984) studied the influence of pressurization on the enzymatic process of rigor which has been supposed to be partial and incomplete. In particular, it is not known if HPP can reduce or attenuate the process of rigor in pre-rigor meat. If such effects were evident, the potential economic benefits of implementing HPP more widely as a processing step in the meat industry and reducing the energy and time spent in employing other methods to improve meat tenderness would be obvious.

Preliminary experiment by the present authors indicated that the application of high pressure to fresh pre-rigor meat resulted in the meat having the texture of pre-rigor muscle with no evidence of rigor. The present study aimed to further investigate the effects of HPP on the degree of fragmentation of pre-rigor meat fibers as well as on the activities of key proteolytic enzymes and the resulting texture of meat.

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https://doi.org/10.1016/j.meatsci.2018.07.013
Received 6 April 2018; Received in revised form 10 July 2018; Accepted 10 July 2018
Available online 11 July 2018

2. Materials and methods

2.1. Meat sample preparation

2.1.1. Post-rigor ageing meat

Aged meat samples were obtained from pork carcasses (one-year-old pigs, 90–100 kg body weight, from a local abattoir) that were hung by the Achilles tendon without packaging for 24 h at 4 °C following slaughter (total of 17 loins). Parts of the loins were boned from the carcasses after hanging for 8 h and thereafter every hour up to 24 h of hanging in the same place of different loins. The loins were cut in parallel to the muscle axis to a size of 8 cm × 5 cm × 5 cm (length × width ×height) and sampling for shear force measurements was done immediately during the process of hanging. After 24 h (when hanging was finished), another three samples were individually vacuum packed in polyethylene bags using a DZ-400 vacuum packaging machine (Hefei Yifei Packaging Machinery Co., LTD, China), and stored at 4 °C until further use. Shear force measurements of the samples were undertaken also at 1.5, 2.5 and 3.5 days. The maximum value of shear force measurement obtained within the first 24 h was taken as that of complete rigor. The values of shear force measurement obtained after 1.5 days were considered to be those of ageing meat. The data presented for each sample are mean values from five measurements.

2.1.2. HPP meat

Pork loins were also obtained from one-year-old pigs and total of 35 samples of the same size as in 2.1.1(obtained from 5 loins) were prepared. Meat samples were individually vacuum packed in polyethylene bags using a DZ-400 vacuum packaging machine and stored at 4 °C until HPP. The preparation of HPP meat samples was completed within 2 h of slaughter (without hanging). Shear force measurements were carried out immediately after HPP, and thereafter at 0.5, 1.5, 2.5 and 3.5 days post-treatment. Additional HPP meat samples were also used for the measurement of the myofibrillar fragmentation index (MFI) and texture analysis 12 h after HPP. The shear force data for each sample are present as a mean value from five measurements, while others were from three measurements.

2.2. Enzyme preparation

An adequate amount of μ-calpain (Sigma.CO, USA), m-calpain (Sigma.CO, USA) and calpastatin (Sigma.CO, USA) were dissolved with 100 mL of normal saline. Each vacuum-packed pouch was then filled with 10 mL of the enzyme solution and kept at 4 °C before HPP. The enzyme samples were used for enzyme activity analysis 12 h after treatment. The data presented for each sample are mean values from three measurements.

2.3. High pressure processing

The meat and enzyme samples were separately transferred to a 1 L pressurization vessel (120 mm diameter × 300 mm length; Inner Mongolia, China) immediately after packaging. Oil (Ester) was used as the pressurization fluid. The samples were treated at 100, 200, 300 and 400 MPa for 10 min, at an initial temperature of 20 °C (± 3 °C) respectively. Pressures were increased and reduced at intervals of 3 and 5 MPa/s, respectively. Untreated samples were retained as a control treatment. All samples were subsequently stored at 4 °C until further analysis following HPP.

2.4. Shear force measurement

Meat tenderness was measured by a meat tenderness meter C-LM3 (Tenovo International Co., Ltd., China) which calculates the Warner-Bratzler Shear Force (WBS). The meat samples were trimmed into 6 cm × 3 cm × 3 cm (length × width ×height) pieces. Samples were cooked at 80 °C (in a water bath) until the center temperature reached 70 °C and they were then chilled and stored at 4 °C until the meat center temperature was 0–4 °C. Measurements were taken immediately and the penetration of the shear blade was set vertical to the direction of the meat fibers.

2.5. Determination of myofibril fragmentation index

The method employed was based on that described originally by Hopkins, Littlefield, and Thompson (2000), with slight modifications. For each sample, 5 g of minced muscle meat was diluted 10-fold in buffer solution (containing 100 mmol/L KCl, 20 mmol/L K-phosphate, 1 mmol NaN_3,1 mmol/L EDTA, and 1 mmol/L $MgCl_2$, adjusted to a pH of 7.0), and homogenized in a homogenizer (FM200, FLUKO, Germany) at 15,000 rpm for 30 s at 2°C. The resulting extracts were centrifuged at 1000 ×*g* for 15 min at 2°C (in a 3 K15 centrifuge, Sigma, Germany). The pellets containing the myofibrils were then re-suspended by diluting 10-fold in the same buffer as before. The suspensions were centrifuged for a second time at 1000 ×*g* for 15 min at 2°C and the pellets re-suspended by diluting 2.5-fold in buffer. The resulting suspensions were filtered through a strainer that was rinsed with another 2.5 volumes (L/g) of buffer to remove any myofibril residue. The protein concentration of the final suspension was determined by the Biuret method (Gornall, Bardawill, & David, 1949). Aliquots of the suspensions were diluted in buffer to a final protein concentration of 0.5 mg/mL. The absorbance at 540 nm was monitored using an ultraviolet spectrophotometer (UV-1600, Beijing Ruili Analytical Instrument Co. Ltd.). To obtain the MFI values, each absorbance was multiplied by 150.

2.6. Determination of enzyme activity

Calpain activity was determined in accordance with the method described by Homma, Ikeuchi, and Suzuki (1995), with slight modifications. The activity was assayed by preincubating the reaction mixture for 5 min at 25 °C before adding 0.5 mL of μ-calpain(1.5 mg/mL) or m-calpain (1.5 mg/mL) solution to start the reaction. The incubation mixture contained 100 mM Tris-HCl buffer adjusted to pH 7.5, 10 mM 2-mercaptoethanol (MCE), 5 mg/mL casein and either 100 μM $CaCl_2$ (for μ-calpain activity measurement) or 5 mM $CaCl_2$ (for m-calpain activity measurement), to achieve a final volume of 2 mL at 25 °C. In the standard samples, calpain was replaced by normal saline at an equal volume. The reaction was stopped at the appropriate time by addition of 5% trichloroacetic acid (TCA) to give a final TCA concentration of 2.5%. The mixture was then filtered, the precipitate removed and the absorbance of the supernatant measured at an optical density of 280 nm using a spectrophotometer. Calpain activity was calculated according to the following formula:

$$\text{Calpain activity}\,(\%) = 100 \times \frac{\text{standard}\,(\Delta\ \text{OD280 nm}) - \text{Sample}(\Delta\ \text{OD280 nm})}{\text{Standard}(\ \Delta\ \text{OD280 nm})}$$

Calpastatin activity was determined in accordance with the method described by Koohmaraie, Seideman, Schoolmeyer, Dutson, and Crouse (1987), with slight modifications. First, the reaction mixture was preincubated with 0.5 mL of m-calpain (1.5 mg/mL) and 1.5 mL of calpastatin for 1 min at 25 °C, before starting the reaction by the addition of 1.5 mL of Tris-HCl buffer (100 mM) adjusted to a pH of 7.5 by addition of 10 mM MCE, 5 mM $CaCl_2$ and 5 mg/mL casein, to achieve a final volume of 3 mL. For the control sample, calpastatin was replaced by an equal volume of normal saline. The reaction process was stopped after 50 min by adding 3.0 mL 5% TCA. Calpastatin activity was calculated according to the following formula: