High-oxygen modified atmosphere packaging induced protein polymerization of myosin heavy chain and decreased tenderness of ovine *M. longissimus* during retail display

Kim Y.H.B., Bødker S. and Rosenvold K.

AgResearch Ltd./Ruakura Research Centre, Hamilton, New Zealand

Abstract— **High-oxygen modified atmosphere packaging (MAP; 80% O_2+20% CO_2) has been widely practiced because it creates bright red meat colour and prevents microbial growth. However, this system can negatively influence meat quality because of elevated oxidative conditions. The objective of this study was to determine the influence of MAP on tenderness of fully-aged lamb loins during display. At 24 hours *post mortem*, loins from 18 lambs were vacuum-packaged and stored for 8 weeks at -1.5°C thereby or to simulating the storage conditions of exporting New Zealand chilled meat. After storage, the loins were cut (6 cm), randomly assigned to either MAP or oxygen-permeable overwrap (PVC), and then displayed for 8 days at 3°C under light. Shear force measurement, SDS-PAGE, and Western blot assays for myosin heavy chain (MHC), desmin and troponin-T were determined. The packaging did not influence (P > 0.05) shear force value after 1 day of display. However, at the end of display, increased shear force values were found in the chops packaged in MAP compared to the chops in PVC. The SDS-PAGE results found a formation of high-molecular weight protein aggregation from the chops in MAP at day 8. The western blot assay further revealed the protein polymerization product as an intermolecular cross-linked MHC due to protein oxidation. There were no significant differences found in desmin and troponin-T degradation between the packaging treatments. The results suggest that MAP can even negatively influence tenderness of fully-tenderized lamb by inducing a protein polymerization of high-molecular weight proteins during retail display.**

Keywords— **High-oxygen MAP, Protein polymerization, Tenderness.**

I. INTRODUCTION

New Zealand is the world's largest exporter of lamb by volume [1]. Generally, it takes seven to nine weeks to ship the meat products to major markets such as the EU. Once arrived, typically, chilled-unfrozen lamb products are repackaged into either a high-oxygen modified atmosphere packaging (MAP) or a conventional overwrapping with polyvinylchloride (PVC) film for the retail display.

MAP incorporating high oxygen (80%) and carbon dioxide (20%) has been extensively used as a retail-ready package, because it provides an extension of shelf life of fresh meat by suppressing microbial growth, and prolongs the bright cherry-red "bloomed" colour during display compared to the PVC [2]. However, oxidation-related quality defects such as greater extents of discolouration, rancid flavour, and reduced tenderness and juiciness of meat packaged in the high-oxygen MAP compared to other low-oxygen utilised packaging systems (e.g. vacuum, PVC, and low-oxygen MAP) have been reported in several studies [3-7]. Further, a recent study found that decreased tenderness of beef muscles packaged in the high-oxygen MAP could be a consequence of a formation of a cross-linked product of myosin heavy chain (MHC) induced by protein oxidation possibly with titin [4].

Due to the extended ageing time of the New Zealand lamb products during shipping (chill-stored at -1.5°C), tenderness development can be optimised through the myofibrillar protein degradation [8, 9]. However, the long-term aged meat might have an inferior anti-oxidation potential, and thus it could be more susceptible to the oxidative condition associated with high-oxygen MAP [10, 11]. Furthermore, although the effect of the high-oxygen MAP on biochemical attributes (particularly protein polymerization with MHC) and tenderness of beef [4] and pork [6] has been determined, its influence on chilled lamb loins for extended periods has not previously been published to our knowledge. Therefore, the objective of this study was to determine the influence of MAP on tenderness of fully-aged lamb loins during display.

II. MATERIALS AND METHODS

A. Raw materials and processing

Eighteen lamb carcasses (10-month-old; average hot-carcass weight 16.5kg) were selected from a commercial abattoir based on measuring an ultimate pH_{24h} of 5.8 or below by inserting a calibrated pH probe between 11th and 12th rib of each carcass. At 24 hours *post mortem*, loins (*M. longissimus lumborum*) from both sides of the long loin saddles were excised (yielding 36 loins in total = 18 lambs x 2 sides), vacuum-packaged and transported to the AgResearch Ruakura campus. The loins were then stored for 8 weeks at -1.5°C by simulating the storage conditions of exporting New Zealand chilled meat. After the ageing period, the loins were removed from the vacuum packages, four 6-cm chops from each side of loins were cut, randomly repackaged into either a high-oxygen modified atmosphere (MAP) or overwrapped with PVC film (PVC), and displayed for 8 days at 3°C under continuous fluorescent natural white light. For HiOx-MAP, the loin cuts were put in a shrinkable bag (BB7L, 30 by 39 cm/ an oxygen-transmission rate of 20 cc O_2/1 cm^3/m^2/24 hours at 23ºC and a water-vapor transmission rate of 10 cc/m^2/24 hours at 23ºC; Cryovac Sealed Air Corporation, Hamilton, New Zealand), and a high-oxygen modified atmosphere (80% O_2/20% CO_2, Certified Standard within ± 5%, BOC GASES; Hamilton, New Zealand) was accomplished by using a Securepak 10 Controlled Atmosphere Packaging Machine (Securefresh Pacific, Auckland, New Zealand) by applying vacuum, then flushing the package with the gas mixture, and sealing. For PVC, trays were wrapped with oxygen-permeable polyvinyl chloride film (23,000 $cc/O_2/m^2$/24 hours at 23ºC). The gas composition of the MAP trays was monitored by using a headspace oxygen/carbon dioxide analyzer (PBI Dansensor, Glen Rock, NJ) confirming that a high-oxygen level (>70% O_2) was maintained during display period.

B. pH measurement

The pH was measured by inserting a calibrated pH probe (Testo 205 pH meter with combined temperature and pH insertion probe, Lenzkirch, Germany) directly into the meat. Duplicate readings were taken for each sample at 24 hours *post mortem*, after 8 weeks of ageing at -1.5°C and during the 8 days of display time.

C. Shear force

The shear force was measured for the loins displayed for 1 and 8 days under either MAP or PVC. The loins were cooked in a water bath set at 99°C to an internal temperature of 75°C (measured by thermocouples). After cooling, 10 mm x 10 mm cross section samples were cut and sheared using MIRINZ Tenderometer [12]. Ten replicates were measured for each sample. The results were expressed as shear force (kgF).

D. SDS-PAGE and Western blot

Whole muscle protein sample preparation for reducing (with β-mercaptoethanol: MCE) and non-reducing conditions (with N-ethylmaleimide: NEM) for the SDS-PAGE and western blot was conducted as described by Kim et al. [4]. The prepared gel samples were loaded at 40 µg of protein per lane on a 7.5% polyacrylamide gel. The gels were silver-stained, and then photographed by using by densitrometry using G-Box: Chemi HR 16 (Syngene, USA) and GeneTools (Syngene, USA). The Western blots to determine cross-linking of myosin heavy chain, desmin and troponin-T of the gels samples were assayed using a 7.5% and 10% gel. After incubating with their respective primary and secondary antibody, protein bands were detected using a chemiluminescent detection kit (Pierce ECL Western Blotting Substrate, Thermo Scientific, Rockford, IL, USA), and quantified by densitrometry using G-Box: Chemi HR 16 (Syngene, USA) and GeneTools (Syngene, USA).

E. Data analysis

The experimental data were analyzed as a split-plot design, where each animal used as the whole-plot to which two packaging methods (MAP or PVC) were randomly assigned to loins from each side of a carcass. For the sub-plot portion, chops from each loin were displayed for 8 days and were analyzed for the biochemical analyses. All statistical analysis was done

using the REML directive of GenStat [13]. Least squares means for each attribute were separated (F test, $P < 0.05$) by using least significant differences.

III. RESULTS AND DISCUSSION

There was no significant change in pH after 8 weeks of vacuum storage at -1.5°C (initial average pH at 24 hours *post mortem* = 5.8). However, a slight increase in pH was observed for the loins in PVC after 8 days of display, which was higher than the loins in MAP at day 8 ($P < 0.05$; Table 1). The pH increase for the loins in PVC might be associated with a microbial growth [14], whereas CO_2 in the MAP is likely to have suppressed microbial growth, resulting in no change in pH during the retail display [2].

The long term ageing of the loins resulted in very low shear force values (below 4 kgF). However, a significant packaging by day interaction was found in shear force values during retail displayed for 8 days (Table 1). The PVC packaged loins had similar shear force values, whilst a significant increase was observed in the loins packaged under MAP at the end of display period.

Table 1 Effects of different packaging methods [high-oxygen modified atmosphere packaging (MAP) and overwrapping with polyvinylchloride film (PVC)] on pH and shear force values of lamb loins during 8 days of display at 3ºC under light. The loins were vacuum-stored for 8 weeks at -1.5ºC prior to the retail display

	MAP		PVC		
Trait	day 1	day 8	day 1	day 8	SED
pH	5.82^{ab}	5.76^{b}	5.85^{a}	5.89^{a}	0.03
Shear force (kgF)	3.4^{a}	3.8^{b}	3.5^{a}	3.3^{a}	0.18

^{ab}Least square means within each trait lacking a common superscript letter indicate significant differences ($P < 0.05$).

Similar results were reported in the beef [4] and pork [6] studies, where increased star probe values and decreased tenderness and juiciness of fresh meat packaged in the high-oxygen MAP were found after storage. However, the *post mortem* storage period of the meat determined in these studies was within 2 weeks, whereas the current study used for the aged meat was for 8 weeks at -1.5°C prior to the retail packaging to MAP.

SDS–PAGE results revealed a clear presence of a high-molecular weight protein polymerization in the loins packaged in MAP after 8 days of retail display period (Fig. 1). Western-blot assay further determined that the high molecular weight protein aggregation in MAP was cross-linked MHC (Fig. 2). A greater extent of intermolecular cross-linked MHC in the loins packaged in MAP compared to the loins in PVC was observed at the end of display. There were no significant effects of packaging treatments in desmin and troponin-T degradation (data not shown).

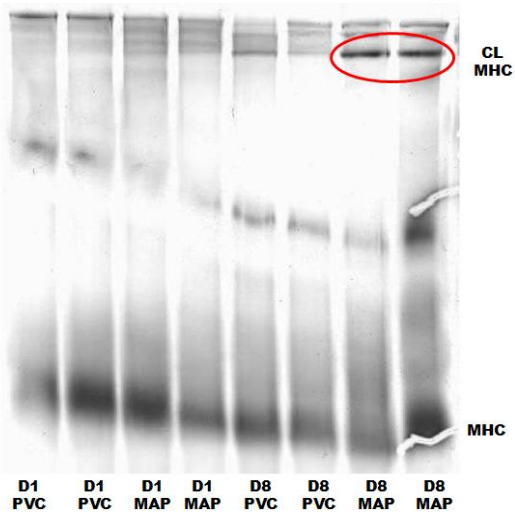

Fig. 1 Representative 7.5% gel SDS-PAGE of whole muscle protein extractions of lamb *M. longissimus lumborum* under non-reducing condition. The loins were vacuum-stored for 8 weeks at -1.5ºC prior to the retail package (MAP or PVC) and display for 8 days at 3ºC under light.; CL-MHC = cross-linked myosin heavy chain; MHC = myosin heavy chain.

These observations indicated that the proteolysis was fully completed during the 8 weeks of ageing (based on no difference in desmin and troponin-T degradation between MAP and PVC), but the occurrence of protein polymerization of MHC with a high molecular protein was the likely factor inducing the significant increase in the shear force values of the loins in MAP during the retail display. Kim et al. [4] reported similar results that oxidative cross-linking/aggregation of myosin and possibly with titin

can be a causative factor for reduced tenderness of beef steaks packaged in MAP, because the MAP environment did not influence the proteolysis and μ-calpain autolysis of beef loins.

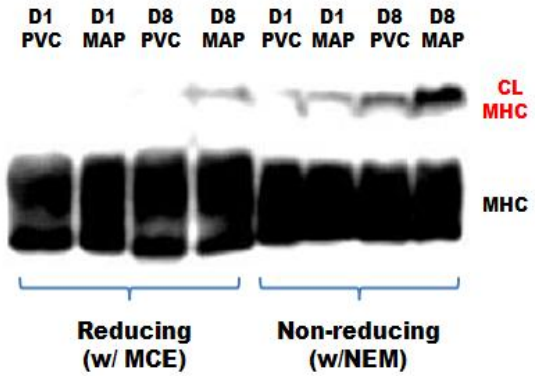

Fig. 2 Representative Western blot of cross-linked myosin heavy chain (CL-MHC) for whole muscle protein extractions of lamb *M. longissimus lumborum* under reducing and non-reducing conditions run on 7.5% gels. The loins were vacuum-stored for 8 weeks at -1.5ºC prior to the retail package (MAP or PVC) and display for 8 days at 3ºC under light.; CL-MHC = cross-linked myosin heavy chain; MHC = myosin heavy chain.

IV. CONCLUSIONS

The results from the present study suggest that a high-oxygen MAP can even negatively influence tenderness of fully-tenderized lamb loins through the oxidative cross-linking/polymerization of MHC with high-molecular weight proteins during retail display.

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