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Relationship between oxygen concentration, shear force and protein oxidation in modified atmosphere packaged pork

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ABSTRACT

Pork loins were stored at 5 °C for 14 days to investigate the effect of oxygen concentration in modified atmosphere packaging (MAP) on shear force and oxidation of lipids and proteins. The modified atmosphere contained 0 to 80% O_2, 20% CO_2, and balanced with N_2. The results showed that shear force and thiobarbituric acid-reactive substances (TBARS) values increased with increasing oxygen concentration. Protein oxidation when measured as loss of free thiol groups, was greater in meat packaged under oxygen (20–80%). Myosin heavy chain (MHC) cross-linking, another marker of protein oxidation, was greater in MAP with 80% oxygen than 0% and 20% oxygen. Desmin degradation was not affected by the presence of oxygen, suggesting that the mechanism of oxygen-induced toughening of meat is through protein oxidation leading to cross-linking of structural proteins rather than through inactivation of proteolytic enzymes leading to reduced proteolysis.

1. Introduction

Protein oxidation is initiated by reactive oxygen species which generate protein radicals in meat. Protein oxidation then progresses through free radical chain reactions, which may lead to carbonylation of amino acid side chains, loss of free thiol groups and formation of protein cross-links (Lund, Heinonen, Baron, & Estevez, 2011). Modified atmosphere packaging (MAP) with high oxygen (70 to 80%) and carbon dioxide (20 to 30%) is widely used for red meat. The high oxygen is used to maintain the desirable bright red color (McMillin, 2008). However, high oxygen MAP promotes oxidation of lipids and proteins, which affect meat quality negatively, leading to off-flavors, and reduced tenderness and juiciness in beef (Clausen, Jakobsen, Ertbjerg, & Madsen, 2009; Cruzen et al., 2015; Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010) and pork (Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007). It is thus becoming clear that high oxygen MAP can cause an increase in meat toughness. The mechanisms involved are not well understood, but are probably related to the oxidative conditions introduced by the high concentration of molecular oxygen.

Oxidation of the cysteine residue in the active site of calpain-1 resulted in loss of activity (Guttmann, Elce, Bell, Isbell, & Johnson, 1997; Lametsch, Lonergan, & Huff-Lonergan, 2008) and calpain-1 is believed to be involved in postmortem meat tenderization. Rowe, Maddock, Lonergan, and Huff-Lonergan (2004) found that strong oxidative conditions caused by irradiation inactivated calpain-1 and reduced the degradation of nebulin, titin, desmin and troponin-T in beef steaks, and in addition the beef steaks showed higher shear force compared to non-irradiated samples. Clausen et al. (2009) showed reduced tenderness of beef in high oxygen MAP accompanied by increased lipid and protein oxidation as well as a lower myofibril fragmentation index. Myofibril fragmentation (Olson & Stromer, 1976) has often been reported to be negatively correlated to shear force and is generally believed to reflect the degradation of proteins within the myofibril. Therefore, reduced proteolysis of meat structural proteins may contribute to toughening of meat in high oxygen MAP.

Oxidation-induced protein cross-linking was suggested as another mechanism for oxygen-induced toughening of meat by Lund et al. (2007). They reported in high oxygen MAP the formation of cross-linked myosin heavy chain (MHC) in pork together with a lower sensory tenderness and larger myofibril fragments measured as higher mean diameter of particles. The larger particle size suggested less myofibril fragmentation. It is reasonable to hypothesize that both protein cross-linking and reduced proteolysis contribute to oxygen-induced meat toughening in high oxygen MAP. However, it remains unclear which of these mechanisms has the greater influence on meat tenderness.

In order to study the effect of oxygen concentration on oxidation reactions and meat toughness, porcine *longissimus thoracis et lumborum* (LTL) muscle was packaged in modified atmosphere with different oxygen concentrations (0, 20, 40, 60 and 80%) together with 20% CO_2 and

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stored at chilled temperature for two weeks. Shear force, oxidation of lipid and protein, and protein degradation were evaluated.

2. Materials and methods

2.1. Sample preparation

The animals belonged to the cross Norwegian Landrace × Swedish Yorkshire × Danish Landrace and were slaughtered at a conventional slaughterhouse in Finland. A total of 6 pork loins (3.29 ± 0.47 kg) from different animals were obtained 1 day postmortem (day 0 of storage). Each loin was trimmed of visible connective tissue and external fat. The LTL muscle was isolated and sliced into 6 pieces with a width of 6 cm. Then these samples were allocated for MAP with different oxygen concentration (0, 20, 40, 60 and 80%) and the remaining piece was used as 1 day postmortem non-aged control. In addition all packages were balanced with N_2 and contained 20% CO_2 to inhibit the microbial growth. The number of meat samples for each packaging system was 6 (one sample from each of the 6 loins).

Packaging was performed on a Multivac D-8941 (Sepp Haggenmüller GmbH & Co., Wolferschwenden, Germany) using a film based on polyamide/polyethylene (CO 80 HFP, Wipak, Nastola, Finland) with an oxygen transmission rate around 4 $cm^3\ m^{-2}\ (24\ h)^{-1}$, 4 °C/50% RH. The ratio of headspace to meat volume was 2:1 and the different gas combinations were controlled by a gas controller Witt-Gasetechnik D-5810 (Witt-Gasetechnik GmbH & Co KG., Witten, Germany) and examined by Checkmate 9900 O_2/CO_2 (Dansensor, Ringsted, Denmark). For each gas combination two packages were checked for changes in composition after storage and no major change was observed. All packages were placed in a 5 °C walk-in cooler for 2 weeks and received light of approximately 2000 lx for 12 h every day from tubular lamps (OSRAM L 36W-76 G13 NATURA, Osram, Munich, Germany) which were positioned at a distance of about 30 cm above the packages.

After storage, each package was opened and the meat piece was sampled for determination of thiobarbituric acid-reactive substances (TBARS), free thiol groups and Allo–Kramer shear force. For the Western blot analysis of desmin and myosin heavy chain (MHC), a sample of about 30 g was taken from each piece and stored in vacuum at −80 °C.

2.2. Allo–Kramer shear force

Determination of shear force was conducted as described by Liu, Ruusunen, Puolanne, and Ertbjerg (2013). After removal of the packages, the meat pieces were cut into cubes (around 7.0 × 7.0 × 6.0 cm) and sealed in individual thin-walled plastic bags and cooked in water bath (75 °C, 30 min). After cooking, they were kept in a cold room (5 °C) overnight. From each sample, 4 to 6 slices of 20 × 20 × 6 mm (fiber axis along the 20 mm direction) were cut and weighed. Then each slice was placed in an Allo–Kramer shear cell and cut across the fiber using an Instron Model 6625 (Instron Co., Canton, MA) with a load cell of 5 kN. The result was expressed as N/g.

2.3. Thiobarbituric acid-reactive substances (TBARS)

TBARS were measured according to the method of Salih, Smith, Price, and Dawson (1987), with some modifications by Utrera, Morcuende, and Estévez (2014).

Briefly, a muscle sample (5.0 g) was homogenized with 15 mL trichloroacetic acid (5%, w/v) and 0.5 mL butylated hydroxytoluene (4.2% in ethanol, w/v) in ice bath. The homogenization was done by IKA Ultra-Turrax T25 homogenizer (Labortechnik, Staufen, Germany) at 13,500 rpm for 30 s. The slurry was filtered with filter paper (Whatman 40, GE Healthcare), and an aliquot of 2 mL filtrate was mixed with 2 mL thiobarbituric acid (0.02 M) in a test tube and boiled in a water bath (100 °C) for 40 min. After cooling, absorbance was read at 532 nm. A standard curve of 1,1,3,3-tetraethoxypropane was used to calculate the amount of malondialdehyde produced. TBARS content was expressed as mg malondialdehyde/kg meat.

2.4. Thiol groups

Protein thiol groups were determined using DTNB (5,5′-Dithiobis(2-nitrobenzoic acid)) (Ellman, 1959) with some modifications based on Lund et al. (2007).

Briefly, 1.0 g meat was homogenized with 25 mL 5% (w/v) SDS in 0.1 M Tris–HCl (pH 8.0) at 13,500 rpm for 30 s. The homogenates were heated in a water bath at 80 °C for 30 min. After cooling, the homogenates were filtered through filter paper (Whatman 40, GE Healthcare). The protein concentration of the filtrate was determined by reading absorbance at 280 nm and calculated from a standard curve prepared from 0 to 2 mg/mL bovine serum albumin (BSA). Thiol groups were measured by mixing 0.5 mL filtrate, 2 mL of 0.1 M Tris–HCl (pH 8.0) and 0.5 mL 10 mM DTNB in 0.1 M Tris–HCl (pH 8.0). The mixture was incubated in the dark at room temperature for 30 min. Absorbance at 412 nm was recorded and the content of thiol groups was calculated using a standard curve prepared from L-cysteine and expressed as nmol/mg protein.

2.5. Western blot against desmin and myosin heavy chain (MHC)

Frozen samples (−80 °C) were thawed and cut into small pieces. Sample filtrates were prepared as for the determination of thiol groups. For gel electrophoresis, NuPAGE (Invitrogen, CA, USA) Novex 12% Bis-Tris gels and 3–8% Tris-Acetate gels were used for desmin and MHC analysis, respectively. For MHC analysis, no reducing agent was added in order to study the protein cross-linking. The amount of protein loaded into each lane was 30 μg. After electrophoresis, proteins in gels were transferred at 30 V for 1 h onto Immobilon-FL Transfer Membrane (Millipore, Bedford, MA). Transfer buffer was prepared from NuPAGE Transfer Buffer (20×) and 10% methanol was added for desmin analysis, while 5% methanol and 0.05% Tween-20 were added for MHC to facilitate transfer of the high molecular weight target proteins. Membranes were probed and quantified according to Liu et al. (2013). Desmin was detected by mouse monoclonal anti-desmin antibody clone DE-R-11 (Santa Cruz, CA, USA) at 1:5000 and MHC was detected by mouse monoclonal anti-myosin (Skeletal, Fast) antibody clone MY-32 (Sigma-Aldrich, Saint Louis, US) at 1:40,000.

2.6. Data analysis

All biochemical analyses were run in duplicate. Data were analyzed by the IBM SPSS Statistics 21 software using general linear model. Packaging atmosphere was included as fixed factor and animal number as random factor. Tukey HSD test was used to find significant differences at a level of $P < 0.05$.

3. Results

3.1. Allo–Kramer shear force

The effect of oxygen concentration in MAP on Allo–Kramer shear force is shown in Fig. 1. After two weeks storage, all the meat samples had lower shear force compared to the day 0 sample ($P < 0.001$). For aged samples, the shear force increased ($P < 0.001$) with increasing oxygen concentration. Aging in 0% oxygen MAP caused a 43% decrease of shear force value from 107 N/g to 62 N/g, while aging in 80% oxygen only decreased shear force by 14% to 92 N/g. At lower oxygen concentrations of 20 and 40%, the shear force was higher compared to 0% oxygen, and the shear force increased further at 60 and 80% oxygen.