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Proteolysis in pork loins during superchilling and regular chilling storage

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ABSTRACT

This study aimed to address the proteolytic phenomena taking place in pork loins during prolonged storage at superchilling (SC) temperature. Loins were stored at either chilling (CH) conditions (2–4 °C) for 4 weeks or at SC temperature (around −1 °C) for 12 weeks. Storage at SC temperatures slowed down the rate of proteolysis in pork loins, so that final levels of most indicators for proteolysis, including after 12 weeks of SC storage were similar to those after 4 weeks at CH conditions. Consequently, the texture of SC pork under extended storage was not so different to that of CH pork. However, total amino acid content peaked by the end of SC storage, pointing out to a potential ongoing exopeptidase activity. Overall, proteolysis seemed to be slowed down in pork at SC conditions, with similar levels for most indicators after 12 weeks of SC storage or 4 weeks at CH conditions.

1. Introduction

Chilled and frozen storage are extensively used to extend shelf-life of different fresh foodstuff. In the last decades, a new cold storage technology, referred to as superchilling, has been introduced and mostly implemented for the fish industry (Kaale, Eikevik, Rustad, & Kolsaker, 2011).

Superchilling is a process where the temperature of foods is lowered slightly below the initial freezing point of the product (Duun, Hemmingsen, Haugland, & Rustad, 2008). During the initial cooling, when the temperature is brought below the freezing point of the food, a thin layer of ice on the surface of the product is formed ("crust-freezing"). The ice equalizes within the product during further storage, until the product reaches a uniform temperature and appears as fresh.

The shelf life of meat is mainly determined by microbial growth (North & Lovatt, 2012). Due to the effect of temperature on microbial growth, spoilage in superchilled meat is delayed compared to conventional chilling, leading to a shelf-life of up to 4 times longer compared to conventional chilling (Jeremiah, Gibson, & Argnosa, 1995). This allows the distribution of fresh meat to distant markets, avoiding freezing and the consequent loss of quality and economical cost related to freeze-thawing (Leygonie, Britz, & Hoffman, 2012).

However, crust freezing during the initial phase of cooling, and subsequent ice crystals formation within the product during storage, may cause structural damages to the cell membranes leading to a decrease in quality of the product (Kaale, Eikevik, Bardal, & Kjorsvik, 2013). Additionally, partial freezing of water causes an increase in the concentration of solutes in the unfrozen solution, which may lead to changes in pH values and denaturation of muscle proteins (Leygonie et al., 2012). In previous studies on Atlantic salmon, it has been shown that ice crystals formed during superchilling where large enough to damage the integrity of fish muscle cells (Kaale et al., 2013), and in superchilled fish muscles, myofibrillar breakages were more evident (Bahuaud et al., 2008) and calpain activity was higher compared to ice-stored fish (Gaarder, Bahuaud, Veiseth-Kent, Mørkøre, & Thomassen, 2012).

Meat tenderness is one of the most important quality attributes to consumers and muscle proteolysis post-mortem is known to improve meat tenderness. Calpains and cathepsins are protease systems that have been shown to be important contributor to proteolytic tenderization of pork during storage (Huff Lonergan, Zhang, & Lonergan, 2010). The calpain system consists of several proteases, of which two, μ- and m-calpain, are involved in the proteolytic degradation of myofibrillar proteins (Pomponio et al., 2008). Both enzymes are heterodimer composed of a distinct 80 kDa catalytic subunit and a common 28 kDa regulatory subunit, both calcium dependent and activated throughout autolysis (Goll, Thompson, Li, Wei, & Cong, 2003). Cathepsins are lysosomal peptidases shown to be involved in the tenderization process of meat, after the disruption of lysosome membranes due to accumulation of lactic acid (Ertbjerg, Henckel, Karlsson, Larsen, & Møller, 1999).

On the other hand, the proteolytic phenomena during cold storage of pork (ageing), lead to the release of free amino acids and a range of peptides of different MW (Moya, Flores, Aristoy, & Toldrá, 2001a). These compounds might affect the flavor of fresh pork, due to the taste notes of different amino acids and peptides, some of which are

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considered very positive due to their umami character (Nishimura, Ra Rhue, Okitani, & Kato, 1988).

It is reasonable to consider that physical and chemical changes in the unfrozen solution during cooling and superchilled storage might have an influence on tenderness and the tenderization process during cooling and storage of meat, and subsequently on the release of free amino acids. Changes in pH and increase in solutes concentration (i.e. Ca2+) can influence the proteases activity, and physical disruption of muscles cells due to ice crystals formation might have an influence on the location of proteases (i.e. release of lysosomal cathepsins into the cytosol) and myofibrillar integrity.

Most of the scientific studies on superchilling have investigated its influence on fish quality and the proteolytic activity of enzymes in fish muscles (Duun & Rustad, 2007; Olafsdottir, Lauzon, Martinsdóttir, Oehlenschläuger, & Kristbergsson, 2006) while for meat, the available information on the effect of superchilling on quality is very limited, and to our knowledge, the potential effect on the overall proteolytic phenomena has not yet been investigated.

This study aimed to gain a deeper understanding of the proteolytic phenomena that take place in pork loins during prolonged superchilled storage and their potential implications on meat quality.

2. Materials and methods

2.1. Experimental design

Samples were obtained from a commercial slaughterhouse. Pork loins ($n = 70$) from a homogeneous batch of pigs were dissected from carcasses the day after slaughter and subsequently vacuum packed. Animals were female pigs from the same farmer and vendor (implying same genetics, feeding and transportation conditions), with a slaughter weight of 83–86 kg, a lean percentage of the carcass around 60% and a final pH ranging from 5.5 to 5.6. Animals were stunned with CO2 and dressing activities were performed within 60 min post-mortem at the abattoir. The average weight of loins was 2.2 ± 0.2 kg. All loins were vacuum packaged (LogiCon EM-628824 - Vacuumpose, Kolding, Denmark). Twenty-one loins were stored at chilling (CH) temperatures (2.5 ± 0.2 °C) and forty-nine were subjected to crust freezing through impingement, followed by superchilling (SC) storage (−1.0 ± 0.3 °C). Impingement cooling is a process based on directing jets of cold air (−20 to −30 °C) at a product surface to rapidly decrease its temperature. The high air speed (20–30 ms^{-1}) disrupts the static surface boundary layer of air surrounding the product and produces a turbulent area around, which makes the heat exchange much more effective.

Sampling was performed throughout storage at 0, 2 and 4 weeks for both CH and SC, and further sampling for SC loins were performed at weeks 6, 8, 10 and 12. At each time point, 7 loins for each treatment were used and samples were collected from the center of the loin. Dry matter of samples was analyzed immediately after sampling. The rest of the sample was vacuum packaged and kept frozen at −80 °C until analysis.

2.2. Warner-Bratzler shear force (WBSF)

Shear-force was measured using the Warner–Bratzler method as described by Honikel (1998). Vacuum packaged fresh samples from each storage system were heat treated for 30 min at 75 °C in a water bath and thereafter chilled in ice water for 10 min. Samples were stored overnight at 4 °C.

Five rectangular shaped blocks (1 × 1 × 5 cm) were cut and each block was sheared three times perpendicularly to the muscle fibre direction with a rectangular-shaped shear blade on an Instron Universal testing machine (High Wycombe, UK). The cutting blade was 1 mm thick and had a speed of 0.83 mm s^{-1} when cutting through strips. The average of 12 shear values represented the WB shear force value for each sample. The maximum load required to shear through the sample was determined.

2.3. SDS-page

At sampling, meat was minced and 0.1 g was homogenized in 0.6 mL (0.027M Tris-Base, 0.021M Tris-HCl, 0.005M EDTA, 0.01M DTT, pH 8.3, 4 °C) and seven stainless steel grinding beads (2 mm of diameter) in a Mixer Mill MM 400 (Retsch GmbH, Haan, Germany) for 2 min, vibrational frequency 30 Hz, and subsequently centrifuged (20,000*g*, 20 min, 4 °C) (Sigma 3K15 centrifuge, Sigma Laborzentrifugen GmbH Osterode am Harz, Germany). The supernatant was stored at −80 °C until analysis (sarcoplasmic proteins solution). The pellet was re-suspended with 0.6 mL (0.1 M Tris-Base, 10% SDS, pH 8.0) by mixing for 3 min (30 Hz) in the Mixer Mill, heat treated in a water bath (80 °C, 30 min) and stored at 4 °C until analysis (myofibrilar proteins solution).

Before protein separation with SDS-PAGE, sarcoplasmic proteins solutions were thawed on ice and centrifuged (20,000*g*, 4 min, 4 °C) (Sigma 3K15 centrifuge). Myofibrilar proteins solutions were heat treated (80 °C, 30 min) and centrifuged (4500*g*, 20 min, 21 °C) (Sigma 3K15 centrifuge). Protein solutions were added with 10 μl of 1 M DTT and 25 μL of NuPAGE LDS sample buffer, and were further separated using NuPAGE® Novex 4–12% gradient Bis-Tris Gels (Novex, Thermo Fisher Scientific Inc.). Electrophoresis was carried out at 200 V for 60 min. Protein concentration in the solutions was determined by measuring the absorbance at 280 nm using a UV–visible spectrophotometer (ND-1000, NanoDrop Technologies, Wilmington, DE, USA). The amount of protein loaded in each lane was 5 μg, and 0.1 M DTT was added directly to samples when reducing conditions was employed during electrophoresis. Gels were stained with colloidal Coomassie Brilliant Blue G (Rabilloud, 2000) and scanned with a SilverFast Ai IT8 Studio (Multi-Exposure) Version 6.6 (Epson Perfection V 750 Pro). Protein bands were analyzed with TotalLab TL120 Nonlinear Dynamics Ltd.

2.4. Cathepsin B+ L activity

Activity of Cathepsins B and L was measured fluorometrically utilizing methylcoumarylamide as substrate (Kirschke, Wood, Roisen, & Bird, 1983). From each samples, duplicates of 2 g of meat were homogenized at 13500 rpm using an Ultra-Turrax T25 Mixer (Ika Labortechnik, Staufen, Germany) in 6 mL of an extraction buffer (100 mM sucrose, 100 mM potassium chloride, 50 mM tris-HCl, 10 mM sodium pyrophosphate, 1 mM EDTA, 0.2%Triton ×100, pH 5.0). These were further centrifuged (15,000*g*, 30 min, 4 °C) (Herolab, HiCen 21, Wiesloch, Germany) and filtered through a 0.45 μm filter. An aliquot of the supernatant was mixed with glycerol to a final concentration of 30% and was stored at −80 °C until analysis for enzyme activity. Samples were thawed at room temperature and analyzed in triplicates on a 96 well microtiter plate using the following procedure: 25 μL of sample was mixed with 100 μL of buffer A (340 mM sodium acetate, 60 mM 100% acetic acid, 4 mM EDTA, 8 mM dithiothreitol, 0.1% Brij 35, pH 5.5) and heated to 37 °C for 5 min. Samples were incubated with 75 μL substrate (12.5 μM *Z*-Phe-Arg-Nmec (Sigma-Aldrich, Steinheim, Germany)) and activity was measured every 10 min up to 30 min at 37 °C, at excitation and emission wavelengths of 355 nm and 460 nm, respectively, using a Fluoroskan Ascent plate reader (Thermo Labsystems, Helsinki, Finland). Standards were made with 7-amino-4-methyl coumarin (Sigma-Aldrich, Steinheim, Germany) and extraction buffer was used as the blank. The activity was expressed in μU/g meat where 1 unit (U) was defined as 1 μmol product produced per minute at 37 °C.

2.5. Calpain activity

From each frozen sample (−80 °C), 1.5 g of meat were homogenized using an Ultra-Turrax T25 Mixer (13,500 rpm) in 9 mL of an extraction