Meat Science 97 (2014) 181–188

Contents lists available at ScienceDirect

Meat Science

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

The effect of protein oxidation on hydration and water-binding in pork packaged in an oxygen-enriched atmosphere☆

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ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 10 January 2014
Accepted 30 January 2014
Available online 8 February 2014

Keywords:
Modified atmosphere packaging
Protein oxidation
Hydration
Water-holding capacity

ABSTRACT

This study investigated the *in situ* oxidative process of myofibrillar proteins in boneless pork loin chops (*Longissimus lumborum*) packaged in an oxygen-enriched atmosphere (HiOx: 80% O_2/20% CO_2), an air-permeable polyvinylchloride (PVC) overwrap, or a partial vacuum (VP) throughout display at 2 °C for up to 14, 7, and 21 days, respectively. Samples stored in HiOx were susceptible to lipid (TBARS) and protein (carbonyls, sulfhydryls, and aggregation) oxidation, while samples in PVC and VP showed lesser oxidative changes. Water-holding capacity of raw muscle decreased ($P < 0.05$) when stored in HiOx but not in PVC and VP. Upon salt and phosphate brine marination, HiOx and PVC muscle samples had improved hydration capacity during display compared with non-stored control, but display generally decreased hydration of VP samples. The result was in agreement with myofibril structural changes. Despite the enhanced hydration, HiOx muscle was least capable of withholding moisture upon cooking.

1. Introduction

Juiciness and tenderness are important quality attributes of fresh meat that are reflective of the water-binding potential and structural integrity of myofibrils and their constituting proteins (Puolanne & Halonen, 2010). The mechanism of water-binding and hydration in muscle is generally described in terms of the structure of the myofibrillar lattice, where the majority of water is imbedded in the interfilament spaces, between the thick and thin filaments (Bertram et al., 2001, Offer & Trinick, 1983). Chemical, physical, or enzymatic processes that cause the myofibril lattice to expand through either the disruption of the actomyosin cross bridges, degradation of cytoskeletal proteins, or enhancement of electrostatic repulsion, can bring about improved water-binding in meat (Huff-Lonergan & Lonergan, 2005; Kristensen & Purslow, 2001; Liu, Chen, & Xiong, 2010).

Recent studies have demonstrated that modified atmosphere packaging (MAP) systems with high levels of oxygen (HiOx) promote oxidation, thereby negatively affecting meat quality (Clausen, Jakobsen, Ertbjerg, & Madsen, 2009, Grobbel, Dikeman, Hunt, & Milliken, 2008, Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007). Myofibrillar proteins are particularly susceptible to oxidative processes, including the destruction of amino acid side chains (deamination, carbonyl formation, etc.), peptide scission, and protein cross-linking (disulfide bonds, dityrosine, etc.), which subsequently reduces water-holding capacity (Xiong, 2000). Kim, Huff-Lonergan, Sebranek, & Lonergan (2010) observed reduced tenderness and juiciness and concurrent intermolecular cross-linking of beef stored in HiOx. Liu, Chen, & Xiong (2009) indicated that protein oxidation was an important restricting factor for the ability of myofibrils to imbibe water, thwarting water-holding capacity of fresh meat. However, the mechanism of water-holding and hydration in fresh pork muscle packaged under oxygen-enriched atmospheres has not been well defined.

In a preceding investigation, we found that fresh ground pork stored in a HiOx MAP progressively lost its water-binding ability, and oxidative aggregation of proteins in the myofilamental lattices was implicated in the functionality change (Delles, Xiong, & True, 2011). In the present study, the impact of protein and lipid oxidation on brine absorption and retention in fresh pork loin muscle stored under HiOx, in comparison with those from ambient and reduced-oxygen packaging systems, was investigated. Our objective was to determine the relationship between *in situ* oxidative processes and water-binding/retention properties of whole pork muscle (chops) so as to contribute to the development of proper packaging and display conditions for consistently high-quality fresh pork products.

2. Materials and methods

2.1. Materials and sample preparation

All procedures used in the study herein were approved by the University of Kentucky Animal Care and Use Committee and the experimental design is presented in Fig. 1. A total of 16 whole loins were obtained from 8 pork carcasses 24 h postmortem. Two random loins were used for each experiment that was replicated two to four times. Because of the large number of analyses, attempts were made to group related

☆ Approved for publication as journal article number 12-07-069 by the Director of Kentucky Agricultural Experiment Station.

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0309-1740/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.meatsci.2014.01.022

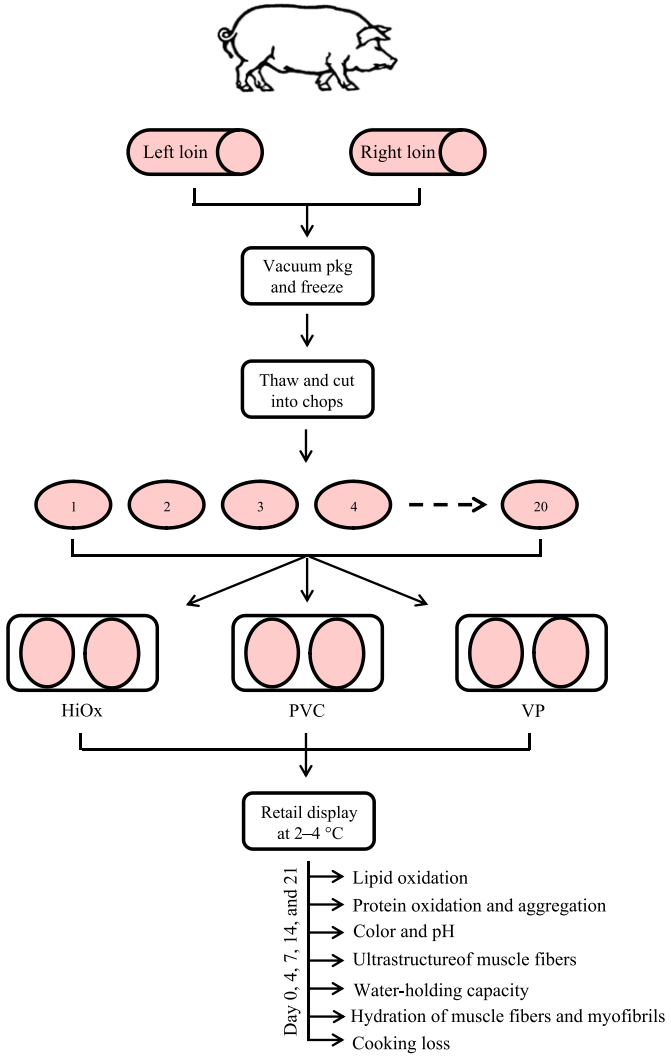

Fig. 1. Schematic representation of the experimental design.

analyses (e.g., tests for whole muscle vs. myofibrils) within the same experiment to ensure all tests were replicated at least two times (up to four times), each experiment was conducted individually and independently from one another. The loins were individually packaged with a vacuum seal (99% vacuum) and stored in a −30 °C freezer until use. All chemicals and reagents were purchased from Fisher Scientific (Pittsburgh, PA, USA) or Sigma-Aldrich (St. Louis, MO, USA) unless specified otherwise.

2.2. Packaging

On the day of packaging, two whole pork loins (per replication) were randomly selected and thawed at 2 °C for 24 h, then sliced into 1.27-cm thick chops, and randomly allotted for either an oxygen-enriched atmosphere (HiOx: 80% O_2/20% CO_2), air-permeable polyvinylchloride (PVC) overwrap, or a partial vacuum (VP) packaging system. A total of 20 chops from the two loins were used for each individual trial replication. Two chops (177 ± 11 g average combined weight) were placed without stacking in Cryovac Family Pack processor trays (7.9 × 31.4 × 26.0 cm in dimension; Sealed Air Corporation, Elmwood Park, NJ, USA) and sealed with a polypropylene–polyethylene film (1.75 mil, 140 cm^3/m^2/24 h oxygen transmission rate, Amcor Flexibles, Abbotsford, Australia) using an ILPRA FoodPack Basic FP372 packaging system (Ilpra Thermosaldatrici, Vigevano, Italy) for HiOx. A gas mixture of 80% O_2 and 20% CO_2 (Scott-Gross Company Inc., Lexington, KY, USA) was used for the HiOx package. For PVC, two unstacked pork chops were placed on #2 supermarket polystyrene trays (2.5 × 21.0 × 14.6 cm in dimension; Genpak; Glens Falls, NY, USA) and overwrapped with an air-permeable polyvinylchloride film (15,500–16,275 cm^3/m^2/24 h oxygen transmission rate; E-Z Wrap Crystal Clear PVC Wrap, North Kansas City, MO, USA). For VP, pork loin chops were packaged using #2 supermarket polystyrene trays and sealed in flexile Cryovac Type B2620 vacuum bags (2.2-mil, 3–6 cm^3/m^2/24 h oxygen transmission rate) under a low-vacuum (60%) condition (Model 600A vacuum machine, Sipromac Inc., St-Germain, Quebec, Canada). The 'hypobaric' condition was about 10 mbar to minimize purge loss.

All packages were placed in a 2 °C walk-in cooler and received approximately 1076 lux of warm white fluorescent light for 24 h a day to simulate retail display conditions. Samples were stored for up to 14, 7, and 21 days for HiOx, PVC, and VP, respectively. The extended display time for HiOx packaging (14 days) was due to the high-level CO_2 that inhibits aerobic bacterial growth, while the furthest extended display time for VP (21 days) was made possible because of the removal of oxygen (McMillin, 2008). However, PVC and HiOx pork chops exhibited significant signs of microbial spoilage (smell) after 7 and 14 days, respectively; therefore, samples beyond these display times were not analyzed. All samples for each test were analyzed on display days 0, 4, and 7 for HiOx, PVC, and VP; day 14 for HiOx and VP; and day 21 for VP only.

2.3. Measurement of lipid oxidation

Duplicate muscle samples from each replication were subjected to the 2-thiobarbituric acid (TBA) test at each display time to measure lipid oxidation according to Sinnhuber & Yu (1977). A malonaldehyde (MDA) standard curve prepared with a series of 1,1,3,3-tetraethoxypropane [Malonaldehyde bis(diethyl acetal)] (Fisher Scientific, Hampton, NH, USA) was used to calculate the amount of MDA produced, and the results were expressed as mg TBA-reactive substances (TBARS) per kg muscle.

2.4. Measurement of protein oxidation

Protein oxidative changes occurring during meat display were assessed by monitoring carbonyl formation (Levine et al., 1990) and sulfhydryl losses (Ellman, 1959) in myofibrillar protein (in the form of myofibrils) isolated from stored muscle samples. Myofibrils were isolated using a rigor buffer containing 0.1 M KCl, 2 mM $MgCl_2$, 1 mM EGTA, and 10 mM K_2HPO_4 (pH 7.0) as described by Xiong, Lou, Harmon, Wang, & Moody (2000). Protein concentration was determined by the Biuret method, and the myofibril pellets were kept on ice and used within 3 d of isolation except for the pellets subjected to phase contrast, which were used within 24 h, and electrophoresis where they were kept in a −80 °C freezer until use.

In the protein carbonyl assay, carbonyl groups in myofibrils were reacted with 2,4-dinitrophenylhydrazine (DNPH) to form protein hydrazones. The carbonyl content (nmol per mg protein) was calculated using an absorption coefficient of 22,000 M^{-1} cm^{-1} for the formed protein hydrazones. For the sulfhydryl analysis, total sulfhydryl content of isolated myofibrils was determined using 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB). Total sulfhydryl content was calculated using the molar extinction coefficient of 13,600 M^{-1} cm^{-1} and expressed as nmol per mg protein.

2.5. Gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on myofibrillar samples in an attempt to detect covalent aggregation of proteins that might be oxidatively modified during meat storage. Previously, isolated myofibrils kept at −80 °C were thawed at room temperature for 5 min. They were then dispersed in a sample buffer containing 4% SDS, 0.125 M Tris–HCl, and 20%