EFFECT OF OXYGEN ON THE OXIDATION OF TWO DIFFERENT MAP RETAIL PORK MEAT PRODUCTS

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Abstract - Fresh meat products are commonly packaged and stored in modified atmosphere packaging (MAP) with high oxygen content (70-80%) in order to retain a red colour which is well-perceived by the consumer at the time of purchase. However, the high oxygen level causes oxidative changes to lipids and proteins and results in more rancid and tough meat. The textural changes are believed to be due to the oxidation of meat proteins, a process that is accelerated by the high oxygen content. In the present study, the effect of oxygen concentration in MAP on protein- and lipid-oxidation was evaluated in two different pork products: mince and chops. The samples were packed in either high oxygen MAP (80% O_2 + 20% CO_2) or non-oxygen MAP (20% CO_2 + 80% N_2), and stored for 7 days under retail conditions. In general, the pork chops were oxidative-stable over the examined storage time; while the mince was more oxidised. In high oxygen MAP, SDS-PAGE revealed a different protein profile at different storage time points for the two products, which originate from muscles with a different physiological profile. Our study suggests that their difference in fat content and fibre-type composition, affect their oxidative stability. It is further suggested that O_2 content of retail products should be differentiated depending on product type and characteristics, in order to effectively control oxidation during storage.

Key Words – MAP, muscle type, protein oxidation

I. INTRODUCTION

MAP is widely used in the packaging of retail pork meat products in order to preserve a bright red colour and extend retail shelf life (1). Specifically, the use of high-oxygen (70-80%) MAP allows the development of a desirable red colour on the product surface (2). This can have an effect on other important quality parameters of muscle foods, as oxygen is known to initiate lipid- and protein-oxidation processes that result in the deterioration of sensorial and nutritional value (3, 4). In addition to undergoing different processing, fresh meat retail products can also originate from muscles with different physiological characteristics. Muscles can therefore differ greatly in fat content, myoglobin content, fibre-type composition, as well as other endogenous factors (5). This has been shown to affect oxidation and it is therefore expected that different products will exhibit dissimilar oxidative stability under identical MAP and storage conditions. The aim of this study was to investigate the development of lipid- and protein-oxidation during storage of two different retail meat i.e. pork chops and mince packed in either anoxic MAP (0%) or high-oxygen (80%) MAP.

II. MATERIALS AND METHODS

Samples

Chops: The left and right *Longissimus dorsi* muscles were excised from three randomly selected Danish pigs, one day after slaughter. Subsequently they were sliced into chops.

Mince: Porcine shoulder (125 Kg) was pre-grinded one day after slaughter at a Danish deboning plant. Afterwards, the meat was transported to an industrial packaging plant and minced finely.

Packaging and storage: The meat samples were placed in trays, sealed with either of two gas mixtures (80% O_2 / 20% CO_2 or 80% N_2 / 20% CO_2) and stored at 5 °C for 7 additional days.

After reaching the target time point, samples were vacuum-packed and stored at -80 °C until the analyses were performed.

Lipid content determination

The meat sample lipid content was determined on 10 g using chloroform/methanol extraction, according to Bligh & Dyer (6). Following evaporation of the solvent, the lipid content of the extract was determined gravimetrically and expressed as percentage of sample weight.

TBARS determination

Meat samples (10 g) were homogenized for 1 min. in 30 mL of TCA solution (7.5% TCA, 0.1% PG and 0.1% EDTA), using an Ultra-Turrax mixer. The mixture was filtrated through Whatman Grade-2 filter paper (Sigma-Aldrich, USA) and 5 mL of 2 mM TBA were added to 5 mL of the filtrate. The solution was then incubated for 40 min. at 100 °C. Following incubation, absorbance at 532 nm was measured against a blank sample with a Shimadzu UV-1800 spectrophotometer and the results were expressed as mg MDA/Kg of sample.

Free-thiol determination

Meat samples (0.5 g) were homogenized with a Polytron PT1200E system in 10 mL of buffer solution (Trizma base 50 mM, EDTA 1mM, pH 7.4) and 100 µL of freshly-prepared BHT solution (1mg/mL in methanol). The homogenate was placed in Eppendorf tubes and centrifuged at 13800 g for 10 minutes in a Heraeus Biofuge Pico centrifuge (Kendro, UK). The supernatant was then filtered through a 0.45 mM wheel filter (Sartorius, Germany). The free-thiol concentration was determined fluorimetrically on 50 µL of the filtrate, using the Amplite fluorimetric Thiol Quantification kit (ATT Bioquest, USA). Fluorescence at Ex/Em of 490/520 nm was measured with a SpectraMAX Gemini fluorimeter (Molecular Devices, USA). The results were expressed as percentage of thiol content of samples stored for two days in 0% O_2, representing 100% of the scale.

SDS-PAGE

Meat samples (0.5 g) were homogenized with a Polytron PT1200E system in 10 mL of Trizma buffer solution and the protein content was adjusted to 1mg/mL. Further, 500 µL were mixed with 400 µL of Laemlli buffer and 100 µL DTT. From the resulting solution, 10 µL were loaded onto a NuPAGE 3-8% Tris-Acetate mini protein gel (Life Technologies, USA) alongside 5 µL of HiMark pre-stained standard. The gel was run in NuPAGE Tris-Acetate running buffer (Life Technologies, USA) at 50V for 5 hours and subsequently stained overnight with AcquaStain protein gel stain (Acquascience, UK). The gel was finally scanned and analysed using the Quantity One 1-D analysis software (Bio-Rad, USA).

Western blotting

Samples run on a NuPAGE 3-8% gel were electrophoretically transferred to a PVDF membrane in a blot module (30V for 1 hour). The membrane was incubated overnight in 5% milk solution in TBS (NaCl 0.8%, Tris-HCl 0.02M, pH 8). After washing with TBS, the membrane was incubated at room temperature for 1 hour with the mouse monoclonal antibody BA-F8 (7) (DSHB, USA), which exhibits specificity against Type-I fibre MyHC and with HRP-conjugated rabbit anti-mouse secondary antibody (Dako, Denmark). This was followed by development with ECL Prime western blotting detection reagent (GE Healthcare, UK). The resulting chemiluminescence was recorded by a DSLR camera.

III. RESULTS AND DISCUSSION

Lipid oxidation

Determination of lipid oxidation revealed that in the pork mince, an increase of secondary oxidation products (TBARS) became apparent at storage day 7 in anoxic and high oxygen MAP, but was more pronounced at 80% O_2 (Table 1). Comparing the two products after 7 days of storage, lipid oxidation was lower in chops compared to the mince. The mince lipid content (10.9% ± 1.1%) was much higher than in the chops (2.5% ± 0.7%), which may explain the higher level of secondary lipid oxidation products in the former. High-oxygen MAP appeared to further increase TBARS in the chops. This increase was equal to that in mince stored in high-oxygen compared to anoxic MAP, for the specific time point.

Table 1. TBARS for pork mince and chops during storage at 5 °C for 7 days.

TBARS (µmol MDA equiv. / Kg meat)			
Sample	Day	Oxygen level in MAP	
		0%	80%
Mince	2	1.4 ± 0.1	1.4 ± 0.4
	5	1.1 ± 0.3	1.2 ± 0.2
	7	2.0 ± 0.3	3.4 ± 0.6
Chops	7	1.4 ± 0.2	2.4 ± 0.4

Protein oxidation

Determination of free thiol groups in the mince revealed a negligible effect of storage time when the product was packaged in anoxic MAP (Figure 1). Similarly, protein oxidation in pork chops remained stable as the thiol-group level did not change considerably over the storage period. While free-thiol levels were retained in chops packaged in high-oxygen MAP, the loss of free thiol content in the mince was much higher, and reached approximately 60% for the high-oxygen MAP samples at Day 7. This represents almost twice the decrease of free thiol determined by Lund (8) in patties formed from similar pork mince (13% fat content) and stored for 7 days. The effect of high-oxygen compared to anoxic MAP on meat protein could be observed after two days of storage for both types of products, with a measurable decrease in free thiols.

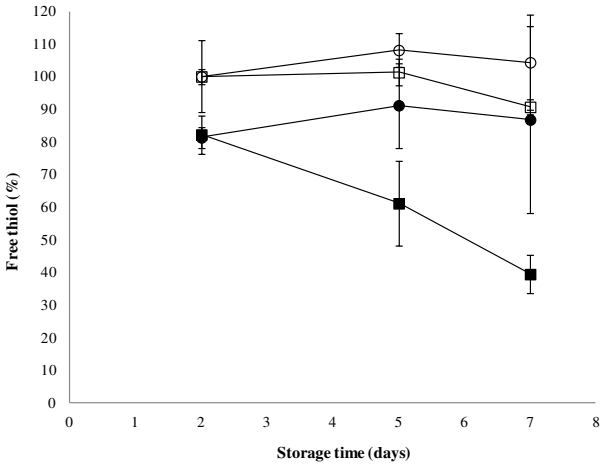

Figure 1. Relative free thiol of pork mince (□: 0% O_2; ■: 80% O_2) and chops (○: 0% O_2; ●: 80% O_2) during storage at 5 °C, compared to two-day storage in 0% O_2, representing 100%.

SDS-PAGE of the samples showed different protein patterns between products and between atmospheres (Figure 2). A battery of additional unidentified protein bands in the range between 117 and 200 kDa were visible in the mince samples, while the intensity of bands over 268 kDa was different between products and MAP. The appearance of high-molecular weight protein above the myosin heavy chain in SDS-PAGE of samples stored in high-oxygen MAP has been previously attributed to the cross-linking of myosin - a process which is believed to be detrimental to meat tenderness (3). The appearance of the bands was further intensified with longer storage time, exhibiting the combinatorial effects of storage time and oxygen presence on oxidation-induced protein modification.

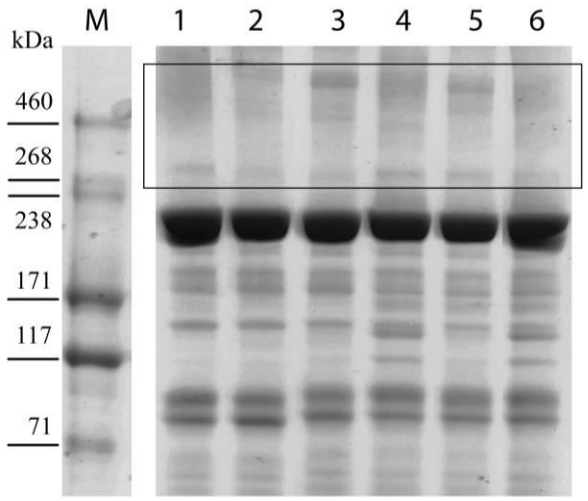

Figure 2. SDS-PAGE. For samples 1-3: Chops: day 2, 0% O_2; day 7, 0% O_2; and day 7, 80% O_2 respectively. For samples 4-6: Mince: day 2, 0% O_2; day 7, 0% O_2; and day 7, 80% O_2. The high molecular-weight region is marked with a rectangle.

Results from fibre typing showed that, beside their fat content and preceding treatment, the examined products also differed regarding their fibre-type composition (Figure 3). The varying intensity of the observed signal suggests a higher prevalence of Slow/Type I fibres in the mince.

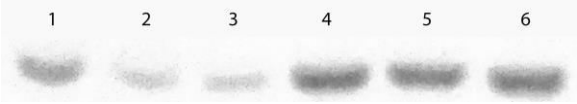

Figure 3. Chemiluminesence signal for anti-Type I MyHC western blot. Numbering is as in Figure 2.

While the chops originate from a single muscle with a high prevalence of the fast/type IIB fibres, the mince was composed of material derived from a mixture of glycolytic fast ("white") and oxidative slow ("red") muscles, which in physiological conditions exhibit a different metabolic capacity for oxygen utilisation. The extent to which these factors affect the oxidative stability of meat products packed in MAP remains undocumented and deserves further investigation.

IV. CONCLUSIONS

- The use of high-oxygen MAP does not affect considerably the oxidative stability of pork chops during seven days of storage.
- In contrast, oxidative changes were observed for minced pork as early as two days of storage, when using high oxygen MAP
- The discrepancy of oxidative stability between product types could be attributed to differences in processing methods (mincing vs. whole cut), lipid content (10.9 % in the mince vs. 2.5 % in the chops), and perhaps also fibre-type composition.
- These factors may impact oxidative stability and should be considered in conjunction to the O_2 concentration. MAP should be optimised for different meat product types.

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