INVESTIGATION OF TENDERNESS AND WATER HOLDING CAPACITY OF AGED PORK LOINS IN TWO PACKAGING SYSTEMS

3A-118

Report prepared for the
Co-operative Research Centre for High Integrity Australian Pork

By

Minh Ha
Frank Dunshea
Robyn Warner

Faculty of Veterinary and Agricultural Sciences
The University of Melbourne
Parkville, VIC 3010

June 2017

Pork CRC

Australian Government
Department of Industry, Innovation and Science

Business Cooperative Research Centres Programme

Executive Summary

High oxygen modified atmosphere packaging (hiOxMAP) is the most common form of retail display of pork in major supermarket chains in Australia. In recent years, both Woolworths and Coles have substantially invested in moving away from the traditional overwrap method to hiOxMAP (Johnston 2011, Condon 2015). However, studies on red meat both in Australia and overseas have shown a negative impact of hiOxMAP on appearance, tenderness, juiciness and flavour. As consistency in eating qualities is a major challenge for the Australian pork industry, identification of issues presented by hiOxMAP and overcoming these with cost-effective and easily adoptable solutions are of significant interest.

This project, using published scientific protocols with instrumental tools, demonstrated a negative effect of hiOxMAP on appearance, water content and texture of Australian pork loins. Biochemical analysis correlated these physical measurements to an increase in lipid and protein oxidation of the loins packed in hiOxMAP.

To overcome the negative impacts of hiOxMAP on Australian pork loins, alternative low oxygen-containing packaging systems, represented by vacuum packaging (VAC), was examined in parallel with hiOxMAP. Compared with loins in hiOxMAP, those packed in VAC had an equivalent fluid loss due to packaging, a reduced fluid loss due to cooking, and an improved texture profile. As expected, lipid and protein, oxidation in VAC packed loins was also lower than hiOxMAP. An increase in lipid oxidation of pork loins in hiOxMAP coincided with higher metmyoglobin content, which in previous studies on beef and lamb to caused browning in meat. Using two different methods to measure protein oxidation, hiOxMAP appeared to cause changes at the molecular level, which ultimately led to detriments in colour, shear force, texture and water holding capacity of Australian pork loins.

Another alternative solution combining the two packaging systems VAC and hiOxMAP was investigated. Pork loins were stored in VAC to enable sufficient tenderisation prior to retail display in hiOxMAP. Both physical and biochemical measurements were indicative of an improvement in eating qualities of the Australian pork loins when the combined packaging system was used. Sensory evaluation using consumer panels is needed to confirm these results. As the current supply, chain involves vacuum packing of the whole pork primal prior to distribution to hiOxMAP facilities; adoption of a combined packaging system may mean an inclusion of a short delay (3-5 days) between vacuum packing of whole pork primal and hiOxMAP packing. Such delays may prove to be a cost-effective and industry adoptable solution to minimise meat oxidation effects ensuring the production of consistently high quality Australian pork.

Table of Contents

1. Introduction

Meat quality attributes tenderness and juiciness are two of the most important determining factors for consumers. Ensuring consistency of these qualities is a challenge for the meat industry. Although extensive research has been conducted, molecular mechanisms of tenderisation and water holding capacity (WHC) contributing to tender and juicy meat cuts is not fully understood. Tenderness and juiciness depends on a variety of factors such as animal genetics, feeding, slaughtering, animal age, muscle type, packaging, post-harvest processing and cooking of meat, and interactions between these factors (Pearce *et al*. 2011). Amongst these, the effect of packaging on meat eating qualities is least understood with some research demonstrate a direct correlation between packaging and sensory results. Packaging of primal cuts at the point of sale serves to display, store, delaying spoilage and allow the meat tenderisation process to occur.

Modified atmosphere packaging (MAP) is a packaging system in which the atmosphere surrounding a product is removed or modified prior to sealing in vapour-barrier materials one of the most common form of packaging of meat and meat products. MAP with 80% O_2 and 20% CO_2 (hiOxMAP) is the most common form of retail display with an average shelf life of 10 days for Australian meat. Due to intrinsic structural and biochemical differences between species and muscles, the effect of hiOxMAP has been shown to vary in different meat cuts. Research with beef longissimus has shown that storage of meat in a high oxygen environment leads to a reduction in meat qualities including tenderness and water holding capacity. Due to these negative effects of hiOxMAP, alternative packaging systems such as vacuum skin packaging (VSP) (similar to MAP except headspace air is completely removed) and vacuum packaging (VAC) (usually for large cuts or whole primal at early stages of the meat distribution chain) are increasingly attracting research attention (Lagerstedt *et al*. 2011a, Geesink *et al*. 2015). In Australia, vacuum packaging is often used in retail for large cuts such as pork and beef roasts and vacuum skin packaging is sometimes used for premium cuts such as fillets.

Tenderisation of meat is a complex process involving multiple factors including regulation of endogenous protease (mainly the calpain family) activity and myofibrillar protein degradation. HiOxMAP can negatively affect texture of meat in comparison with low oxygen storage conditions (Smulders *et al*. 2006, Clausen *et al*. 2009, Zakrys-Waliwander *et al*. 2012, Chen *et al*. 2015). Reduction in tenderness of meat in hiOxMAP was confirmed with sensory evaluation, which showed hiOxMAP resulted in meat with lower score for tenderness trait (Lund *et al*. 2007, Lagerstedt *et al*. 2011a, Lagerstedt *et al*. 2011b).

Although the exact mechanism in which hiOxMAP reduces meat tenderness is not fully understood, it has been shown hiOxMAP induces protein oxidation, resulting in a reduction of calpain activity and an increased cross-linking of myofibrillar proteins. As a result, proteolysis of meat structural proteins, which contributes to meat tenderisation, is reduced. Comparison of pork and beef packed in hiOxMAP and VAC showed an increase in protein oxidation indicated by a reduction in the level of free thiol and/or an increase in carbonyl content in hiOxMAP packed meat (Zakrys-Waliwander *et al*. 2012, Bao and Ertbjerg 2015, Chen *et al*. 2015, Fu *et al*. 2015). Similarly, Lund *et al*. (2007) compared hiOxMAP and VSP packed pork samples and found hiOxMAP produced meat with a lower level of free thiol. The authors, however, found no significant difference in carbonyl

content between pork samples packed in hiOxMAP and VSP. Using immunoblotting, Chen *et al.* (2015) correlated protein oxidation represented as a reduction in free thiol in hiOxMAP packed pork with less μ-calpain autolysis and therefore its activation. In the same study, an elevated level of protein oxidation in hiOxMAP packed pork was shown to concur with less desmin degradation and a higher shear force, suggesting that hiOxMAP inhibited pork tenderisation by lowering myofibrillar protein degradation. This finding is consistent with results of other studies in which a decrease in myofibrillar fragmentation index or solubility was found in hiOxMAP pack pork when compared with VAC (Lund *et al.* 2007, Chen *et al.* 2015). Similarly, a study by Fu *et al.* (2015) comparing the effect of VAC and hiOxMAP on beef longissimus tenderisation demonstrated that ageing of primal beef in MAP for 7 days resulted in less degradation of desmin and troponin-T. Intriguingly, the study by Kim *et al.* (2010) comparing the effects of hiOxMAP and VAC on tenderisation of beef longissimus dorsi showed that although hiOxMAP, produced beef with significantly higher shear force, it did not affect degradation of desmin and troponin-T after 9 days of ageing. The difference in effect of hiOxMAP on myofibrillar protein degradation observed in the two studies is currently not understood. Differences in animal genetics are proposed to play a role.

Apart from tenderness, juiciness (measured as water holding capacity and sensory data) of meat has been shown to be affected by packaging. Meat aged for 6 days in hiOxMAP had a lower purge loss and higher centrifuge loss compared to those aged in VAC for the same period (Chen *et al.* 2015). A study (Lund *et al.* 2007) comparing pork aged up to 14 days in VSP and hiOxMAP found a significant drip loss in hiOxMAP packed pork (5.5% as opposed to 1% in VSP packed pork) which attracted a lower sensory score for juiciness. Similar results were also observed in beef longissimus in which total loss (purge, freezing and cooking losses combined) was higher in 21 day ageing in hiOxMAP compared to 21 day VAC or VSP ageing (Lagerstedt *et al.* 2011a). Individual loss in this study varied depending on packaging method and ageing time. Furthermore, the study of Lagerstedt *et al.* (2011b) examined the effect a combination of VAC and MAP and found evidence for toughening in hiOxMAP packed beef. Evidence of meat toughening has not been demonstrated for pork. A study examining hiOxMAP and VAC using three different beef muscles (longissimus, semimembranosus and adductor) found that only the adductor muscle showed a difference in sensory score with VAC beef attracting a higher score after a 9 day display period, indicating that response to packaging method varies with muscle types (Kim *et al.* 2010).

Although extensive research of different post-mortem treatments in meat exists, the exact mechanism of water holding capacity is not understood. As approximately 85% of meat, total water content is thought to be contained within myofibrils, denaturation and degradation of myofibrillar proteins leading to structural changes in muscle cells has been hypothesised to affect water-holding capacity. A correlation between changes in myofibrillar proteins, such as desmin, and purge loss in meat has been established. Desmin, a substrate protein of μ-calpain, is an integral protein of intermediate filament connecting adjacent myofibrils. Degradation of desmin as an indicator of WHC is well established. A study by Davis *et al.* (2004) reported greater purge loss in pork loins with less desmin degradation. Similar results were observed for PSE pork in which significant centrifuge and drip losses were found to correlate with a lower amount of desmin being hydrolysed compared with RFN pork (Yin *et al.* 2014). Similar to desmin, other structural proteins in pork including vinculin and talin are thought to be involved in the ability of meat to hold water (Kristensen and Purslow 2001, Melody *et al.* 2004). Degradation of

these cytoskeletal proteins has been hypothesised to result in later shrinkage of laterally connected myofibrils and therefore of a whole muscle fibre causing water to be squeezed out. However, a recent study comparing WHC of VAC packed pork from different pig breeds showed that Meishan pork, although exhibiting a similar level of desmin degradation, had a significantly lower centrifuge and drip losses compared to pork from a crossbreed at 1 and 3 day post-mortem (Wang *et al*. 2016). Similarly, experimental evidence of varying denaturation degree of structural proteins titin and nebulin did not explain significantly low WHC for PSE pork (Warner *et al*. 1997). Results of these studies indicate that WHC is controlled by mechanisms other than degradation/denaturation of myofibrillar structural proteins. Limited results have suggested denaturation of sarcoplasmic proteins and their association with denatured myofibrillar proteins to play a role in WHC and colour development of PSE and RSE pork (Joo *et al*. 1999, Laville *et al*. 2005). However, further research is needed to elucidate the role of sarcoplasmic proteins in this complex process.

Another aspect of WHC observed in numerous studies is an increase of losses with ageing time. Cayuela *et al*. (2004) measured weight loss of pork chops packed in hiOxMAP and VAC and showed that there was a gradual increase in loss in both treatments. Similarly, a study by Zakrys-Waliwander *et al*. (2012) on beef longissimus showed that drip loss of beef samples increased with ageing time and a significant difference was observed between day 14 post-mortem samples and those at 1, 4 and 8 day post-mortem. Increase in purge loss and centrifuge loss have been correlated with ageing time in both MAP and VAC packed meat (Chen *et al*. 2015). The mechanism behind the relationship between ageing and WHC is currently not understood. It is possible that rate and extent of early post-mortem pH decline and protein denaturation/degradation play a role as limited evidence has suggested (Zhang *et al*. 2006, Bee *et al*. 2007). As mentioned previously, packaging methods such as hiOxMAP and VAC alter protein denaturation/degradation during meat ageing, which may explain the influence of packaging on WHC of meat during the ageing process. However, the study by Cayuela *et al*. (2004) observed a correlation between meat weight loss and ageing in air packaging (in which meat is packed in an oxygen permeable material) as with meat packed in hiOxMAP and VAC. Further research is required for a more concrete conclusion.

This study aimed to investigate the effect of VAC and hiOxMAP on tenderness and water holding capacity of pork loins at different ageing time. We also investigated, ageing of pork loins in VAC prior to display in hiOxMAP as a solution to maximise ageing effect and colour retention while minimising drawbacks of hiOxMAP.

2. Methodology

2.1. Carcass information and loin collection

In order to minimise carcass differences contributing eating qualities, all animals were from the same breed and sex, and had the same feeding, housing and slaughtering method. Carcass grading information is in Table 1. Loins (longissimus) were obtained from female pig carcasses (n=12) from Rivalea Australia (Corowa, NSW). Ultimate pH (upH) was taken at 24-hour post mortem. Following boning, the loins were transferred to the Faculty of Veterinary and Agricultural Sciences, the University of Melbourne in refrigerated conditions.

Table 1: Pig carcass grading information

BODY	DATE	TIME	LOT	SEX	FED	P2	MD	HSCW	GRADE	upH
37633	9/05/2016	11:00:56	2197	F	Yes	13.2	49.2	76.1	N11	5.41
37634	9/05/2016	11:01:11	2197	F	Yes	11.6	66.4	80.8	N6	5.4
37651	9/05/2016	11:05:12	2197	F	Yes	13.2	56.8	80.7	N12	5.45
37671	9/05/2016	11:09:54	2197	F	Yes	10.8	48.4	84.2	N4	5.38
37676	9/05/2016	11:11:05	2197	F	Yes	14.8	47.6	85	N12	5.4
37696	9/05/2016	11:15:51	2197	F	Yes	10	60.4	83.8	N4	5.46
37698	9/05/2016	11:16:16	2197	F	Yes	12.8	66	78.1	N5	5.43
37708	9/05/2016	11:18:37	2197	F	Yes	10	60.4	82.5	N4	5.48
37715	9/05/2016	11:20:16	2197	F	Yes	10.4	62.4	81.1	N4	5.41
37718	9/05/2016	11:21:02	2197	F	Yes	13.2	63.2	84.1	N12	5.42
37720	9/05/2016	11:21:28	2197	F	Yes	13.2	54.8	84	N12	5.37
37722	9/05/2016	11:21:55	2197	F	Yes	10.4	53.2	79.3	N4	5.41

From left and right loin of each of the 12 carcasses at approximately 28-hour post-mortem, 11 steaks measuring 4cm in thickness were excised and allocated to 11 treatments outlined below:

- Control, 0 days ageing
- Vacuum packing for 1 day (1VAC)
- HiOxMAP packing for 1 day (1MAP)
- Vacuum packing for 5 days (5VAC)
- HiOxMAP packing for 5 days (5MAP)
- Vacuum packing for 10 days (10VAC)
- HiOxMAP packing for 10 days (10MAP)
- Vacuum packing for 10 days followed by HiOxMAP for 5 days (10VAC-5MAP)
- Vacuum packing for 15 days (15VAC)
- Vacuum packing for 10 days followed by HiOxMAP for 10 days (10VAC-10MAP)
- Vacuum packing for 20 days (20VAC)

Note on above; each high oxygen MAP (HiOxMAP) treatment is matched with equivalent days in vacuum, as a control.

2.2. Meat packaging and storage

The 0 day aged steaks (control) were frozen (-20°C) immediately until analysis. Steaks allocated to vacuum packaging were placed in polyethylene vacuum pouches PA/PE 70 with an oxygen permeability less than 65 cc/m2 (24h) and water transmission less than 5 g/m2 (24h) and packaged with a MultiVac C200 chamber vacuum packer (Sepp Haggenmüller GmbH & Co., Wolferschwenden, Germany). HiOxMAP packaging was conducted with a Multivac T200 (Sepp Haggenmüller GmbH & Co., Wolferschwenden, Germany) connected to a gas mixer to achieve a final O2:CO2 ratio of 80%:20%. Steaked were placed on a cello pad positioned in Cryovac black trays (170mm × 223mm, Sealed Air, Australia). The trays were sealed with a biaxially Oriented PolyAmide / Polyethylene / Ethylene vinyl alcohol based film (LID-1050, OTR 10 cm3/m2/24). The ratio of meat to headspace was approximately 1:2 as recommended previously (McMillin 2008).

Retail packs were randomly distributed on shelves of a retail cold display cabinet with LED lighting (Bromic Pty Limited). The packs were rotated daily to minimise uneven illumination on samples.

2.3. pH and colour measurement

After 1, 5, 10, 15 or 20 days storage, packaging materials were removed and blooming was allowed to occur at 4°C for 30 min. The pH of the interior of meat steaks was measured using a spear-head IJ44C pH probe with a temperature compensation WP-80 pH-mV probe (TPS Pty Ltd., Brisbane, Australia). The pH meter was calibrated using pH 4 and pH 7 buffers.

Instrumental colour measurement on the surface of meat was conducted using a Hunterlab Miniscan EZ (Hunter Assoc. Labs Inc., Virginia, USA) calibrated against white and black reference tiles. At designated ageing time (1, 5, 10, 15 and 20 days), samples were removed from packaging material and allowed to bloom at 4°C for 30 min. Duplicate surface colour measurements were taken with D65 illuminant and 10° observer angle. CIE L* (lightness), a* (redness) and b* (yellowness) values were obtained from the average values of two readings on the surface of loin samples. The ratio of oxymyoglobin: metmyoglobin (oxy: met) was calculated using reflectance values at wavelength 630nm and 580nm as described by Khliji *et al.* (2010).

2.4. Purge and cooking loss

Control samples were stored at -20° on day 0 ageing (day 1 post mortem), thus purge loss of control sample was not applicable. Other samples were weighed prior to packaging. After the designated ageing time (1, 5, 10, 15 and 20 days), steaks were removed from packaging material and patted dry with paper towels. The purge loss was calculated as the percentage of weight loss.

Cooking loss is the loss (mostly water) as a result of heating. Cooking of pork samples were performed according to procedures outlined by Channon et al. (2013) with modifications. Before freezing, pork samples were prepared into 2 x 70g±5g blocks, one for WBSF and one for compression, where the weight was recorded. Prior to cooking, samples were removed

from vacuum bags and individually placed in plastic bags. A metal rock was used to suspend bags with samples and samples were cooked in a water bath (F38-ME, Julabo, 77960 Seelbach/Germany) which was preheated to 70°C. Samples were cooked from frozen to an internal temperature of 70°C (approximately 35 minutes). The temperature was recorded using a Grant thermometer equipped with T-type thermocouples. After cooking, samples were cooled in iced water for 30 minutes to prevent further cooking. Samples were dried with paper towels and weighed to calculate cooking loss presented as the percentage of weight reduction. After weighing, samples were wrapped in plastic to minimise moisture loss and stored at 4°C overnight for WBSF and compression assessments the next day.

2.5. Warner-Bratzler (WB) peak force

Toughness of cooked samples were measured using an established method outlined by (Honikel 1998) with modifications. From each sample, six rectangular strips of 1 cm^2 were cut parallel to the direction of muscle fibres. WBSF was measured by using a shear blade (V-shaped) adapted to a texture analyser (Lloyd Instruments Ltd., Largo, FL, USA) with a 500N load cell, and the shearing speed was set at 300mm/min. The peak of the shear force were recorded and the mean was calculated from 6 sub-samples and used as an estimate of toughness.

2.6. Compression

Compression analysis was conducted according to a method previously established (Channon *et al*. 2014). A 0.63 cm diameter flat-ended probe was adapted to a texture analyser (Lloyd Instruments Ltd., Largo, FL, USA). A total of 2 penetrations were applied to meat cut parallel to the direction of muscle fibres. Lloyd instrument settings were 50 mm/min, 80% of 1 cm thick samples. The force work from the initial and second penetration was recorded. A total of 5 measurements were taken for each sample and presented as means. Parameters measured from compression were:

- Hardness: the force work required for the initial penetration
- Cohesiveness (or called ease of break down): the reduced force work required for second penetration from work needed for the initial penetration
- Chewiness: the force required to achieve harness and cohesiveness

2.7. Myofibrillar fragmentation index

Myofibrillar fragmentation index measurement was conducted according to the method of (Culler *et al*. 1978) in duplicates for all sample treatments (n = 12, total = 264). Frozen raw muscle tissue (4 g) was weighed and homogenised using a Polytron PT 10-35GT homogeniser (Kinematica AG, Switzerland) in 7 mL of ice-cold extraction buffer (50 mM Tris/HCl; 10 mM EDTA, pH 8.3) at 13,000 rpm for 10 s. The tubes were centrifuged at 1500 × g for 10 min at 2°C and the supernatant was discarded. The pellet was re-suspended in 25 mL of extraction buffer. This process was repeated twice. After the third wash, the pellet was re-suspended in 5 mL of extraction buffer. The suspension was filtered through a tea strainer to remove fat and connective tissue, followed by addition of another 5 mL of extraction buffer to wash the strainer. Protein concentration was determined using Biruet assay with bovine serum albumin as standard. The myofibrillar suspension was diluted to a

final protein concentration of 0.5 mg/mL with extraction buffer and the absorbance at 540 nM was determined in triplicate. MFI was calculated as A540 × 200.

2.8. Lipid oxidation assay

Lipid oxidation for all treatments was examined in duplicate (n = 6, total = 132) using an established thiobarbituric acid reactive substances (TBARS) assay (Sorensen and Jorgensen 1996) with modifications. Pork samples (4 g) were homogenised in 7.5 mL of 10% TCA solution containing 0.1% EDTA and 0.1% PG using a Polytron PT 10-35GT (Kinematica AG, Switzerland) at 19,000 rpm for 60 s. The samples were then centrifuged at 2°C at 2000 g for 8 min in a Rotina 380R Hettich Centrifuge. The supernatant was filtered through no. 1 Whatman filter paper. Equal amount of filtrate (1 mL) and 0.02 M TBA solution (1 mL) were mixed in screw cap tube and incubated in water bath at 95°C for 60 min. After incubation, the samples were cooled in ice and 200 µL of each sample was transferred into clear 96-well plate. The absorbance of samples was measured at 532 nm using a Multiskan Spectrum spectrophotometer (Thermo Scientific, Vic, Australia) and subtracted with absorbance at 600 nm for correction of nonspecific turbidity.

A standard calibration curve was prepared from 16.7036 µM 1,1,3,3-tetraethoxy-propane in Milli-Q water. The standards were mixed with 1 mL of 20 mM TBA and subjected into the same analytical procedures as the beef samples. Results were expressed as mg MDA/kg of meat.

2.9. Carbonyl content assay

Carbonyl content assay for all treatments (n = 6, total = 132) was conducted according to the method outlined by Lund *et al*. (2007). Samples (1 g) were homogenised in 5 mL pyrophosphate buffer (pH 7.4) consisting of 100 mM potassium chloride, 2 mM sodium pyrophosphate, 2 mM EGTA, and 100 mM potassium chloride using a Polytron PT 10-35GT (Kinematica AG, Switzerland) at 15,000 rpm for 40 s. Aliquots of samples were divided into two equal aliquots (0.5 mL each). To remove chromophores, those aliquots were washed using HCl: Acetone (3:100) (v/v) three times, followed by washing with 10% TCA for twice. Out of two identical samples: i) one pellet was derivatised with 0.5 mL of 10 mM DNPH dissolved in 2 M HCl; ii) one pellet was incubated with 0.5 mL of 2 M HCl for protein concentration estimation. Both samples were left on a rocker in the dark for 30 min. After that, the samples were washed once with 0.5 mL of 20% TCA and three times with 1 mL of ethanol: ethyl acetate (1:1) (v/v) with gentle agitation. The tubes were centrifuged for 5 min (12,000 *g*; 4°C) after each washing step. Supernatants from all samples were decanted. The pellets were added with 1 mL of 6.0 M guanidine hydrochloride dissolved in 20 mM potassium dihydrogen phosphate (pH 2.3) and were left on a rocker overnight at 4°C.

For each sample, absorbance of pellet containing DNPH was measured at 370 nm, while that of the other pellet was measured at 280 nm. Protein concentration of samples was determined using a standard curve prepared from 0-3 mg/mL bovine serum albumin. The carbonyl content was calculated using an absorption coefficient at 370 nm for the formed hydrazones (21,000/mM.cm) and was expressed as nmol/mg protein.

2.10. Free thiol content assay

Pork samples (2.0 g) in duplicates for all treatments (n = 6, total = 132) were homogenised in 50 mL of 0.1 M Tris buffer (pH 8.0) containing 5% SDS using a Polytron PT 10-35GT at 16,000 rpm for 40 s. The homogenates were heated at 80°C in a water bath for 30 min and centrifuged for 20 min in a Rotina 380R Hettich Centrifuge. The supernatants were filtered through Whatman no. 114 filter paper. Protein concentration was determined by measuring the absorbance of filtrate at 280 nm using a standard curve prepared from 0-3 mg/ml BSA. The filtrates were diluted with homogenisation buffer into a concentration of 1.5 mg/mL by adding 2.0 mL of 0.1 M Tris buffer (pH 8.0) and 0.5 mL of 10 mM DTNB (dissolved in 0.1 M Tris buffer pH 8.0) into 0.5 mL sample. The mixture was incubated at room temperature and in the dark condition for 30 min. Absorbance was measured at 412 nm and subtracted with the absorbance of the blank containing 0.5 mL of 5% SDS, 2.0 mL of 0.1 M Tris buffer (pH 8.0), and 0.5 mL of 10 mM DTNB. Concentration of thiol was determined from the standard curve, which was constructed using L-cysteine diluted in 5.0% SDS in 0.1 M Tris buffer (pH 8.0) to cover a concentration range of 0.165 to 0.825 mM cysteine. The content of thiol was presented as nmol L-cysteine/mg protein.

2.11. Statistical analysis

There were ten treatments of packaging method, days aged and extra packaging (either VAC or MAP) by day aged combinations along with a control (no packaging or ageing applied). To ensure the appropriate examination of these combinations the following structure was used. First, a control treatment structure was established with the levels being yes or No (the control was yes, all other combination No). Then there are the initial treatment packaging method (MAP & VAC) and days aged (0, 5, 10). Finally, the additional MAP and /or VAC days aged were called extra map (5 & 10 days) and extra vac (5 & 10 days). This then gives the following nested model that explains all eleven treatments involved, control/(packaging method*ageing)/(extra vac.extra map) where the "/" shows terms nested within other terms i.e. packaging method nested within control.

All parameters were analysed by the method of restricted maximum likelihood (REML). The treatment structure used, as explained above, was control/packaging method/ageing/ (extra vac.extra map) fitted as fixed effects while carcass/sample (sample nested within carcass) were fitted as random effects. Data for WBSF, chewiness, TBARS and free thiol required natural log transformation while purge loss required angular transformation. All statistical analyses were performed using GENSTAT (18th Edition, VSN International Ltd, Hemel Hempstead, UK).

3. Outcomes

3.1. Testing gas concentration of commercial packaging of meat

Gas concentration in commercial packaging of meat bought from major retailers was tested (Figure 1). Pork was purchased from various Woolworths and Coles supermarkets in Victoria between 27th September 2017 and 2nd October 2017. The gas concentration inside each pack was tested on the day of purchase using an Oxybaby® Gas Analyzer (Witt, Germany). The O_2 concentration used for pork packaging varied between 70-80% with the rest of the gas being CO_2.

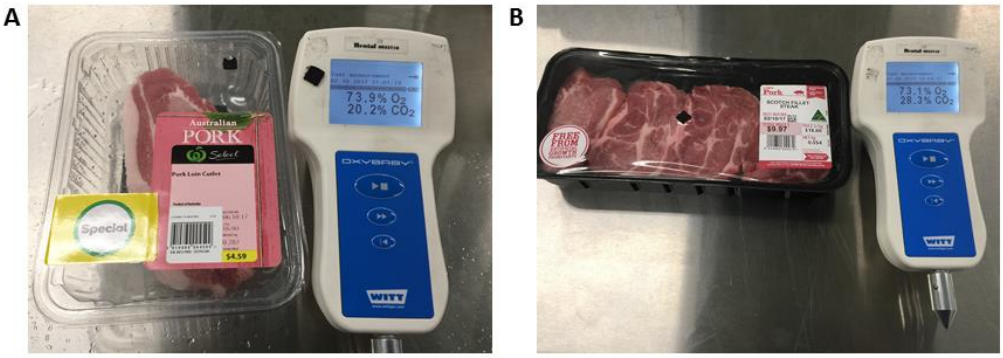

Figure 1. Representative photos of gas concentration testing of pork packaging from (A) Woolworths and (B) Coles.

3.2. pH

Ultimate pH of pork loins at 24 h post mortem was recorded (Table 1) to ensure no dark cutting and PSE pork was included in the study. Ultimate pH of meat is well established to influence biochemical changes of muscle tissue during ageing (Yu and Lee 1986) which determine eating qualities such as tenderness, juiciness and flavour of meat. Ultimate pH has been proposed to cause elevation in ionic strength with a correlation reported to be about 0.9 (Koohmaraie 1994). Ionic strength such as that of calcium ions has been shown to affect structural changes such as Z-disks weakening of Z-disks, weakening of rigor linkages formed between actin and myosin, splitting of titin filaments, and fragmentation of nebulin filaments, regardless of endogenous enzymatic activities (Takahashi 1996).

Also important is the change in pH of meat during ageing due to pH dependent endogenous enzymes such as calpains, capthepsins and multicatalytic proteinase complex whose catalytic activities contribute to the tenderisation process (Koohmaraie 1994). Results in Table 2 showed a significant shift in pH because of packaging and ageing. However, it is worth noting that the pH of all the meat samples after packaging and ageing were between 5.4 - 5.5 which is well within the optimal ageing pH range 5.4 - 5.7.

3.3. Surface L*a*b*

3.3.1. Lightness

Surface lightness values (L*) of pork loins were obtained after packaging and ageing treatments (Table 3). The results showed a general increase in lightness in both packaging

system as ageing occurred. However, differences were observed between VAC and MAP at each of the ageing time points. While there was no difference in L* values between VAC and MAP at 1 day ageing, pork loins in hiOxMAP were significantly lighter in colour compared to those in VAC after days 5 and 10 days ageing. Interestingly, loins that were aged for 5 day in hiOxMAP following 10 ageing in VAC displayed similar L* value to those aged in MAP only for 5 or 10 days. Together, these results suggest hiOxMAP resulted in an increase in paleness compared to VAC.

3.3.2. Redness

Surface redness values (a*) presented in

Table 4 showed that as ageing occurred a decrease in redness was observed in hiOxMAP whereas that in VAC redness increased up to 15 days of ageing. In addition, compared to VAC, packaging of loin in hiOxMAP appeared to result in redder meat up to 10 day of ageing. There was no difference in redness between hiOxMAP and VAC at day 10. Intriguingly, it appeared that the redness of pork loins in packaging combination (10 days in VAC followed by 5 or 10 days in hiOxMAP) at day 15 or 20 was similar to that of loins in hiOxMAP alone at day 5 or 10. Together, these results suggest a combination of both packaging system helped to maximise redness retention in pork loins.

The high L* and low a* values that was measured on hiOxMAP loins especially after 5 days of ageing indicated that the colour of the steak was very light pink, and paler compared to 1 day storage. These colour measurement values were similar to those seen in PSE meat which exhibited L* values of 57 and above (Lesiow and Xiong 2013). A consumer evaluation of the colour of pork loins in hiOxMAP is needed to translate these instrumental results to consumer perception.

3.3.3. Yellowness

Yellowness (b*) (**Table 5**) was higher in hiOxMAP than VAC packed loins at any of the ageing time point up to 10 days. Combining the two packaging did not affect the b* values either at 15 or 20 days.

The chroma (saturation) and the hue angle are both based on the a* and b* values. However, while a* is mostly affected by both the pigment content and the myoglobin form, b* value is mainly influenced by the forms of myoglobin present in pork evident by a very low correlations (r = 0.02 to 0.22) between yellowness and pigment content (Tam *et al*. 1998, Lindahl *et al*. 2001). A higher metmyoglobin content in hiOxMAP packed loins in this study was proposed to result in higher b* values when compared to VAC packed loins.

The colour of pork is strongly associated with meat quality and consumers prefer intensely pink pork (Bredahl *et al*. 1998, Brewer and McKeith 1999). It was suggested that categorising meat colour on the basis of L*, a* and b* values to varying degrees of pink colour is possible (Brewer and McKeith 1999). L*, a* and b* had a strong interaction with one another with each of the three parameters contributing to the overall appearance quality of pork. Pork meat which is generally considered to be very light pink and pale in appearance are associated with high L*, low a* and high b* values, whilst pork meat which are dark pink are associated with low L*, high a* and high b* values (Brewer and McKeith 1999). As part of overall appearance, other sensory traits such as wetness/dryness and general consumer acceptability were also correlated with colour. It was also suggested that consumers discriminated heavily against pork meat, which is very light pink in colour (Bredahl *et al*. 1998, Brewer and McKeith 1999). Pork meat with lower L* value and higher a* value are generally more desirable in appearance.

Table 2. pH of 1 day post mortem pork loins before and after packaging and ageing.

	Days in packaging						Statistics					
	0	1	5	10	15	20	S.E.D.	Con	Con × Packaging	Con × Ageing	Con × Packaging × Ageing	Con × Packaging × Ageing × Combo
Control	5.419						0.016	<0.001	0.786	<0.001	<0.001	<0.001
VAC		5.435	5.510	5.528	5.499	5.475						
MAP		5.417	5.477	5.544								
COMBO					5.448	5.473						

Con = Control (1 day post mortem meat not treated with packaging or ageing). VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

Table 3. Surface lightness (L*) of 1 day post mortem pork loins before and after packaging and ageing.

	Days in packaging						Statistics					
	0	1	5	10	15	20	S.E.D.	Con	Con × Packaging	Con × Ageing	Con × Packaging × Ageing	Con × Packaging × Ageing × Combo
Control	55.19						0.518	<0.001	0.204	<0.001	0.001	<0.001
VAC		55.66	55.42	56.46	57.59	58.15						
MAP		56.29	58.29	58.98								
COMBO					58.65	60.79						

Con = Control (1 day post mortem meat not treated with packaging or ageing). VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. VAC samples were allowed to bloom (exposure to atmosphere) for 30 min at 4°C prior to surface colour measurement. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

Table 4. Surface redness values (a*) of 1 day post mortem pork loins before and after packaging and ageing treatments.

	Days in packaging						Statistics					
	0	1	5	10	15	20	S.E.D.	Con	Con × Packaging	Con × Ageing	Con × Packaging × Ageing	Con × Packaging × Ageing × Combo
Control	6.015						0.345	<0.001	<0.001	0.009	<0.001	<0.001
VAC		5.930	7.821	7.235	7.719	6.563						
MAP		9.390	8.679	7.512								
COMBO					7.984	6.108						

Con = Control (1 day post mortem meat not treated with packaging or ageing). VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

Table 5. Surface yellowness values (b*) of 1 day post mortem pork loins before and after packaging and ageing treatments.

	Days in packaging						Statistics					
	0	1	5	10	15	20	S.E.D.	Con	Con × Packaging	Con × Ageing	Con × Packaging × Ageing	Con × Packaging × Ageing × Combo
Control	15.82						0.286	0.024	<0.001	<0.001	<0.001	<0.001
VAC		14.80	16.72	15.63	16.34	16.10						
MAP		16.91	17.09	16.00								
COMBO					16.94	16.41						

Con = Control (1 day post mortem meat not treated with packaging or ageing). VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

3.4. Browning effect

The ratio of oxymyoglobin/metmyoglobin calculated from reflectance values at 630 nm and 580 nm (Table 6). While R630/R580 remained relatively stable over the 20-day ageing period for VAC packed loins, those in hiOxMAP exhibited a significant decrease in this ratio, indicating an increase in myoglobin oxidation. Combination of packaging appeared to delay oxidation. Together, these results confirm acceleration of myoglobin oxidation in the high O_2 environment.

Extensive use of HiOxMAP in the meat industry is to promote the 'fresh' colour of meat for longer. However, surface colour and R_{630}/R_{580} ratio in this study showed that HiOxMAP leads to an increase in lightness and yellowness. It also leads to reduced redness and accelerated myoglobin oxidation compared to VAC. Together, the results in this study suggest that, apart from initial (2 days post mortem) appearance on retail display shelves, HiOxMAP does not provide further advantage in pork loin appearance compared to VAC.

3.5. Water holding capacity

3.5.1. Purge loss

Purge loss was recorded for all ageing and packaging treatments (

Table 7). Apart from the initial difference at 1-day storage, there was no difference in purge loss in pork aged in VAC or hiOxMAP. Combined packaging resulted in a higher purge loss compared to VAC at either day 15 or 20 ageing. In both VAC and hiOxMAP, significant loss due to ageing was only during the first 4 days of packaging.

Purge loss is the fluid loss from meat during storage. It is an indicator of juiciness of meat and potentially associated with tenderness and flavour of meat (Nam *et al*. 2009). The study of Lund *et al*. (2007) showed that pork loins from Danish pigs displayed a higher purge loss in hiOxMAP compared to those aged in vacuum, in agreement with results in the current project. However, Cayuela *et al*. (2004) showed that VAC caused a significantly higher purge loss than hiOxMAP for pork loins from Spanish pigs. Similarly, loins of Duroc × Landrace × Yorkshire crossbred pigs from Denmark were observed to have a higher purge loss in VAC compared to hiOxMAP at 1, 4 and 6-day storage (Chen *et al*. 2015). Differences in pig genetics contribute to varying purge loss in these studies.

3.5.2. Cooking loss

Cooking loss is the loss of water with soluble substances from meat during cooking. It correlates inversely with sensory tenderness and juiciness (Aaslyng *et al*. 2003, Nam *et al*. 2009).

This study provided mixed results for cooking loss (Table 8) in both VAC and hiOxMAP. Cooking loss was most significant at 2 days post mortem, however, a decrease in cooking loss occurred after 5 days in packaging. Combination of VAC for 10 days and MAP for 5 days did not affect cooking loss of pork loins.

Table 6. Oxymyoglobin/metmyoglobin (R630/580) of 1 day post mortem pork loins before and after packaging and ageing treatments.

	Days in packaging						Statistics					
	0	1	5	10	15	20	S.E.D.	Con	Con × Packaging	Con × Ageing	Con × Packaging × Ageing	Con × Packaging × Ageing × Combo
Control	2.322						0.051	<0.001	<0.001	<0.001	<0.001	<0.001
VAC		2.236	2.451	2.312	2.343	2.162						
MAP		2.423	1.992	1.776								
COMBO					1.983	1.699						

Con = Control (1 day post mortem meat not treated with packaging or ageing). VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

Table 7. Purge loss (%) of 1 day post mortem pork loins before and after packaging and ageing treatments.

	Days in packaging					Statistics				
	1	5	10	15	20	S.E.D.	Packaging	Ageing	Packaging × Ageing	Packaging × Ageing × Combo
VAC	11.11	14.64	16.07	16.77	16.56	0.748	<0.001	<0.001	0.04	<0.001
MAP	9.08	15.24	16.21							
COMBO				18.71	19.33					

VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. Purge loss of control (frozen at 1 day post mortem) was not applicable. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

Table 8. Cooking loss (%) of 1 day post mortem pork loins before and after packaging and ageing treatments.

	Days in packaging						Statistics					
	0	1	5	10	15	20	S.E.D.	Con	Con × Packaging	Con × Ageing	Con × Packaging × Ageing	Con × Packaging × Ageing × Combo
Control	24.31						0.625	<0.001	0.762	<0.001	<0.001	<0.001
VAC		28.83	23.33	26.74	28.55	28.59						
MAP		29.97	24.53	25.29								
COMBO					23.72	27.06						

Con = Control (1 day post mortem meat not treated with packaging or ageing). VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

3.6. Warner Bratzler peak force

WB peak force, together with compression texture profiles and juiciness, is well established as an indication of meat tenderness. WB shear force is the resistant force to shearing and is influenced mostly by myofibrillar structure (Bouton *et al*. 1975). A reduction in WB peak force is therefore associated with ageing of meat. Figure 2 shows that tenderisation of pork loins was most significant between days 1 and 5 in packaging (days 2 to 6 post mortem) for both VAC and hiOxMAP. At any of the other ageing time points, a lower WB peak force value existed for pork loins in VAC compared to those in hiOxMAP, indicating more significant myofibrillar structural changes of pork loins in VAC compared to hiOxMAP. The lower shear- force of loins in VAC compared to hiOxMAP is in agreement with results from a previous Pork CRC honours project in our group. In that study, untrained consumer panels showed loin packaged in hiOxMAP had inferior consumer acceptability for tenderness relative to samples vacuum packed for seven days.

An increase in peak force of pork loins in hiOxMAP only between days 5 and 10 and in combined packaging indicates toughening of meat, most likely due to oxidation-induced cross-linking of degraded proteins. The study of Kim *et al*. (2010) using SDS-PAGE, immunoblotting, and diagonal-PAGE revealed cross-linking of myosin heavy chain in different beef muscles packaged in hiOxMAP. Specific protein cross-linking in pork loins because of oxidation remains to be elucidated.

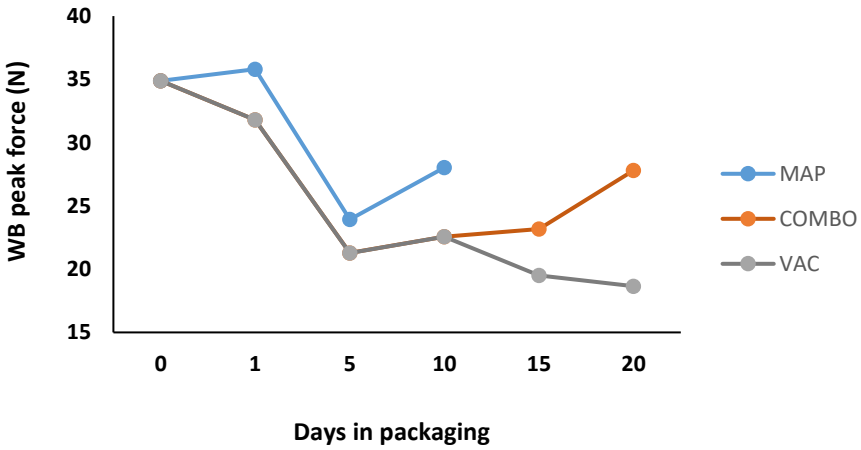

Figure 2. Predicted means for Warner Bratzler (WB) peak force of pork loins at various ageing times in different packaging systems. MAP = high oxygen modified atmosphere packaging; VAC = vacuum packaging; COMBO = 10 day in VAC followed by 5 or 10 days in MAP.

Table 9 showed that while packaging and ageing individually affected peak force ($P < 0.001$ for both treatments), no significant interaction between packaging and ageing was observed ($P = 0.428$).

It is also worth noting that loins packed in combination of VAC and hiOxMAP at either 15 or 20 days did not significantly differ in WB peak force compared to those in hiOxMAP only at day5 (

Table 9), suggesting loins packed in packaging combination improved tenderness of loins compared to hiOxMAP only.

Table 9. WB peak force (N) of 1 day post mortem pork loins before and after packaging and ageing treatments.

	Days in packaging						Statistics					
	0	1	5	10	15	20	S.E.D.	Con	Con × Packaging	Con × Ageing	Con × Packaging × Ageing	Con × Packaging × Ageing × Combo
Control	34.88 (3.55)						(0.09)	<0.001	<0.001	<0.001	0.428	<0.001
VAC		31.79 (3.46)	21.28 (3.06)	22.56 (3.12)	19.49 (2.97)	18.65 (2.93)						
MAP		35.80 (3.58)	23.93 (3.18)	28.02 (3.33)								
COMBO					23.17 (3.14)	27.80 (3.33)						

Con = Control (1 day post mortem meat not treated with packaging or ageing). VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. Values within parentheses were from natural log transformation which were back transformed to obtain predicted means. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

3.7. Compression texture profile

3.7.1. Hardness

Texture profile analysis was used as a quantitative tool to measure texture changes of meat. Hardness expressed as Newton is the resistant force required to compress meat, mimicking the chewing process.

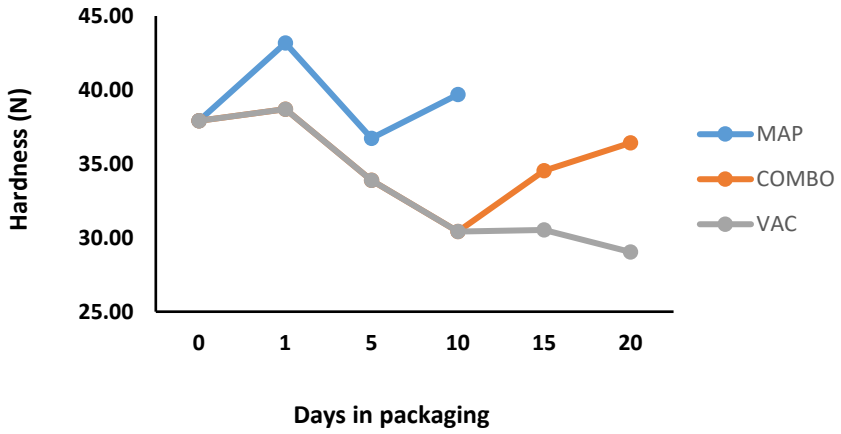

Figure 3. Predicted means for hardness of pork loins at various ageing times in different packaging systems. MAP = high oxygen modified atmosphere packaging; VAC = vacuum packaging; COMBO = 10 day in VAC followed by 5 or 10 days in MAP.

The hardness value of VAC packed loins continuously decreased throughout period of ageing up to 20 days (Figure 3). A significant P value (<0.001) of ageing (

Table 10) was also observed. However, the hardness of pork loins in hiOxMAP underwent a three-phase change. Within the first day of packaging, a significant increase in hardness occurred, indicating toughening of meat. The next 4 days of packaging saw a drop in hardness followed by another increase. Although the exact mechanism of the three-phase change is not understood, it is proposed a combination of (i) protein oxidation and denaturation, (ii) calpain activity leading to protein degradation and (iii) cross-linking or denatured/degraded proteins was at play.

Intriguingly, changing of packaging from VAC to hiOxMAP led to a significant increase in hardness of loins compared to the equivalent (in terms of days aged) VAC packed samples, confirming evidence for toughening of pork loins due to hiOxMAP. However, additional ageing of loins in hiOxMAP for either 5 or 10 days resulted in a lower hardness values compared with loins in hiOxMAP only treatment at 5 or 10 days, suggesting the combined packaging system alleviated some of the toughening effect caused by hiOxMAP.

Table 10. Hardness (N) of 1 day post mortem pork loins before and after packaging and ageing treatments.

	Days in packaging						Statistics					
	0	1	5	10	15	20	S.E.D.	Con	Con × Packaging	Con × Ageing	Con × Packaging × Ageing	Con × Packaging × Ageing × Combo
Control	37.91						1.68	0.037	<0.001	<0.001	0.075	<0.001
VAC		38.70	33.89	30.42	30.53	29.04						
MAP		43.17	36.73	39.68								
COMBO					34.54	36.42						

Con = Control (1 day post mortem meat not treated with packaging or ageing). VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

3.7.2. Cohesiveness

Cohesiveness of loins decreased in both VAC and hiOxMAP (Table 11) throughout the ageing period of 20 and 10 days, respectively. The most significant change in cohesiveness observed within the first day in hiOxMAP. The combined packaging system appeared to have no effect on cohesiveness of the loins.

3.7.3. Chewiness

In VAC, chewiness values of pork loins decreased significantly (improved) within the first day of packaging and continued to drop through the period of ageing up to 20 days. On the other hand, tenderisation of pork loins was abolished after 1 day in hiOxMAP evidenced by a significant increase in chewiness value.

In the combined packaging system, exposure of loins to high O_2 after 10 days of ageing in VAC neither increased nor decreased the chewiness value of meat. A lower chewiness value of loins in combined packaging system at 15 or 20 days compared to those in hiOxMAP only at 5 and 10 days indicated that the combined packaging system was able to lessen the toughening effect caused by hiOxMAP.

Table 11. Cohesiveness of 1 day post mortem pork loins before and after packaging and ageing treatments.

	Days in packaging						Statistics					
	0	1	5	10	15	20	S.E.D.	Con	Con × Packaging	Con × Ageing	Con × Packaging × Ageing	Con × Packaging × Ageing × Combo
Control	0.451						0.021	<0.001	<0.001	<0.001	<0.001	<0.001
VAC		0.321	0.377	0.259	0.192	0.198						
MAP		0.254	0.372	0.317								
COMBO					0.255	0.240						

Con = Control (1 day post mortem meat not treated with packaging or ageing). VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

Table 12**. Chewiness of 1 day post mortem pork loins before and after packaging and ageing treatments.**

	Days in packaging						Statistics					
	0	1	5	10	15	20	S.E.D.	Con	Con × Packaging	Con × Ageing	Con × Packaging × Ageing	Con × Packaging × Ageing × Combo
Control	12.58 (2.53)						(0.15)	<0.001	<0.001	<0.001	<0.001	<0.001
VAC		6.81 (1.92)	8.56 (2.15)	3.48 (1.25)	1.87 (0.63)	1.95 (0.67)						
MAP		5.30 (1.67)	9.40 (2.24)	6.88 (1.93)								
COMBO					3.78 (1.33)	3.77 (1.33)						

Con = Control (1 day post mortem meat not treated with packaging or ageing). VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. Values within parentheses were from natural log transformation which were back transformed to obtain predicted means. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

3.8. Lipid oxidation

Lipid oxidation and production of free radicals is correlated to colour and shelf life of meat (Faustman *et al*. 2010). Lipid oxidation leads to a decrease in surface redness and in oxymyoglobin: metmyoglobin ratio because of myoglobin oxidation (Lin and Hultin 1977). Rancidity is a flavour, which creates undesirable and unacceptable odour and taste in meat. An increase in lipid oxidation also leads to more rancidity, thus reducing shelf life. The molecular connection of lipid oxidation and rancidity has not been fully established. Campo *et al*. (2006), attempting to link TBARS values with sensory qualities of beef, found that rancid overpowered beef flavour at the TBARS threshold of 2 mg MDA/kg meat. In another study, Tarladgis *et al*. (1960) suggested TBARS value of 1 mg MDA/kg food is unacceptable to consumers, and MDA is only a part of the total odour complex. However, TBARS results varied widely due to different assay procedures, and panellists' personal preferences and experience. It is also noted that other volatile odour compounds produced by mechanisms other than lipid oxidation also contribute to the rancid flavour in pork (Casaburi *et al*. 2015).

Lipid oxidation, expressed as the amount (mg) of MDA per kg of meat, was determined (**Table 13**). Oxidation of lipids in VAC appeared to occur mostly in the first day of ageing and remained stable for up to 20 days while lipid oxidation in HiOxMAP packed loins increased over time to 10 days ageing. This is in agreement with previous studies (Lund *et al*. 2007, Kim *et al*. 2010). The level of MDA in loins in combined packaging at day 15 did not significantly differ to that in hiOxMAP packed only loins at days 5 or 10, suggesting that this packaging combination did not induce further rancidity compared to hiOxMAP packaging only. However, combined packaging of loins at day 20 significantly increased lipid oxidation. We propose that because of 10 days ageing in VAC, extensive generation of metabolites occurred. Combination of these metabolites and high O_2 environment for a further 10 days in hiOxMAP led to acceleration of lipid oxidation. In addition, there was a significant interaction between lipid oxidation and ageing time ($P < 0.001$,

Table **13**). Hence, reducing the number of ageing days in VAC prior to hiOxMAP may reduce lipid oxidation in the combined packaging system. As seen with WB peak force data (Table 9), significant tenderisation occurred within the first 5 days of ageing in VAC. Thus, a new combined packaging system (5 days in VAC followed by hiOxMAP) may enable a reduction lipid oxidation for pork loins.

It is noted that the level of MDA of all samples in this study was well below the 2 mg/kg meat recommended by Campo *et al*. (2006), suggesting an acceptable lipid oxidation level for shelflife. However, an increase in lipid oxidation in hiOxMAP may influence sensory attributes such as flavour and appearance. Consumer panel studies are required to confirm.

Table 13. Lipid oxidation (mg MDA/kg meat) of 1 day post mortem pork loins before and after packaging and ageing treatments.

	Days in packaging						Statistics					
	0	1	5	10	15	20	S.E.D.	Con	Con × Packaging	Con × Ageing	Con × Packaging × Ageing	Con × Packaging × Ageing × Combo
Control	0.06 (-2.82)						(0.153)	<0.001	0.009	<0.001	0.047	<0.001
VAC		0.13 (-2.08)	0.11 (-2.23)	0.09 (-2.36)	0.14 (-2.00)	0.12 (-2.09)						
MAP		0.15 (-1.89)	0.21 (-1.54)	0.28 (-1.29)								
COMBO					0.32 (-1.13)	0.62 (-0.48)						

Con = Control (1 day post mortem meat not treated with packaging or ageing). Pac = Packaging. VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. Values within parentheses are log transformed which were used to back transform predicted mean values. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

3.9. Protein oxidation

3.9.1. Carbonyl content

Protein carbonylation is promoted by reactive oxygen species. Primary protein carbonylation such as oxidation of side chains of L, R, P, and T amino acids produces DNPH detectable protein product. DNPH derivatizable protein adducts can also be formed via the addition of aldehydes such as those generated from lipid peroxidation (Suzuki *et al*. 2010). An increase in carbonyl content is equivalent to an increase in protein oxidation.

No significant difference in protein carbonylation occurred for VAC packed loins up to 20 days of ageing. However, hiOxMAP pack loins displayed a rising trend for carbonyl content over the ageing period of 10 days. The amount of carbonyl group significantly differed (two fold) between VAC and hiOxMAP at day 10. The combined packaging system appeared to eliminate the increase in protein oxidation created by hiOxMAP at both days 15 and 20 of ageing.

3.9.2. Free thiol group content

The level of three thiol groups in extracted protein samples is another indication of protein oxidation. As the endogenous calpains belong to thiol protease family, free thiol group content is also indicative of calpain activation/activity in meat. A decrease in free thiol content is equivalent to an increase in protein oxidation.

While ageing did not appear to affect the level of free thiol in VAC packed loins except at 20 days ageing, the loins in hiOxMAP exhibited a significant drop in free thiol content within the first day of ageing. There was no further significant change in free thiol content in hiOxMAP samples up to 10 days of ageing. These results suggest that an increase in disulphide bond formation and inactivation of calpain occurred rapidly when loins ware exposed to the high O_2 environment. The use of combined packaging system at both days 15 and 20 appeared to reduce the effect of hiOxMAP on thiol groups.

Modified amino acid side chains as a result of oxidation has been shown to induce cross-linking of myofibril proteins, especially myosin (Lund *et al*. 2008, Xiong *et al*. 2008), which may explain a reduction in tenderness and increase in hardness. Intriguingly, oxidation of myofibrillar proteins has also been linked to a decrease in WHC thus a decrease in juiciness in pork (Liu *et al*. 2010, Delles and Xiong 2014).

Table 14. **Carbonyl content (nmol DNPH/mg protein) of 1 day post mortem pork loins before and after packaging and ageing.**

	Days in packaging						Statistics					
	0	1	5	10	15	20	S.E.D.	Con	Con × Packaging	Con × Ageing	Con × Packaging × Ageing	Con × Packaging × Ageing × Combo
Control	1.711						0.569	0.113	0.003	<0.001	0.023	0.345
VAC		1.448	1.666	2.362	2.51	1.777						
MAP		1.954	2.31	4.58								
COMBO					2.949	2.237						

Con = Control (1 day post mortem meat not treated with packaging or ageing). VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

Table 15. Free thiol content (nmol L-cysteine/mg protein) of 1 day post mortem pork loins before and after packaging and ageing.

	Days in packaging						Statistics					
	0	1	5	10	15	20	S.E.D.	Con	Con × Packaging	Con × Ageing	Con × Packaging × Ageing	Con × Packaging × Ageing × Combo
Control	67.22 (4.21)						(0.12)	0.002	0.003	0.629	0.006	0.003
VAC		63.05 (4.14)	59.15 (4.08)	64.78 (4.17)	54.43 (4.00)	41.64 (3.73)						
MAP		42.52 (3.75)	42.73 (3.76)	52.61 (3.96)								
COMBO					49.40 (3.90)	49.45 (3.90)						

Con = Control (1 day post mortem meat not treated with packaging or ageing). VAC = vacuum packaging. MAP = high oxygen modified atmosphere packaging. COMBO = VAC for 10 days followed by MAP for 5 or 10 days. Values within parentheses are log transformed which were used to back transform predicted mean values. S.E.D = Standard error difference. Significant difference ≥ 2 × S.E.D.

4. Application of Research

This project, using published scientific quantitative tools, identified an increase in browning and a reduction in instrumental tenderness and water holding capacity of Australian pork loins packed in the widely used hiOxMAP system. Comparing vacuum-packed loins with those in hiOxMAP presented an alternative packaging method with predicted superior eating qualities. These findings suggest that Australian pork loins are not suited for packaging in hiOxMAP. They suggest a low oxygen-containing packaging system, such as VAC and vacuum skin pack (VSP), a recent innovation in packing for retail shelves, might be the preferred option over hiOxMAP.

The results could be implemented by industry to ensure that high quality pork is delivered to the consumers. This can be achieved at minimal cost by reducing the use of hiOxMAP while increasing the use of alternative packaging systems that are already in place such as vacuum and vacuum skin packaging. Also reducing the time of storage in hiOxMAP to less than 5 days by limiting hiOxMAP to only pork cuts with a high turnover would benefit eating qualities. At the same time, allowance for sufficient ageing of pork loins in VAC prior to hiOxMAP may alleviate some of the negative impacts. Lipid and protein oxidation results suggest that if use of hiOxMAP continues further investigation in reducing oxidation such as different gas mixtures and concentrations, and edible antioxidant coatings. Delivering consistently high quality Australian pork that is superior in appearance, juiciness and tenderness while being low in rancidity will better satisfy consumers and may potentially induce repeat purchases.

5. Conclusion

From the results of this study, it is concluded that:

- HiOxMAP had negative impacts on appearance, tenderness and juiciness of Australian pork loins because of increased lipid and protein oxidation.
- Low oxygen-containing packaging systems such as vacuum packaging and the more recent innovation vacuum skin packaging - are recommended.
- Should hiOxMAP continue to be used, delaying distribution to maximise tenderisation in VAC prior to hiOxMAP may partly reverse some detrimental impacts on eating qualities of hiOxMAP.

6. Limitations/Risks

An increase in lipid oxidation was observed for loins packed in the combined packaging system for 20 days (VAC for 10 days followed by hiOxMAP for 10 days). Experimental design of storing loins in VAC for 10 days was to ensure sufficient ageing of meat. The results in this project showed sufficient ageing in VAC after 5 days. Thus, in the combined packaging system varying storage time in VAC to 5 days prior to hiOxMAP may reduce lipid oxidation of pork loin.

As shown by previous research, due to differences in muscle tissue structure, lipid and protein compositions, eating qualities of meat can differ significantly between muscles. The findings for pork loins in this project may not be applicable to other pork cuts/muscles. Similar investigation is recommended.

The current project used instrumental techniques to quantify various parameters, which have been shown to affect eating qualities of pork. However, pork consumers whose preferences have been shown to vary widely ultimately decide eating qualities. Therefore, there is a need for an evaluation by consumer panels of pork eating qualities should recommendations in this report be adopted.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

- Further research is needed for alternative packaging systems, such as vacuum skin packaging, and recommendation of blooming to consumers for a more appealing appearance of pork in low oxygen-containing packaging systems.

- HiOxMAP should be limited to pork cuts with a high turnover to reduce the amount of time meat is exposed to the high oxygen environment to less than 5 days.

- If usage of hiOxMAP was to be continued, sufficient ageing (approximately 5 days post slaughtering in VAC) is needed prior to distribution to packaging facilities.

- Research of different packaging systems including a combined VAC and hiOxMAP on pork cuts/muscles other than longissimus (loin).

- Research of sensory qualities using consumer panels of different packaging systems including a combined VAC and hiOxMAP on Australian pork.

8. References

Aaslyng, M. D., Bejerholm, C., Ertbjerg, P., Bertram, H. C. and Andersen, H. J. (2003) 'Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure', *Food Quality and Preference*, 14(4), 277-288.

Bao, Y. L. and Ertbjerg, P. (2015) 'Relationship between oxygen concentration, shear force and protein oxidation in modified atmosphere packaged pork', *Meat Science*, 110, 174-179.

Bee, G., Anderson, A. L., Lonergan, S. M. and Huff-Lonergan, E. (2007) 'Rate and extent of pH decline affect proteolysis of cytoskeletal proteins and water-holding capacity in pork', *Meat science*, 76(2), 359-365.

Bouton, P., Ford, A. L., Harris, P. and Ratcliff, D. (1975) 'Objective-subjective assessment of meat tenderness', *Journal of Texture Studies*, 6(3), 315-328.

Bredahl, L., Grunert, K. G. and Fertin, C. (1998) 'Relating consumer perceptions of pork quality to physical product characteristics', *Food Quality and Preference*, 9(4), 273-281.

Brewer, M. S. and McKeith, F. K. (1999) 'Consumer-rated quality characteristics as related to purchase intent of fresh pork', *Journal of Food Science*, 64(1), 171-174.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D. and Richardson, R. I. (2006) 'Flavour perception of oxidation in beef', *Meat Science*, 72(2), 303-311.

Casaburi, A., Piombino, P., Nychas, G.-J., Villani, F. and Ercolini, D. (2015) 'Bacterial populations and the volatilome associated to meat spoilage', *Food Microbiology*, 45, Part A, 83-102.

Cayuela, J. M., Gil, M. D., Banon, S. and Garrido, M. D. (2004) 'Effect of vacuum and modified atmosphere packaging on the quality of pork loin', *European Food Research and Technology*, 219(4), 316-320.

Channon, H. A., Taverner, M. R., D'Souza, D. N. and Warner, R. D. (2014) 'Aitchbone hanging and ageing period are additive factors influencing pork eating quality', *Meat science*, 96(1), 581-590.

Chen, L., Zhou, G. H. and Zhang, W. G. (2015) 'Effects of high oxygen packaging on tenderness and water holding capacity of pork through protein oxidation', *Food and Bioprocess Technology*, 8(11), 2287-2297.

Clausen, I., Jakobsen, M., Ertbjerg, P. and Madsen, N. T. (2009) 'Modified atmosphere packaging affects lipid oxidation, myofibrillar fragmentation index and eating quality of beef', *Packaging Technology and Science*, 22(2), 85-96.

Condon, J. (2015) *Woolies new $150m Melbourne meat facility geared for growth* [online], available: https://www.beefcentral.com/processing/woolies-new-150m-melbourne-meat-facility-designed-for-growth/ [accessed 1st May 2016].

Culler, R. D., Parrish, F. C., Smith, G. C. and Cross, H. R. (1978) 'Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle', *Journal of Food Science*, 43(4), 1177-1180.

Davis, K. J., Sebranek, J. G., Huff-Lonergan, E. and Lonergan, S. M. (2004) 'The effects of aging on moisture-enhanced pork loins', *Meat Science*, 66(3), 519-524.

Delles, R. M. and Xiong, Y. L. (2014) 'The effect of protein oxidation on hydration and water-binding in pork packaged in an oxygen-enriched atmosphere', *Meat Science*, 97(2), 181-188.

Faustman, C., Qun, S., Mancini, R. and Suman, S. P. (2010) 'Myoglobin and lipid oxidation interactions: Mechanistic bases and control', *Meat Science*, 86(1), 86-94.

Fu, Q. Q., Liu, R., Zhang, W. G., Li, Y. P., Wang, J. and Zhou, G. H. (2015) 'Effects of different packaging systems on beef tenderness through protein modifications', *Food and Bioprocess Technology*, 8(3), 580-588.

Geesink, G., Robertson, J. and Ball, A. (2015) 'The effect of retail packaging method on objective and consumer assessment of beef quality traits', *Meat Science*, 104, 85-89.

Honikel, K. O. (1998) 'Reference methods for the assessment of physical characteristics of meat', *Meat Science*, 49(4), 447-457.

Johnston, B. (2011) *Western Meat Packers opens $7m facility* [online], available: http://www.farmweekly.com.au/news/agriculture/cattle/general-news/western-meat-packers-opens-7m-facility/2086202.aspx [accessed 1st May 2016].

Joo, S. T., Kauffman, R. G., Kim, B. C. and Park, G. B. (1999) 'The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle', *Meat Science*, 52(3), 291-7.

Khliji, S., van de Ven, R., Lamb, T. A., Lanza, M. and Hopkins, D. L. (2010) 'Relationship between consumer ranking of lamb colour and objective measures of colour', *Meat Science*, 85(2), 224-229.

Kim, Y. H., Huff-Lonergan, E., Sebranek, J. G. and Lonergan, S. M. (2010) 'High-oxygen modified atmosphere packaging system induces lipid and myoglobin oxidation and protein polymerization', *Meat Science*, 85(4), 759-767.

Koohmaraie, M. (1994) 'Muscle proteinases and meat aging', *Meat Science*, 36(1-2), 93-104.

Kristensen, L. and Purslow, P. P. (2001) 'The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins', *Meat Science*, 58(1), 17-23.

Lagerstedt, A., Ahnstrom, M. L. and Lundstrom, K. (2011a) 'Vacuum skin pack of beef - A consumer friendly alternative', *Meat Science*, 88(3), 391-396.

Lagerstedt, A., Lundstrom, K. and Lindahl, G. (2011b) 'Influence of vacuum or high-oxygen modified atmosphere packaging on quality of beef M. longissimus dorsi steaks after different ageing times', *Meat Science*, 87(2), 101-106.

Laville, E., Sayd, T., Sante-Lhoutellier, V., Morzel, M., Labas, R., Franck, M., Chambon, C. and Monin, G. (2005) 'Characterisation of PSE zones in semimembranosus pig muscle', *Meat Science*, 70(1), 167-172.

Lesiow, T. and Xiong, Y. L. L. (2013) 'A simple, reliable and reproductive method to obtain experimental pale, soft and exudative (PSE) pork', *Meat Science*, 93(3), 489-494.

Lin, T.-S. and Hultin, H. O. (1977) 'Oxidation of myoglobin in vitro mediated by lipid oxidation in microsomal fractions of muscle', *Journal of Food Science*, 42(1), 136-140.

Lindahl, G., Lundstrom, K. and Tornberg, E. (2001) 'Contribution of pigment content, myoglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs', *Meat Science*, 59(2), 141-151.

Liu, Z., Xiong, Y. L. and Chen, J. (2010) 'Protein oxidation enhances hydration but suppresses water-holding capacity in porcine longissimus muscle', *Journal of Agricultural and Food Chemistry*, 58(19), 10697-10704.

Lund, M. N., Lametsch, R., Hviid, M. S., Jensen, O. N. and Skibsted, L. H. (2007) 'High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine longissimus dorsi during chill storage', *Meat Science*, 77(3), 295-303.

Lund, M. N., Luxford, C., Skibsted, L. H. and Davies, M. J. (2008) 'Oxidation of myosin by haem proteins generates myosin radicals and protein cross-links', *Biochemical Journal*, 410(3), 565-574.

McMillin, K. W. (2008) 'Where is MAP Going? A review and future potential of modified atmosphere packaging for meat', *Meat Science*, 80(1), 43-65.

Melody, J. L., Lonergan, S. M., Rowe, L. J., Huiatt, T. W., Mayes, M. S. and Huff-Lonergan, E. (2004) 'Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles', *Journal of Animal Science*, 82(4), 1195-1205.

Nam, Y. J., Choi, Y. M., Lee, S. H., Choe, J. H., Jeong, D. W., Kim, Y. Y. and Kim, B. C. (2009) 'Sensory evaluations of porcine longissimus dorsi muscle: Relationships with postmortem meat quality traits and muscle fiber characteristics', *Meat Science*, 83(4), 731-736.

Pearce, K. L., Rosenvold, K., Andersen, H. J. and Hopkins, D. L. (2011) 'Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes--a review', *Meat Science*, 89(2), 111-24.

Smulders, F. J. M., Hiesberger, J., Hofbauer, P., Dogl, B. and Dransfield, E. (2006) 'Modified-atmosphere storage under subatmospheric pressure and beef quality: II. Color, drip, cooking loss, sarcomere length, and tenderness', *Journal of Animal Science*, 84(9), 2456-2462.

Sorensen, G. and Jorgensen, S. S. (1996) 'A critical examination of some experimental variables in the 2-thiobarbituric acid (TBA) test for lipid oxidation in meat products', *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung*, 202(3), 205-210.

Suzuki, Y. J., Carini, M. and Butterfield, D. A. (2010) 'Protein carbonylation', *Antioxidants & Redox Signaling*, 12(3), 323-325.

Takahashi, K. (1996) 'Structural weakening of skeletal muscle tissue during post-mortem ageing of meat: The non-enzymatic mechanism of meat tenderization', *Meat Science*, 43, S67-S80.

Tam, L. G., Berg, E. P., Gerrard, D. E., Sheiss, E. B., Tan, F. J., Okos, M. R. and Forrest, J. C. (1998) 'Effect of halothane genotype on porcine meat quality and myoglobin autoxidation', *Meat Science*, 49(1), 41-53.

Tarladgis, B. G., Watts, B. M., Younathan, M. T. and Dugan, L. (1960) 'A distillation method for the quantitative determination of malonaldehyde in rancid foods', *Journal of the American Oil Chemists' Society*, 37(1), 44-48.

Wang, J., Yan, X. l., Liu, R., Fu, Q. q., Zhou, G. h. and Zhang, W. g. (2016) 'Differences in calpain system, desmin degradation and water holding capacity between commercial Meishan and Duroc × Landrace × Yorkshire crossbred pork', *Anim Science Journal*, 87(1), 109-116.

Warner, R. D., Kauffman, R. G. and Greaser, M. L. (1997) 'Muscle protein changes post mortem in relation to pork quality traits', *Meat Science*, 45(3), 339-352.

Xiong, Y. L., Park, D. and Ooizumi, T. (2008) 'Variation in the cross-linking pattern of porcine myofibrillar protein exposed to three oxidative environments', *Journal of Agricultural and Food Chemistry*, 57(1), 153-159.

Yin, Y., Zhang, W. G., Zhou, G. H. and Guo, B. (2014) 'Comparison of protein degradation, protein oxidation, and mu-calpain activation between pale, soft, and exudative and red, firm, and nonexudative pork during postmortem aging', *Journal of Animal Science*, 92(8), 3745-3752.

Yu, L. P. and Lee, Y. B. (1986) 'Effects of postmortem pH and temperature on bovine muscle structure and meat tenderness', *Journal of Food Science*, 51(3), 774-780.

Zakrys-Waliwander, P. I., O'Sullivan, M. G., O'Neill, E. E. and Kerry, J. P. (2012) 'The effects of high oxygen modified atmosphere packaging on protein oxidation of bovine M. longissimus dorsi muscle during chilled storage', *Food Chemistry*, 131(2), 527-532.

Zhang, W. G., Lonergan, S. M., Gardner, M. A. and Huff-Lonergan, E. (2006) 'Contribution of postmortem changes of integrin, desmin and mu-calpain to variation in water holding capacity of pork', *Meat Science*, 74(3), 578-585.