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Effect of oxygen level on the oxidative stability of two different retail pork products stored using modified atmosphere packaging (MAP)

Dimitrios Spanos [a,*], Mari Ann Tørngren [b], Mette Christensen [c], Caroline P. Baron [a]

[a] National Food Institute, DTU, Kgs. Lyngby, Denmark
[b] Department of Meat Technology, Danish Meat Research Institute, Taastrup, Denmark
[c] Carometec A/S, Hassellunden 9, 2765 Smørum, Denmark

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ABSTRACT

The characteristics and the oxidative stability of pork steaks and of pork mince were investigated during 2, 5 and 7 days of refrigerated storage using oxygen (O_2) levels of 0%, 20%, 50% and 80% in modified atmosphere packaging (MAP). Steaks stored during 7 days were not affected by an increase in O_2 concentration, as revealed by lipid and protein oxidation markers. In contrast, the mince was characterised by an altered protein profile, loss of free thiol groups and increased protein oxidation, early during storage. The oxidative stability of pork mince was improved by using intermediate (50%) O_2 MAP. The results show that fresh pork products are affected differently by the MAP O_2 concentration and strongly indicate that optimisation of MAP based on the retail product type would be of considerable benefit to their oxidative stability.

1. Introduction

The use of high concentrations (70–80%) of O_2 in the modified atmosphere packaging (MAP) of fresh retail meat products is a common practise to maintain a bright red colour which is attractive to consumers (Belcher, 2006; Gill, 1996). However, it has been demonstrated that high O_2 levels impact the product shelf life and quality aspects of commercial importance other than colour (Faustman & Cassens, 1990). This is due to lipid- and protein-oxidation initiated by O_2, which is known to result in the deterioration of sensorial and nutritional value of meat (Zhang, Xiao, & Ahn, 2013). Lipid oxidation has been shown to lead to gustative and olfactory defects, such as perceivable rancidity i.e. warmed-over flavour (Kanner, 1994), while modification of protein under an oxidative environment is known to affect the mechanical properties of meat (Lund, Christensen, Fregil, Hviid, & Skibsted, 2008).

Beef stored in high-O_2 MAP exhibits increased thiobarbituric acid reactive substances (TBARS) values (Zakrys-Waliwander, O'Sullivan, Walsh, Allen, & Kerry, 2011) while elevated thiol levels and an altered protein profile were detected in pork (Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007). Furthermore, beef packaged in an oxygen-rich environment has been shown to have increased Warner–Bratzler shear values compared to vacuum packed meat (Zakrys-Waliwander, O'Sullivan, O'Neill, & Kerry, 2012), confirming the effect of MAP oxygen on several meat quality aspects. While the specifics of the structural changes that underlie those effects have not been fully elucidated, mechanisms have been hypothesised to include conformational changes to myosin and cross-linking between sulphide-containing residues (Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010). This process has been postulated to involve the heme protein myoglobin as a key prooxidant in the modification of myosin (Frederiksen, Lund, Andersen, & Skibsted, 2008; Lund, Luxford, Skibsted, & Davies, 2008).

However, the physiological characteristics of meat cuts can greatly vary (Min, Nam, Cordray, & Ahn, 2008) and some muscles contain more myoglobin than others. This indicates that different retail products might not be equally affected by high-oxygen MAP. Furthermore, muscles can be subjected to very different processing conditions (mincing, etc.) during preparation of different retail pork products and this could influence protein oxidation and final product quality. It can thus be expected that different retail pork products will exhibit dissimilar oxidative stability under identical MAP and storage conditions.

Therefore, different retail products may benefit from individual optimisation of the gas composition, through control of the MAP O_2 concentration. Oxygen levels below 60% have been previously shown to result in better sensory scores for beef juiciness and toughness compared to high-oxygen (Zakrys, O'Sullivan, Allen, & Kerry, 2009). Zhang and Sundar (2005) demonstrated that even small differences in the MAP O_2 concentration can have an impact on pork quality while in beef, a myoglobin rich system, changes of as little as 10% in O_2 levels significantly affected oxidation (Resconi et al., 2012). Clausen, Jakobsen, Ertbjerg, and Madsen (2009) reported that an intermediate (50%) oxygen MAP influenced some of the beef quality attributes in a different

* Corresponding author.
E-mail address: disp@food.dtu.dk (D. Spanos).

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manner than a high (80%) oxygen MAP, while Zakrys, Hogan, O'sullivan, Allen, and Kerry (2008) showed that reduction of oxygen levels under 50% O_2 resulted in beef steaks that were preferentially selected by a sensory panel, compared to other O_2 concentrations.

However, investigations have not been extensively performed in chilled pork and therefore, examination of pork packed with different O_2 concentrations would be of interest for the optimisation of retail pork quality. Furthermore, the impact of oxygen concentration on lipid and protein oxidation on different retail pork products has not yet been fully documented.

The aim of this study was therefore to investigate oxidation, and especially protein oxidation, of two common retail pork products, i.e. Longissimus thoracis et lumborum (LTL) steaks and minced pork, packaged in MAP with a step-wise increasing oxygen concentration, under conditions mimicking retail packaging and storage. Furthermore, the retail products were characterised in terms of fat and α-tocopherol content, myoglobin content, fatty acid composition and presence of oxidative type-I muscle fibres. Protein oxidation markers (free thiol, SDS-PAGE, myosin blot, and oxidised protein blot) and secondary lipid oxidation markers (TBARS) were determined for different O_2 concentrations and compared between LTL steaks and pork mince.

2. Materials and methods

2.1. Reagents

All chemicals and reagents were of analytical grade and purchased from either Merck (Darmstadt, Germany) or Sigma (St. Louis, MO, USA), unless otherwise specified.

2.2. Processing and packaging of pork samples

2.2.1. LTL steaks

The left and right Longissimus thoracis et lumborum muscles were excised from three randomly selected female pigs, one day after slaughter, at a Danish slaughter house. Each loin was subsequently trimmed and sliced into sixteen 2 cm-thick chops. LTL steaks (12) cut from the left loin were used for measuring oxidation during storage (day 2, 5 and 7). The loins were packed in four different gas mixtures (Table 1) with one slice per tray. The three pigs were used as replicates.

2.2.2. Mince

Porcine shoulder (125 kg, boneless, trimmed to approx. 2 mm, fat with shank and shoulder chap) was pre-grinded one day after slaughter at a Danish deboning plant. Afterwards, the meat was transported (0–2 °C during 1 h) to a large retail butcher, minced to approx. 3–4 mm particle size and portioned into 450 g per tray. LTL steaks and pork mince originated from different animals.

2.2.3. Packaging and storage

The retail pork samples were placed in M71-51A Black MAPET trays (oxygen permeability: 0.63 cm^3/tray/24 h/atm) sealed with either of four gas mixtures (Table 1) under a TOPSEAL.PET.MAP.P.B.AF.62 film (oxygen permeability: 0.3 cm^3/m^2/24 h/atm) and stored at 5 °C for 2, 5 and 7 days under 1200 lx fluorescent light (Certus T5, Riegens, Denmark). Trays and film were from Færch Plast (Holstebro, Denmark). Upon reaching the target time point, samples were vacuum-packed and stored at −80 °C until further analyses were performed.

Table 1
Gas composition of MAP pork mince and LTL steaks.

Gas	Anoxic	Low-oxygen	Intermediate	High-oxygen
O_2	0%	20%	50%	80%
CO_2	20%	20%	20%	20%
N_2	80%	60%	30%	–

2.3. Lipid analyses

2.3.1. Lipid content extraction

The lipid content of 10 g pork mince and chop samples stored for 2, 5 and 7 days was determined using chloroform/methanol extraction, according to Bligh and Dyer (1959), modified for using less amount of solvent. Following evaporation of the solvent, the lipid content of the extract was determined gravimetrically and expressed as percentage of the initial sample weight.

2.3.2. Fatty-acid composition of the lipids

Fatty acids in lipid extracts from the mince and chop samples stored at 0% O_2 for 2 days were trans-esterified to methyl esters using a boron trifluoride catalysed transesterification according to the AOCS official method Ch 1–91 (Firestone, 2009). The methyl esters were then dissolved in n-heptane and determined with an HP 5890 gas chromatograph (GC) (Hewlett-Packard, Palo Alto, CA), as described by Baron et al. (2013). Fatty acids were identified with commercial standards (68D, Nu-Check-Prep, US) and expressed as percentage of total fatty acids.

2.3.3. Determination of tocopherol content

α-Tocopherol content was determined in duplicate from the lipid extracts using an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA), according to the AOCS official method Ce 8-89 (Firestone, 2009). A fraction of the lipid extract was evaporated under nitrogen and re-dissolved in 2 mL of n-heptane before injection. For tocopherol analysis, an aliquot (20 μL) of the n-heptane fraction was injected on a Spherisorb column (150 mm × 4.6 mm) (Waters, Ireland) and eluted with an isocratic mixture of n-heptane/2-propanol (100:0.4, v/v) at a flow of 1 mL min^{-1}. Detection was performed using a fluorescence detector with excitation at 290 nm and emission at 330 nm. Concentration of α-tocopherol was calculated using authentic standards from Merck Chemicals Ltd. (Darmstadt, Germany).

2.3.4. Determination of secondary lipid oxidation products (TBARS)

TBARS was analysed according to the method described by Vyncke (1970) and as modified by Sørensen and Jørgensen (1996). In short, pork samples (10 g) from LTL steaks and mince stored for 2, 5 and 7 days were homogenised for 1 min at approximately 12,000 rpm in 30 mL of trichloroacetic acid (TCA) solution (7.5% TCA, 0.1% PG and 0.1% ethylenediaminetetraacetic acid (EDTA)), using an Ultra-Turrax mixer (Janke & Kunkel IKA-Labortechnik, Staufen, Germany). The mixture was filtrated through Whatman Grade-2 filter paper (Sigma-Aldrich, USA) and 5 mL of 20 mM thiobarbituric acid were added to 5 mL of the filtrate. The solution was then incubated for 40 min at 100 °C in closed test tubes. Following incubation, absorbance at 532 nm was measured against a blank sample with a Shimadzu UV-1800 spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, USA) and quantified using a standard curve. Results were expressed as μmol malondialdehyde (MDA) equivalent/kg of sample.

2.4. Protein analyses

2.4.1. Determination of free thiols of the sarcoplasmic fraction

Meat samples (0.5 g) from LTL steaks and mince stored for 2, 5 and 7 days were homogenised at high-speed for 1 min with a Polytron PT1200E system (Kinematica AG,Lucerne, Switzerland) in 10 mL of buffer solution (Trizma base 50 mM, EDTA 1 mM, pH 7.4) and 100 μL of freshly-prepared BHT solution (1 mg/mL in methanol). The homogenate was placed in Eppendorf tubes and centrifuged at 13,800 *g* for 10 min in a Heraeus Biofuge Pico centrifuge (Kendro, UK). Subsequently; the supernatant was filtered through a 0.45 μm cellulose acetate wheel filter (Minisart, Sartorius, Germany). The free-thiol