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High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine *longissimus dorsi* during chill storage

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Abstract

The effect of modified atmosphere packaging (70% O_2/30% CO_2) and skin packaging (no oxygen) on protein oxidation and texture of *longissimus dorsi* was investigated during storage for 14 days at 4 °C. High oxygen atmosphere resulted in reduced tenderness and juiciness and SDS–PAGE revealed cross-linking of myosin heavy chain through disulfide bonding, and the content of protein thiols was reduced indicating protein oxidation. Myofibril fragmentation was reduced in meat stored in high oxygen atmosphere indicating less proteolysis and/or cross-linking of proteins. Protein carbonyl content was not affected by the packaging atmospheres. This study shows that packaging in modified atmosphere containing a high level of oxygen can result in protein cross-linking and reduced tenderness and juiciness of the meat.

Keywords: Pork; Protein oxidation; Modified atmosphere packaging; Cross-linking; Texture

1. Introduction

Modified atmosphere retail packaging with a high level of oxygen (70–80%) find an increasing use for fresh meat as high oxygen concentrations preserve the bright red colour of fresh meat and increase shelf-life by reducing microbial growth (Okayama, Muguruma, Murakami, & Yamada, 1995). However, high concentration of oxygen may increase levels of oxidation in meat which has been shown for meat lipids to cause rancidity (Cayuela, Gil, Bañón, & Garrido, 2004; Monahan, 2003; Okayama et al., 1995). It is yet unknown how protein oxidation and texture of pork are affected by high oxygen atmosphere.

In meat, protein oxidation may lead to decreased eating quality such as reduced tenderness and juiciness, flavour deterioration, and discoloration (Xiong, 2000). Physical and chemical changes in oxidized proteins include amino acid destruction, decreases in protein solubility due to protein polymerization, loss of enzyme activity, and formation of amino acid derivatives including carbonyls (Meucci, Mordente, & Martorana, 1991; Stadtman & Oliver, 1991; Starke-Reed & Oliver, 1989; Uchida, Kato, & Kawakishi, 1992).

One of the consequences of protein oxidation is the formation of protein aggregates through both non-covalent and covalent intermolecular bonds. The oxidation-mediated formation of protein–protein cross-linked derivatives in meat can occur by several reactions which include oxidation of cysteine thiol groups to form disulfide bonds, formation of dityrosine, and reaction between a carbonyl in one protein and with an ε-amino group of a lysine residue

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in another protein (Xiong, 2000). Additionally, intermolecular cross-links formed in highly oxidative conditions can make proteins less susceptible to enzymatic proteolysis (Davies, 2001) reducing development of tenderness by proteolysis in meat.

In a recent study, protein oxidation and proteolytic activity of the tenderizing enzyme calpain was investigated in beef steaks after irradiation and subsequent aging for up to 14 days (Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004a, 2004b). The oxidative treatment was found to decrease tenderness of the beef, increase protein carbonyl content, and decrease calpain activity indicating a connection between tenderness, protein oxidation, and proteolysis in meat.

In the present study we describe the consequences for the texture and protein oxidation of the use of commercial high oxygen atmosphere packaging compared to anaerobic skin packaging for storage of LD slices for up to 14 days at 4 °C.

2. Materials and methods

2.1. Chemicals

Potassium dihydrogenphosphate, dipotassium hydrogenphosphate, trichloroacetic acid (TCA), trifluoroacetic acid (TFA), ethylenediaminetetraacetic acid (EDTA), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), L-cysteine, tris(hydroxyamino)methane (tris), hydrochloric acid, and sodium chloride were obtained from Merck, Darmstadt, Germany. Acetone, ethyl acetate, and acetonitrile were obtained from Lab-scan Ltd., Dublin, Ireland. 2,4-Dinitrophenylhydrazine (DNPH), ammonium bicarbonate, and sodium dodecylsulphate (SDS) were obtained from Bie & Berntsen, Rødovre, Denmark. Sodium pyrophosphate, tris-maleate, ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA), dithiothreitol (DTT), and iodoacetamide were from Acros Organics, Geel, Belgium. Guanidine hydrochloride, magnesium chloride, and bovine serum albumin (BSA) were from Sigma Chemical Co., St. Louis, USA. Potassium chloride was from J.T. Baker, Holland. Absolute ethanol was obtained from Danish Distillers, Aalborg, Denmark. NaOH was obtained from Merck Eurolab (Albertslund, Denmark). Water was purified through a Millipore Q-plus system (Millipore Corp., Bedford, MA). All chemicals were of analytical grade.

2.2. Preparation, packaging, and storage of LD slices

Twelve female pigs were chosen on the basis of their weight, meat percentage, and pH (78.0–82.0 kg, 59.0–61.0% meat, and pH 5.50–5.70), in order to limit biological variation, and were obtained from Danish Crown, Marsvej 43, Randers, Denmark. Meat percentage (meat content of carcass) was classified according to EU regulations. pH was measured in the left *longissimus dorsi* (Knick Portamess 751 pH meter with an Ingold LOT glass electrode) at Danish Crown, Randers.

De-boning of the pig carcasses and packaging was performed on day 1 (min 22 h) after slaughter. After de-boning the LD muscles were cooled for 30 min at 0 °C and subsequently sliced into slices of 100 g (1.5–2.0 cm thickness). The exact weight of the LD slices was noted for calculation of drip loss after storage. LD slices from each of the 12 pigs were commercially packed in either skin pack (without oxygen) or modified atmosphere with 70% O_2/30% CO_2 with two LD slices in each packaging. Skin packaging is a commercially used type of vacuum packaging consisting of an upper co-extruded film with barrier properties and a bottom semi-rigid film in the form of a tray that maintains the original form of the product (Cryovac, Sealed Air Corporation, NJ, USA), max. O_2 transmission rate (upper film and bottom): 2 cm^3/m^2/24 h/atm. Packaging was performed on a Multivac R70 (Sepp Haggenmüller GmbH and Co., Germany) with 5–10 mbar vacuum. For modified atmosphere packaging PP trays type 71–43A (Færch Plast, Denmark) were used with TOPSEAL™ PP MAP AF 57 top film (Færch Plast, Denmark), O_2 transmission rate: <100 cm^3/m^2/24 h/atm. Each side of a pig was used for either skin packaging or modified atmosphere packaging. Day 1 LD slices were vacuum packed and kept at −80 °C until analysis.

The LD slices were stored at 4 °C in a walk-in cooler for 4, 8, or 14 days after slaughter with display light on (Philips Fluotone TLD 18 W/830 yielding 1200 lx at the packaging surface) for 12 h every day. The gas composition in the modified atmosphere packs was measured with a CheckMate 9900 (PBI Dansensor, Ringsted, Denmark) prior to sample take-out. The weight of the LD slices was noted, and drip loss (%) was calculated by difference in weight between day 1 and each storage time (days 4, 8, and 14). Sensory analysis was performed on days 4, 8, and 14 after slaughter and the LD slices were kept at 4 °C until heat treatment and subsequent sensory evaluation. For the rest of the analyses, the LD slices were divided into smaller pieces, vacuum packed in separate bags and kept at −80 °C until analysis.

LD slices from day of slaughter (day 0) and day 14 for both packaging atmospheres were tested for aerobic growth, psychrotrophs, lactic acid bacteria, *Enterobacteriaceae*, and *Pseudomonas*. No microbial growth was found in modified atmosphere packaged meat. A slight increase in the microbial count for aerobic growth, psychrotrophs, and lactic acid bacteria was found for the skin packaged meat but no count was above risk level.

2.3. Sensory analysis

The panel consisted of nine assessors, all non-smokers recruited from the professional sensory panel at the Danish Meat Research Institute. All assessors had undergone a basic training program in sensory assessment in accordance with ISO 4121, ASTM-MNL 13, DIN 10964 and were familiar with sensory assessments of meat.