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Impact of high oxygen packaging on MSA lamb loin and topside eating quality

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Executive Summary

Highly centralised meat processing, packaging and distribution chains require new solutions for “retail ready” or “case ready” packaging formats for lamb. Hi oxygen modified atmosphere packaging (Hi-Ox-MAP) – where typically the gas mix is 80% O_2 and 20% CO_2 - has been the most widely used retail ready solution for the last decade in Australia, as a “fresh” bright red meat colour is maintained for a long period in the pack. However, recent overseas studies (mainly in beef) have shown that the eating quality of Hi-Ox-MAP packaged meat is less than optimal due to toughening of the meat and negative flavour changes. Vacuum or Cryovac® packaging (VP) has been used successfully for the storage of meat primals; however VP meat has traditionally been unacceptable for retail display due to the presence of the dark purple deoxymyoglobin in meat stored under this anaerobic environment. Thus retailers have generally avoided using VP, because the purple colour is perceived negatively by consumers. Industry (Sealed Air Inc.) has recently developed new technologies for retail ready meat; Cryovac Darfresh® Barrier vacuum skin packaging (VSP) and Cryovac Darfresh® Bloom (Hi-Ox-DB) and now other packaging formats such as UltraDarfresh®. VSP uses an advanced barrier system which takes advantage of a novel composite polymer with two layers. The Hi-Ox-DB system incorporates elements of VSP, within a Hi-Ox-MAP tray; e.g. the headspace gas composition is also 80% O_2 and 20% CO_2 .

Lamb topside and backstrap muscles from animals of known genetics were sourced from Victorian producer demonstration sites. The muscles were directly cut into steaks without any prior vacuum wet aging and evenly allocated to either Hi-Ox-MAP, VSP or Hi-Ox-DB retail packaging treatments and stored at 1 ^{o}C for either 5 or 10 days in a simulated retail display. After storage, the raw meat colour (L*a*b* tristimulus values) and pH were measured and meat was repackaged and frozen for later consumer sensory testing according to Meat Standards Australia (MSA) protocols. After 5 and 10 days storage both Hi-Ox-MAP and Hi-Ox-DB maintained a bright red colour in contrast to a characteristic purple colour for the VSP packaged lamb.

The effects on eating quality (CMQ4 scores) were consistent for each packaging format regardless of the muscle type, backstrap or topside. All sensory attributes were rated significantly lower for the topside samples compared to the backstrap. After 5 and 10-days storage CMQ4 scores indicated that Hi-Ox-MAP and Hi-Ox-DB systems were able to maintain “good everyday” quality, whereas VSP packaged backstrap was rated “better than everyday” quality. CMQ4 scores were significantly lower for topside samples, with Hi-Ox-MAP stored topsides falling into an “unsatisfactory” quality category after 10 days. Overall, the MSA consumer testing clearly showed that the eating quality of VSP packaged lamb was better than Hi-Ox-MAP, despite the purple coloured meat. Hi-Ox-MAP and Hi-Ox-DB samples had low

tenderness and flavour scores compared to VSP after 5 days of storage. The Hi-Ox-DB samples had only marginally better eating quality than the Hi-Ox-MAP samples at 10 days, despite the maintenance of a red meat colour in the pack.

All sensory attributes were rated significantly lower for the topside samples compared to the backstrap. It was concluded that direct packaging of lamb under high oxygen MAP (Hi-Ox-MAP or Hi-Ox-DB) should not generally be recommended for retail ready packaging of lamb at 1 day post-slaughter. Although Hi-Ox-DB provided some modest gains in eating quality, it is uncertain whether it can be recommended as a viable solution for retail packaging of lamb. It should be noted that current Australian guidelines require that lamb in first wet aged under vacuum for 5 days before repackaging into other retail ready formats.

There were minimal differences in the volatile profiles and odour quality of raw samples stored for 10 days under VSP, Hi-Ox-DB or Hi-Ox-MAP. This confirmed that no discernible or characteristic “off-odours” developed during storage for any of the evaluated packaging systems. Finally, there was little evidence of differences in proteolysis between the packaging treatments, as measured by concentrations of free amino acids or peptides. This result was consistent with the hypothesis that the toughening of meat under Hi-Ox-MAP occurs primarily due to intermolecular cross-linking or other mechanisms, rather than inhibition of proteolytic enzymes.

Recommendation for MSA SMEQ pathways committee:

- The data in this project has clearly shown that packaging of backstrap or topside in a high O_2 MAP system (Hi-Ox-DB or Hi-Ox-MAP) at 1 day post-slaughter results in inferior eating quality, due to a failure to exhibit normal ageing. Thus in order for sheep meat to be eligible for MSA grading, it is essential that the recommended ageing period for the particular carcass/cut is undertaken in anaerobic (vacuum packaged, VSP) environment. Current guidelines require a minimum 5-day aging period

- There was no data collected on whether there is deterioration in quality of sheep meat which has undergone prior ageing in vacuum before high O_2 packaging. Thus it is recommended that further research is undertaken to collect this data.

- Industry will require flexible options for packaging in the future. Thus it is recommended that methods to ameliorate the deterioration in eating quality during Hi-Ox-MAP should be included in the research in point 2 above. Possible strategies could include, nitrite/antioxidants embedded in the packaging material or using CO in the gas mixture.

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1 Background

1.1 Use of modified atmosphere packaging for meat

1.1.1 A short history of MAP for red meat packaging

Modified atmosphere packaging can be defined as; “the removal and/or replacement of the atmosphere surrounding [meat] before sealing in with vapour-barrier materials” (McMillin *et al.* 1998; McMillin 2008). MAP is commonly understood to mean the replacement of the headspace gas with another gas mixture before sealing with barrier materials, although McMillin *et al.* (1998; 2008) states that MAP formats may also include the compete removal of air, as in vacuum packaging (VP). In this report, VP will be considered and discussed as a separate packaging system to MAP formats. The goals of MAP are to extend the shelf life of the meat, maintaining a microbiologically safe product with high visual appeal and acceptable eating quality. Conversely, the goals of VP are to provide an environment which allows the ageing of meat to occur, with no compromise in microbiological safety. Thus VP was introduced as an alternative to the traditional ageing method of carcass ageing. Carcass ageing became obsolete due to the lack of space in the chillers of processing plants. MAP was developed for shelf-life extension on retail shelves, and as an alternative to the over-wrap system.

Safety is a non-negotiable factor; the meat must be wholesome to eat and free of microbiological risk. The decision to purchase meat is based on visual appearance and the overall visual consumer appeal, which is influenced by meat and fat colour, presence of weep in tray and fat content. The consumer satisfaction with their purchase decision is made after they have cooked the meat and have considered the eating quality traits of odour, flavour, juiciness and tenderness. For consumers, initial selection in the supermarket is mainly based on the presence of an acceptable meat colour, with some variations from pale, to bright red being acceptable, and any dark, purple, green or brown hues being considered unacceptable. Whereas after cooking, tenderness, flavour and juiciness become the major determinants of eating quality (Dransfield *et al.* 1998; Mancini and Hunt 2005). Traditionally, the optimal system for delivering safe, visually acceptable and highly desirable eating quality red meat is to age the meat in VP for 5-14 days, followed by slicing and over-wrapping for display on retail shelves. Using this system, the shelf-life of the over-wrapped meat is approximately 3 days, before it needs to be removed from the shelves due to the development of undsirable brown colours in the meat surface. The brown colour is due to the oxidative conversion of oxy- or deoxy- myoglobin (red and purple colour re) to metmyoglobin (brown colour). High oxygen MAP (Hi-Ox-MAP) was introduced in order to obtain greater shelf-life. In fact the shelf-life of lamb meat is only about 2-3 days for over-wrapped product and up to 8-10 days for Hi-Ox-MAP product (Warner 2014). Overseas studies (in beef and pork mainly) have shown that meat does not age optimally in Hi-Ox-MAP.

Thus to date, no single packaging format has been able to simultaneously deliver on the visual and eating quality traits as well as on long shelf-life and safety. Plastics and packaging manufacturers continue to devise novel solutions for the meat industry. These may include MAP variations, with novel headspace gas compositions (e.g. high CO_2 or CO) and new barrier materials as well as active packaging and intelligent packaging (McMillin 2008).

Vacuum packaging (VP) effectively removes oxygen from the sample and creates an anaerobic environment (McMillin 2008). VP has been widely used to extend the storage life (but not shelf-life) of meat and improve the quality of primal and sub-primal meat cuts. The familiar Cryovac® (Sealed Air) vacuum bag packaging systems have been successfully in use for decades (McMillin 2008; Delmore 2009). VP barrier materials are generally co-extruded films with very low O_2 permeability (McMillin 2008). In the meat supply chain, primals are generally first "wet-aged" in VP for a specified period of time, before breaking down into consumer or retail ready packs. VP allows very long shelf-life for beef primals (traditionally 60-90 days but now up to 20 weeks; (Hughes *et al.* 2015) and 45-60 days of shelf-life for minced or ground beef depending on the barrier film used (McMillin 2008).

Lamb primals under VP can attain long storage times (up to 10 weeks , possibly longer; (McPhail *et al.* 2014d) under very well controlled temperatures (-1.5 ± 0.5 ^oC) (Kiermeier *et al.* 2013; Mills *et al.* 2014). Such long storage times have enabled successful export of packaged lamb to distant markets (Mills *et al.* 2014). The storage shelf life of VP lamb and beef is highly temperature dependent and is also dependent on the pH (McPhail *et al.* 2014d; Hughes *et al.* 2015) . At higher storage temperatures, the shelf-life of VP lamb can decrease dramatically; 40 days at 0 ^oC and only 10 days at 2 ^oC (Mills *et al.* 2014). VP barrier films typically have very low oxygen transmission rates, e.g.; 18.6 cm^3/m^2/24 h at 23 °C (Kiermeier *et al.* 2013).

The oxygen transmission rate (OTR) of the VP material can also influence shelf-life. Lamb primals packed in VP with high OTR (36 cm^3/m^2/day) has been to have significantly reduced storage life relative to lamb primals packed in material of lower OTR (9.9 cm^3/m^2/day) (McPhail *et al.* 2014a). Oxygen transmission rates decrease at low temperatures, especially below 0 ^oC. Thus meat in VP material of low OTR and stored below 0 ^oC have a longer storage life than meat in VP material of high OTR and stored above 0 ^oC (McPhail *et al.* 2014a). After the primal is removed from vacuum, cut and re-packaged in a case ready format such as Hi-Ox-MAP, a retail shelf life of up to 10 days can be expected (R. Warner, *pers.comm.*)

Increasingly, the industry supply chain requires centralised packaging solutions, where consumer–sized portions are directly packaged into "retail-ready" or "case-ready" formats with extended shelf-life. Hi-Ox-MAP, with a headspace composition of 80% O_2, 20% CO_2 remains the most popular packaging option for retail-ready fresh red meat. The high concentration of O_2 helps retain a bright red meat colour and the CO_2 inhibits bacterial growth (Eilert 2005; McMillin 2008). The high O_2 levels used in

Hi-Ox-MAP can promote oxidative changes in meat, potentially leading to oxidation of lipids and flavour and colour changes. A low muscle content of the natural anti-oxidant, vitamin E, or the application of irradiation to meat, have been shown to induce oxidative conditions and reduced tenderisation during ageing (Warner *et al.* 2005b). The first evidence for a detrimental effect of Hi-Ox-MAP on meat tenderisation was in 1994 (see (Warner *et al.* 2005). Since the review by (Warner *et al.* 2005a), there has been many articles reporting reduced tenderisation and even toughening during the ageing period in Hi-Ox-MAP (Lund *et al.* 2007b; Lund *et al.* 2008; Kim *et al.* 2010b; Estevez 2011; Kim *et al.* 2012).

MAP gas combinations using N_2, CO and higher levels of CO_2 have been described (Wilkinson *et al.* 2006; Brooks *et al.* 2008). The addition of low concentrations of carbon monoxide (CO) to MAP gas mixtures has been of particular interest, because the binding affinity of CO to myoglobin is much higher than oxygen. The presence of CO in the headspace gas at concentrations as low as 0.4% irreversibly forms carboxymyoglobin and a stable bright cherry red colour, even in the presence of residual O_2. CO is often mixed with 60-70% CO_2 and 30-40% N_2, with low residual oxygen, a format known as CO-Lo-Ox-MAP. The high CO_2 concentration effectively extends the meat shelf life from a microbiological perspective and the CO maintains the bright red colour well beyond a safe shelf life (Brooks *et al.* 2008).

Although CO-Lo-Ox MAP was declared safe for use by the US Food and Drug Administration in (2004) and generally recognised as safe by the European Union (2001), the use of CO-Lo-Ox-MAP has subsequently been banned in the European Union. Other countries such as Canada and Singapore have also banned the use of CO-Lo-Ox-MAP for meat packaging.

Consumer advocate groups in the US and Europe have been vocal in raising concerns of food safety, because the bright red colour denies the consumer the normal visual cues of meat spoilage and potentially masks the presence of food spoilage pathogens. It is fair to say that CO-Lo-Ox-MAP has attracted widespread controversy (rightly or wrongly) and there is significant consumer resistance to this gas being used in MAP (Grebitus *et al.* 2013). There is currently no legal restriction on the use of CO-Lo-Ox-MAP for red meat in Australia, however the current use of this technology in Australia is estimated to be low and most large retailers are not eager to adopt this technology. It is worth noting that the recent standard 1.3.3 of the *Australia New Zealand Food Standards Code* (the Code) does not permit carbon monoxide to be used to fix or alter the colour of the flesh of fish to be sold as food. (http://www.foodstandards.gov.au/code/proposals/Pages/proposalp1019carbonm5490.aspx).

There is evidence that Food Standards Australia & New Zealand is likely place formal restrictions on the use of CO-Lo-Ox-MAP for red meat applications in the future. Interestingly, similar to the consumer advocates' reasons for being concerned about the use of carbon monoxide, it is possible that a high-Ox MAP system results in the bright red colour of oxymyoglobin masking the normal cues of oxidation in meat, thus masking the chemical deterioration and development of inferior eating quality.

Vacuum skin packaging (VSP) is regarded as an improvement on conventional VP, as it is a flexible low oxygen permeability skin that is shrink-wrapped around the meat onto a rigid base. VSP minimises the formation of air pockets and wrinkles in the packaging creating a visually appealing retail format (Vázquez et al., 2004). VSP also has other advantages for retailers, as outlined in Appendix 1.

A major disadvantage of VP/VSP systems is that the surface of the meat has the purple deoxymyoglobin (DeoxyMb) predominating in the surface, with negligible formation of oxymyoglobin (OxyMb). The meat will "bloom" and develop a red colour after removal from vacuum and re-exposure to air, although longer storage periods in VP/VSP, result in a shorter shelf-life. In the past, many consumers found the purple colour of VP meat unacceptable, to the extent that retailers have avoided using VP for retail packs altogether (Mancini and Hunt 2005). Although VP meat has not traditionally been put onto the retail shelves, recently VSP packaged meat has become more common in retail settings overseas and in Australia, mainly for high value meat cuts and quality brands. This implies that any intrinsic consumer resistance to the appearance of VP meat is lower than previously thought.

Another potential disadvantage of VP/VSP packaged meat is the development of "confinement" or a slight "off" odour, due to the activity of anaerobic bacteria on the surface of the meat. But once the VP/VSP is opened, this odour rapidly dissipates. Consumers may find this off-putting and (falsely) associate the odour with spoiled product and a subsequent bad eating experience. To avoid these issues, Hi-Ox-MAP has been the packaging system of choice for retailers. Hi-Ox-MAP maintains a bright red fresh meat colour and minimises odour development while providing a shelf life (for beef and lamb) of up to 10 days at 4 ^{o}C. However, a significant negative trade-off, includes decreased tenderness, which has been reported overseas (McMillin 2008) as well as potential oxidative flavour changes.

Because meat texture (tenderness and juiciness) is such a fundamental part of a good meat eating experience, new packaging solutions that can enhance tenderness and retain desirable fresh red meat colour would provide the industry and consumers with valuable new options. A number of published research trials have compared Hi-Ox-MAP with alternative packaging formats such as the studies by (Lagerstedt *et al.* 2011; Li *et al.* 2012) where they investigated the tenderness of beef steaks packed in Hi-Ox-MAP, VP and VSP. In both studies, the tenderness of Hi-Ox-MAP beef was significantly lower than either VSP or VP samples.

There has been little published data on the effect of Hi-Ox-MAP on Australian meat products and also there is not much published data world-wide on the effects of Hi-Ox-MAP on sheep meat quality. Finally, MSA is the system used in Australia for assuring eating quality of beef and sheep meat. Thus, reliable data on the effect of Hi-Ox-MAP on MSA consumer eating quality scores is required. This will enable the effects of Hi-Ox-MAP (and other retail packaging formats) to be incorporated into MSA models for assuring sheep and beef eating quality.

Sheep meat is known to have a shorter shelf-life than beef. Although the shorter storage life of sheep meat is attributed to higher initial pH, recent data shows that the storage temperature and OTR are the most critical factors for determining storage life, with no effect of pH (McPhail *et al.* 2014a). Vacuum packaged lamb stored at 0 ^{o}C should have a storage life of 6-10 weeks.

No studies have examined the effect of different case ready packaging formats on lamb eating quality as most studies have focussed on beef and pork. In order to address the knowledge gap, we evaluated two new commercially available barrier films and packaging formats developed by Sealed Air and compared them to traditional Hi-Ox-MAP. Cryovac Darfresh® Barrier vacuum skin packaging (VSP) uses a novel composite polymer with two layers. The Cryovac Darfresh® Bloom (Hi-Ox-DB) is a triweb system that incorporates elements of VSP, within a Hi-Ox-MAP tray.

In the current study we compared three case ready options for direct packaging of fresh lamb under simulated retail conditions; conventional Hi-Ox-MAP, Darfresh® Bloom (Hi-Ox-DB) and VSP (Darfresh® Vacuum Skin Packaging (VSP). A summary of the main specifications, advantages and disadvantages of these and other retail ready MAP formats is shown in **Table 1**.

A list of product features, benefits and specifications provided by the manufacturer (Sealed Air) are shown in **Appendix 1**.

Table 1: **Summary of main specifications, advantages and disadvantages of MAP packaging formats. Table adapted from (McMillin 2008)**

Packaging Type	Air permeable overwrap	vacuum skin packaging	Low O_2 with CO_2 and N_2	Low O_2 with CO	High O_2	High O_2 VSP
Nomenclature used in this report	**overwrap**	**VSP, Darfresh VSP**	**Low O_2- CO_2 /N_2 MAP**	**CO-Lo-Ox-MAP**	**Hi-Ox-MAP**	**Darfresh Bloom Hi-Ox-DB**
System description	Air-permeable film overwrap of product on tray; product displayed in package	Flexible film shrunk wrapped around product on rigid base web; product displayed in package	Thermoformed or preformed trays with lidding film; product displayed in package	Thermoformed or preformed tray with lidding film; product displayed in package	Thermoformed or preformed tray with lidding film; product displayed in package	Flexible film shrunk wrapped around product on rigid base web; product enclosed in MAP tray
Headspace gases	Atmospheric air	No headspace	$CO_2$and/or N_2	CO (0.5%) with CO_2 and/or N_2	O_2 and CO_2; often 80% O_2:20% CO_2	Low oxygen transmission barrier film Headspace 80% O_2:20% CO_2
Meat colour for display	red	purple	red	red	red	red
Whole muscle shelf life, d at 4 °C	5-7	30-60	20-40	35	12-16	121-16?
Mince shelf life, d at 4 °C	2-7	45-60	15-40	28	10-12	10-12?
Drip loss (%)	8-10	2-5	1-5	1.7	0-5	?
Advantages	High product visibility; lowest cost; multiple sizes on same equipment	Long storage life before display; high product visibility. Long storage	Long storage life before display	Long red colour stability and no lipid oxidation; high product visibility with VSP	Moderate red colour stability	Moderate red colour stability
Disadvantages	Short display life; leaky package if bottom sealed rather than tube sealed at ends	Display purple colour Confinement odour	Purple display colour in MAP; scavengers increase costs; bloom may be inconsistent on exposure to air after removal from MAP; increased cost with master pack	Negative image by consumers; concern red products may be spoiled in other factors; scavengers increase costs; cooked meat colour may be pink	Lipid oxidation; bone darkening, decreased tenderness; headspace required; premature browning of cooked meat	Slower lipid oxidation?; slower decreased tenderness?; headspace required; premature browning of cooked meat

2 Projective Objectives

2.1.1 Potential of new retail MAP formats for Australian lamb

As per the research agreement, the objectives of the project were to:

1. Identify current packaging materials, structures and systems used for Australian retail-ready MSA Beef and Lamb.

2. Develop and implement experimental design to assess MAP, Darfresh® VSP, Darfresh® Bloom and combinations – ensure compliance to current MSA Lamb procurement, pre- and post-packaging ageing treatments and equipment set-up to simulate commercial practices.

3. Develop and implement experimental design to assess consumer acceptability of meat for raw lamb loin and leg packed under MAP, Darfresh® VSP, Darfresh ® Bloom and combination systems.

4. Determine MSA lamb loin and leg eating quality profiles and colour for various packaging treatments.

3 Methodology

3.1 Materials & Methods

3.1.1 Experimental design

Extensive discussions were conducted with industry packaging experts (Sealed Air and Multivac), to identify appropriate retail ready packaging formats for the storage trial; Hi-Ox-MAP (default industry standard format) and two new systems, Darfresh® Bloom (Hi-Ox-DB) and Darfresh® VSP (VSP). The original design (**Figure 1**) shows the main packaging experiment and an additional experiment (designated by dashed circles). The design and the level of replication were discussed with a biostatistician to ensure sufficient statistical power to adequately answer the main research questions. According to the design, lamb steaks from each carcass (n=88, both sides, topside and backstrap muscles) were equally allocated to each packaging and time treatments. In the main experiment, the effects of Hi-Ox-DB and VSP could be directly compared to Hi-Ox-MAP after 5 and 10 days storage. Replicate (n=22) experimental samples (retail trays of meat), were prepared for each treatment and time point. For the additional experiment, samples were vacuum packaged (VP) and wet aged for 5 days as primals. Packs were then opened and samples cut into steaks and repackaged into retail ready formats under either Hi-Ox-MAP or HI-Ox-DB for a further 5 days. A comparison between all samples after a total of 10-days storage, would allow an understanding of whether the vacuum packaging period as a primal would affect the eating quality differently compared to direct packaging of cut steaks. It would also help answer questions regarding the reversibility of any initial tenderisation that occurs prior to repackaging in retail packs.

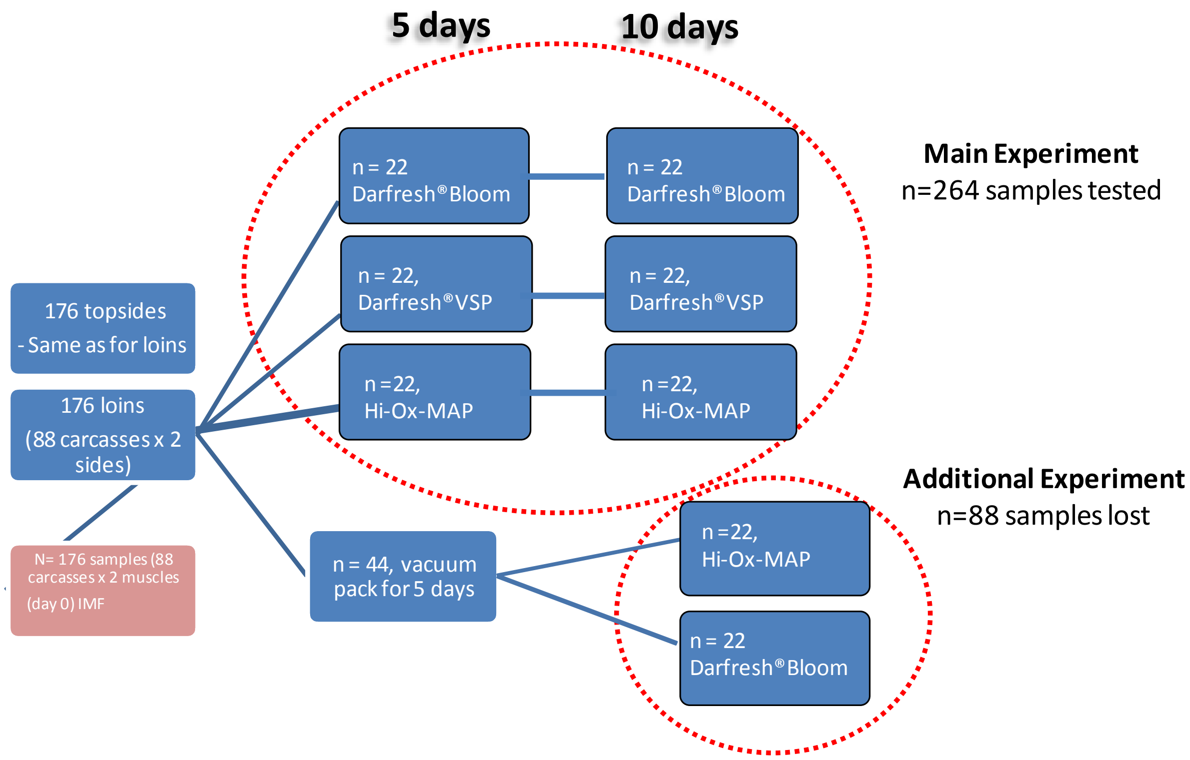

Figure 1: Original experimental design. The main experiment is circled by the dashed red line. Unfortunately the samples in the additional experiment were lost due to a storage refrigeration failure and there is no further reference to these samples in this report.

3.1.2 Animals & carcass collection

Wethers (n=111) of known genetics from a larger cohort of lambs raised by the Victorian Department of Environment, Land, Water & Planning (DELWP-Vic), at Rutherglen (Melbourne University's Dookie Field station in Victoria) were used in the packaging experiment, following direction from MLA. The animals were sourced from ongoing producer demonstration site experiments coordinated by DELWP. The study was covered by an animal ethics permit number DELWP-2012-29 registered within the state of Victoria. Further details are available in the MLA final report – B.SCC.0181 "LMY & EQ Producer Demonstration Sites – Victorian Sites Carcase Measurements". No violations of the protocol occurred. Lambs were selected to represent progeny from 8 sire groups with known Australian Standard Breeding Values (ASBVs). Animals were killed as part of a commercial slaughter at JBS Abattoir (Brooklyn, Victoria) and carcasses were electrically stimulated and chilled overnight.

The following day both backstrap and both topside muscles were removed from each side of all carcasses. The side of the carcass from which the primal was taken was not recorded or used in the experimental design. The hot carcass weight (HCW) was recorded for each animal. Carcass quality parameters were measured by DELWP-Vic and CSIRO staff. Measurements included: eye muscle area (EMA), eye muscle depth (EMD), eye muscle width (EMW) and fat depth at the GR-site. A small meat sample was taken from each carcass for further texture and chemical analyses. A 15 cm midsection of the right backstrap was taken by DEPI-Vic for Warner-Bratzler shear force measurement at day 5 (SF-5) and intramuscular fat (%IMF) determination. Further details of these measurements are available in MLA final report B.SCC.0181. Meat colour (CIE L*a*b* tristimulus values) and pH were determined in each muscle after 5 and 10 days of storage as described below.

3.1.3 Lamb packaging and storage

At 24 hrs post-slaughter, the meat primals were bagged, labelled and transported in a refrigerated van (2 ± 1°C) to the facilities of Multivac Pty. Ltd. (Keilor East, Vic) and cut and packaged according to the experimental design (**Figure 1**). T-200 tray sealing machines were used for packaging of product. MAP packs and Darfresh Bloom packs used a gas blend of 80%O_2 /20% CO_2.

Each backstrap and topside was manually cut into 15-mm steaks before allocating to packaging treatment. The meat collected was taken from 8 different sire groups or genotypes, with different ABVs (**Table 2**). Meat from the different sire groups were allocated to the different packaging formats and coded according to the Meat Standards Australia (MSA) consumer testing protocols (Watson *et al.* 2008a; Watson *et al.* 2008d). After packaging into retail packs at Multivac, the trays were transported at 2 ± 1°C to CSIRO (Werribee, Vic) and placed in two retail cold display cabinets; 2 m wide, 4 shelves, top and bottom fluorescent illumination (Saturno 200, Oscartielle S.p.A., Italy). The cabinets were set at 1 ± 1°C and the temperature was monitored and logged using a thermocouple. The position of packages was rotated daily within the cabinets so that cases received similar average exposure to illumination and temperature. After removal of meat packages after 5 or 10 days storage the colour and pH was measured, before individually wrapping steaks in plastic film and freezing for later "pick and post" preparation for MSA consumer testing. Any remaining meat from the packaging experiment was repackaged in plastic screw lid tubes, labelled and frozen for further testing.

3.1.4 Colour and pH measurement

Muscle pH was measured by directly inserting a spear head pH probe (IJ44C probe, Ionode, Pty Ltd., Australia) and temperature probe connected to a WP-80 pH-mV-Temperature meter (TPS Pty Ltd, Australia), into the muscle, immediately after the pack was opened. The unit was calibrated with pH 4 and pH 7 buffer standards prior to measurement each day.

Tristimulus L*, a* and b* values were measured directly on the raw meat surface using a Minolta Chromameter CR-300 with an aperture of 8 mm (Minolta Co., Ltd, Japan). The chromameter was calibrated with a standard white plate under D65 illumination and 0 º viewing angle (Y = 92.0, x = 0.3163, y = 0.3328) before each use. Triplicate colour measurements were taken per pack immediately after opening (at ~5 ^{o}C). In the CIELAB colour space, L* represents lightness (0 = dark black, 100 = brightest white), a* represents the red/green axis (negative green, positive red) and b* the yellow/blue axis (negative blue, positive yellow). Hue angle and Chroma were mathematically calculated from a* and b* values. Hue angle = $\tan^{-1}(b^*/a^*)$, measured in degrees (0 - 360^{o}), represents the base colour (0^{o} = red, 90^{o} = yellow, 180^{o} = green, 270^{o} = blue). Chroma = $(a^{*2}+b^{2})^{1/2}$ (colourfulness, saturation) is defined as the difference between pure grey (chroma = 0) to very high colour purity (chroma = 100).

3.1.5 Consumer testing

Human ethics approval for conducting the consumer trial was granted by the CSIRO low risk ethics committee (CSIRO-LRRP 2014-13). The consumer testing of samples was performed using Meat Standards Australia standard protocols and was conducted by the consumer research company "TastePoint". Consumer testing (7 picks, n=420 consumers) was completed between October – November 2014. The testing was performed in Metropolitan Melbourne according to established MSA protocols, in collaboration with Rod Polkinghorne. A copy example of the paper ballot used to assess the grilled lamb samples is included in **Appendix 2**.

3.1.6 Volatile analysis and informal odour analysis

Backstrap samples from day-10 of the packaging experiment were thawed and a 4 g portion was weighed into a glass headspace vial. The sample was macerated using an Ultra Turrax (T-25, Janke and Kunkel, 1 cm diameter head) with 2 mL of Milli-Q water and 20 µL of 4-methyl pentanol internals standard was added mixed and the vial sealed. Duplicate samples were placed in the auto-injector (AOC-5000 Shimadzu). Headspace volatiles were extracted with divinylbenzene/Carboxen/PDMS 23 gauge, 2 cm solid phase microextraction (SPME) fibres (Supelco) for 60 minutes at 40 °C with sample agitation. After extraction the volatiles were desorbed into a hot injector (250 °C) in splitless mode and analysed by gas chromatography-mass spectrometry (QP 2010 Plus GC-MS, Shimadzu). Volatiles were separated on a Sol-Gel Wax column (SGE, Australia, 30 m, 0.25 id, 0.25 µm film) using temperature programming; initial temperature 35 ^{o}C (held 5 minutes) and then heated at 5 °C/min to 250 °C. The mass spectrometer was programmed to scan the mass range *m/z* 40-250. Semi-quantitative data were generated using the Shimadzu proprietary software "LabSolutions" (Version 2.53). Integrated area data were normalised to the IS and expressed as a percentage of the IS. Mass spectral matches were conducted with the NIST Mass Spectral Search database.

3.1.7 Free amino acid analysis

Postmortem proteolysis of meat during ageing involves the degradation of various muscle structural proteins, the predominant proteins degraded by the proteases calpain being titin, nebulin and troponin-T (Koohmaraie 1994) with some evidence also for degradation of myosin by the action of the proteases cathepsins (Ertbjerg *et al.* 1999). An increase in free-amino acids and peptides is generally associated with increases in taste and flavour (Watanabe *et al.* 2004). Changes in free amino acids during storage may indicate proteolysis (Moya *et al.* 2001), however bacterial metabolism could also contribute to changes in the free amino acid level in the meat (Watanabe *et al.* 2004).

Notwithstanding the above discussion, in the current experiment, a lower concentration of free amino acids in Hi-Ox-MAP packaged lamb compared to VP after storage could be interpreted as evidence of inhibition of proteolysis enzymes. Semi-quantitative measurement of free amino acids (and other non volatile compounds) was achieved using a chemical derivatisation technique with subsequent analysis by gas chromatography mass spectrometry (GC-MS) (Smart *et al.* 2010; Leggio *et al.* 2012) Thawed raw lamb backstrap muscle from 10-days packaging (5g) was immediately suspended in methanol (3mL) and macerated into fine slurry using an Ultra-Turrax. The samples were centrifuged and a volume of the supernatant was used for analysis. A 500µL volume of the meat suspension was transferred to a reaction vial and a 50µL volume of norvaline internal standard solution was added. A semi-quantitative estimation of the concentration of free amino acids (mg/kg) was achieved against the norvaline internal standard. After derivatisation, the volatile derivatives were extracted into 500 µL of chloroform and dried with anhydrous sodium sulphate for analysis by gas chromatography-mass spectrometry (QP 2010 Plus GC-MS, Shimadzu). 1 µL of sample was injected at 250 ^{o}C in splitless mode. Derivatives were separated on a Sol-Gel Wax column (SGE, Australia, 30 m, 0.25 id, 0.25 µm film) using temperature programming; initial temperature 45 ^{o}C (held 2 minutes) and then heated at 9 °C/min to 180 °C (held 5min), 40°C/min to 220°C (held 5 minutes). The mass spectrometer was programmed to scan the mass range *m/z* 40-300. Reference compounds were used to establish retention times of most compounds and quantified using a characteristic ion fragment. Integration of peaks was achieved using the Shimadzu proprietary software “LabSolutions” (Version 2.53). The identity of most analytes was established by reference compounds. In most cases published mass spectral data was used (Smart *et al.* 2010; Leggio *et al.* 2012).

3.1.1 Statistical analysis

The Restricted Maximum Likelihood (REML) procedure in Genstat (Version 15.1) was performed on the replicate carcass data to test for sire-group related differences; SIRE as a fixed effect and carcass number a random effect Mean clipped consumer scores of tenderness, juiciness, flavour, odour and overall liking as well as a composite score (C-MQ4; composite of all consumer eating quality traits) for each sample were calculated. The clipped consumer data were analysed using the Restricted Maximum Likelihood (REML) procedure in Genstat (Version 15.1). The fixed effects of meat cut (CUT), packaging (PCK) and days aged (DA) and the random effects of Carcass number + sire group (SIRE) were tested. In order to ascertain the potential significance of SIRE on meat quality traits, the deviance information criterion or log 2 likelihood values were compared after successive removal of SIRE interaction terms with cut, packaging type and days aged. A difference of less than the critical value for a Chi-squared distribution and the appropriate degrees of freedom was interpreted as a non-significant effect. For the volatile and amino acid statistical analysis, data for each packaging treatment were analysed using the multivariate analysis of variance (MANOVA) procedure in Genstat (Version 15.1).

4 Results

4.1 Carcass characteristics

A number of carcasses were under the commercial HCW target of 20 kg. The HCW ranged from 14.1 to 24.7 kg, mean = 18.8 kg, median = 19.1 kg, standard deviation = 2.1 kg (**Table 2**). There were significant sire-related differences for eye muscle depth (EMD), eye muscle width (EMW), hot carcass weight (HCW), GR-fat depth, intramuscular fat (IMF) and colour (L*, a* & b*) were measured. Significant sire-related differences in IMF were observed. Because IMF is related to meat tenderness, it was hypothesised that IMF would be the parameter most likely to affect eating quality. As shown in **Table 2**, the range of IMF values between the sire groups was small e.g.; 3.67%-4.79%. It is of note that the corresponding shear-force values (SF5) did not differ significantly according to sire group, showing that these small IMF differences did not result in measurable instrumental texture changes.

4.1.1 Storage trial

Backstrap and topside samples from the different sire groups were allocated in a balanced fashion to each of the packaging treatments according to the experimental design (MAP, n=79, VSP, n=78, HI-OX-DB n= 80), meat cut (BCK, n=119; TOP, n=118) and days aged (5, n=119, 10, n=118). The SIRE effect was considered a random experimental variable in the REML analyses (**Figure 1**).

Unfortunately, due to refrigeration failure, temperature control of the samples in the “additional” experiment was lost overnight, resulting in the meat samples reaching a temperature of ~12 °C for a number of hours. At the conclusion of the storage period, a number of samples had a greenish tinge and an ‘off’ odour indicating microbial spoilage. Three representative samples were promptly transported under chilled conditions for microbiological testing to Food Laboratories (Aust) Pty. Ltd. (Abbotsford, Vic). Total plate count per g were 570,000, < 1,000, and less < 4,000 respectively. Pseudomonas spp. per g were 10,000,000, 130,000 and 63,000 respectively.

Because of the high microbiological counts it was decided that samples were unfit for human consumption. As these samples could not be subjected to consumer testing, the additional experiment was abandoned and these samples are not further discussed in this report. **Figure 2,** shows the colour differences between lamb samples stored in the different packaging formats after 24 hours storage at 1 °C. The purple colour of the VSP (middle) samples was visually apparent after only 24 hours storage. This colour persisted throughout the storage period.

Table 2: Average carcass parameters for each of the 8 sire groups used in this study

Sire	1	2	3	4	5	6	7	8	P value $_{sire}$
	n=11	n=12	n=13	n=18	n=14	n=11	n=13	n=19	
	Kilfeera Park 95444	**Kilfeera Park** 060205	**Kilfeera Park** 1000024	Leahcim Poll 110282	**OneOak** 000-6R114	**Toland Poll** 020W69	**Toland Poll** 091187	**Pooginook** 08-1290	
EMA	11.18	10.01	10.89	11.09	11.07	10.75	11.44	11.68	0.09
EMD	24.18	22.65	23.31	23.15	24.3	21.86	24.58	25.21	0.007
EMW	57.73	55.27	58.38	59.89	57	61.6	58.08	57.74	0.004
L*	35.3	35.5	35.45	34.58	34.95	35.07	33.96	34.67	0.33
a*	16.39	15.82	16.09	17.19	15.82	15.84	16.77	17.24	0.009
b*	7.78	7.424	7.519	8.105	7.48	7.061	8.106	8.231	0.006
HCW	18.05	17.2	18.08	19.4	18.2	16.57	19.75	19.63	<0.001
HGR-Fat	3.27	3.75	3.39	4.32	4.00	3.94	5.95	5.69	<0.001
IMF (%)	4.44	4.13	4	4.79	4.04	3.67	4.9	4.73	0.002
pH	5.692	5.582	5.602	5.649	5.575	5.667	5.596	5.663	0.53
SF5	28.13	27.65	29.62	29.53	29.35	29.92	29.99	26.76	0.72

Figure 2: Colour differences after 24 hours at 1 ºC in lamb packaged under conventional MAP (left), Darfresh®-VSP (middle) and Darfresh® Bloom (right).

4.1.2 Consumer data

The retail ready packs were able to maintain a microbiologically safe product up to at least 10 days post packaging in a simulated retail environment, regardless of the packaging system used. Results from the REML analysis of the clipped consumer data are summarised in

Table 3. The main effects of meat cut and packaging type were significant for every consumer sensory attribute. Sire group and carcass number were used as random terms. For all consumer attributes the backstrap muscle was rated significantly higher than topside, regardless of packaging type or number of days aged. It is well known that the eating quality of different muscles varies considerably (Polkinghorne *et al.* 2008; Watson *et al.* 2008a). The “cut × packaging” and the “cut × days aged” interaction terms were not significant for any of the consumer scores, indicating that the effects of packaging and days aged were independent of the meat cut. Regardless of the CUT, the VSP treatment was consistently rated higher than the Hi-Ox-MAP or Hi-Ox-DB for all consumer attributes.

Tenderness was rated highest in the VSP packaged meat at all time points regardless of CUT. In most cases the Hi-Ox-DB lamb was marginally better than the Hi-Ox-MAP, but significantly lower than VSP. Juiciness was highest for the VSP samples at both time points. Juiciness was rated similarly for the Hi-Ox-MAP and Hi-Ox-DB samples at 5 days; however a significant decline was measured by day-10 for the Hi-Ox-MAP samples. The significant “packaging × days” aged interaction demonstrated that changes in juiciness over time were packaging dependent.

Flavour liking was rated significantly higher for VSP at both time points. Flavour liking changes over time were similar to tenderness and juiciness changes; VP and Hi-Ox-MAP were similar at day 5, but a significant decline for Hi-Ox-MAP was measured by day-10. The interaction of “packaging × days aged” was significant e.g. for flavour and juiciness. The flavour of the Hi-Ox-MAP packaged lamb decreased significantly from day 5 to 10; whereas no flavour changes were found for the other packaging types. The decrease in flavour may have been confounded with the corresponding decrease in tenderness that occurred over the same period. The sensory “halo effect”, where consumers tend to rate one attribute (such as flavour) lower, when another attribute (such as tenderness) is also low — or the other way around, is a common phenomenon in sensory evaluation (Lawless 1999) and well documented in meat consumer assessment (Hunt *et al.* 2014; Corbin *et al.* 2015). Meat that is tender is generally always given a higher flavour liking score than meat that is not tender, even when flavour and tenderness are not logically related (O'Quinn *et al.* 2012). It was possible that oxidative processes may have generated off flavours, under Hi-Ox-MAP conditions. We attempt to check this in a later section, where the samples were screened for the presence of typical oxidation volatiles. Similarly the juiciness declined from 5 to 10 days, but once again only for the Hi-Ox-MAP samples. Tenderness and juiciness are often highly correlated. The “packaging × days aged” interaction was significant for some effects; e.g. juiciness, flavour and overall liking. This was reflected in the fact that the largest decrease in most consumer parameters was most pronounced at day 10 for the Hi-Ox-MAP samples only.

Liking of smell scores showed a broadly similar pattern to the other attributes; however, the differences between the BK and TOP were relatively smaller. Liking of smell was generally highest in the VSP > Hi-Ox-DB and lowest in the Hi-Ox-MAP. Overall liking and overall satisfaction scores were clearly higher for backstrap compared to topside and highest for the VSP samples at 5 and 10 days.

The CMQ4 – consumer meat quality score is a weighted composite of four sensory attribute scores; tenderness (0.3), juiciness (0.1), flavour (0.3) and overall liking scores (0.3). The CMQ4 forms the basis of the Meat Standards Australia quality grading system (Watson *et al.* 2008a; Watson *et al.* 2008d). According to MQ4 star system cut-off scores, CMQ4 < 42. 3 = "unsatisfactory", CMQ4 > 42.3 < 64.7 = "good everyday", CMQ4 > 64.7 < 79 = "better than everyday" and CMQ4 > 79 = "premium" quality.

The CMQ4 scores for the backstrap (left) and topside (right) are plotted graphically in **Figure 3**. It can clearly be seen that backstrap samples had better overall eating quality compared to topside, regardless of the packaging system used. CMQ4 scores indicated that the backstrap samples were at the high end of the "good everyday" quality, whereas the topside samples were at the lower end. It was also clear that the eating quality of VSP lamb was highest after both 5 and 10-days. The data also indicated that the overall quality of the MAP samples declined from day 5 to 10. The VSP packaged backstrap samples attained "better than everyday" quality whereas all the other treatments were rated "good everyday". The Hi-Ox-MAP packaged TOP would have attracted an "unsatisfactory rating" (< 42.3 CMQ4) after 10-days storage. The effects of different retail packaging types on meat eating quality are currently not taken into consideration in the MSA grading system.

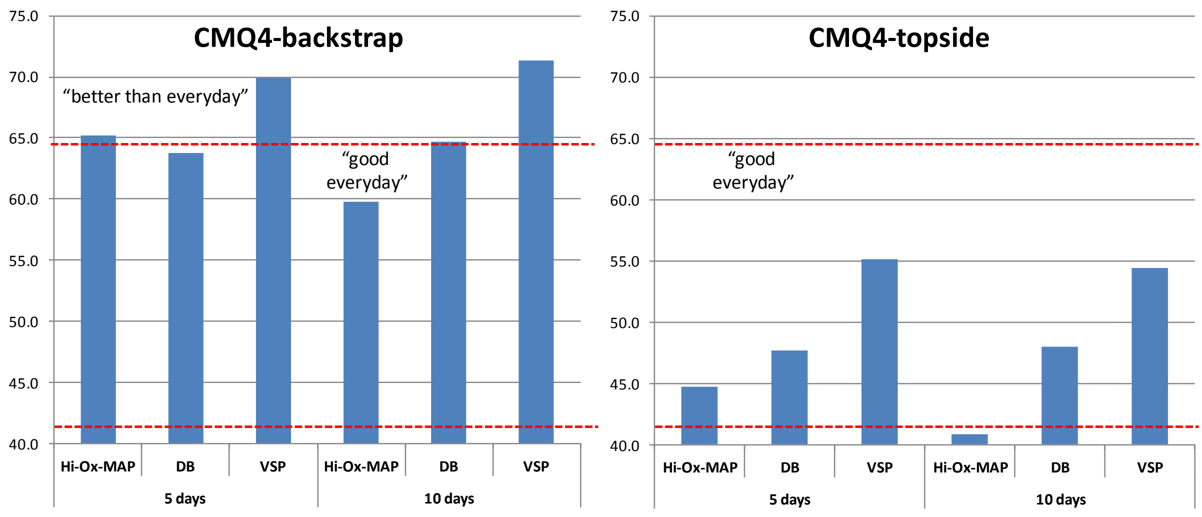

Figure 3: Graphical representation of the CMQ4 scores for backstrap and topside samples after 5 and 10 days storage in Hi-Ox-MAP, Darfresh® Bloom (Hi-Ox-DB) or Darfresh® vacuum skin packaging (VSP). The lower dashed red line shows the boundary between "good everyday" and "unsatisfactory" quality and the top red line shows the boundary between "better than everyday" and "good everyday" quality.

4.1.3 Colour data

The colour (Minolta L* a* b*, Hue and Chroma) and pH were measured after chilled storage immediately after the pack was opened (before significant blooming), to capture the product colour the consumer would visually see in the pack. The effects of CUT, PCK and DA on colour parameters and pH were assessed by REML, with the random factors SIRE and carcass number (

Table 4). The effect of CUT had no significant effect ($P < 0.05$) on most colour measures, except the hue angle, where the TOP muscle was higher, especially for the Hi-Ox-DB samples. In general the TOP muscle had lower, but not significantly lower, L* and a* values compared to the backstrap. For L* a* b*, both packaging and days aged were significant. Most noticeably, the VSP samples had very low or negative b* values, indicating purple colour, consistent with the formation of DeoxyMb. This colour was already apparent 24 hours after packaging in VSP (**Figure 2**). The formation of DeoxyMb in vacuum packed beef is well known (Mancini and Hunt 2005; McMillin 2008; Delmore 2009; Lagerstedt *et al.* 2011; Li *et al.* 2012). Chroma decreased from day 5 to 10 for Hi-Ox-MAP and HI-OX-DB, but did not change for VSP. The “cut × packaging” and “packaging × days aged” interactions were also significant. Neither packaging type nor days aged had a significant effect on the meat pH (data not shown).

4.1.4 Sire effects

Although there were initial sire-related differences in a number of carcass parameters, the effect of SIRE as a random variable was not significant for any of the main effects in the packaging experiment. Variations in the amount of IMF in the meat from the different sire groups were found to be small (~ 1%) and not related to instrumental tenderness measurements. Hence the lack of significant effect of SIRE on the sensory attributes was not surprising.

Table 3: Predicted MSA consumer scores for MAP, HI-OX-DB& VSP samples after storage at 1 ºC for 5 and 10 days. Statistical significance given for main effects and significant interactions only ($P < 0.10$). BK = backstrap, TOP = topside. Samples in the same trait sharing different superscripts are significantly different ($P<0.05$?). All differences between BK and TOP were significant ($P <0.001$); hence no superscripts used to denote differences between muscle types. PCK= packaging, DA= days aged.

		5 Days Hi-Ox-MAP	5 Days Hi-Ox-DB	5 Days VSP	10 days Hi-Ox-MAP	10 days Hi-Ox-DB	10 days VSP	Effect	P value
Tenderness	**BK**	66.2^{a}	65.1^{a}	70.7^{b}	63.9^{a}	67.7^{a}	73.4^{b}	**Cut**	<0.001
	TOP	39.9^{a}	43.4^{b}	51.9^{c}	37.9^{a}	45.1^{b}	51.3^{c}	**Packaging**	<0.001
								Days Aged	0.77

		5 Days Hi-Ox-MAP	5 Days Hi-Ox-DB	5 Days VSP	10 days Hi-Ox-MAP	10 days Hi-Ox-DB	10 days VSP	Effect	P value
Juiciness	**BK**	61.5^{a}	61.8^{a}	66.0^{b}	55.9^{c}	60.3^{a}	68.0^{b}	**Cut**	<0.001
	TOP	47.2^{a}	47.7^{a}	53.0^{b}	40.1^{c}	46.2^{a}	54.3^{b}	**Packaging**	<0.001
								Days Aged	0.07
								PCK.DA	0.019

		5 Days Hi-Ox-MAP	5 Days Hi-Ox-DB	5 Days VSP	10 days Hi-Ox-MAP	10 days Hi-Ox-DB	10 days VSP	Effect	P value
Flavour	**BK**	65.3^{a}	62.8^{a}	70.1^{b}	58.2^{c}	63.4^{a}	70.8^{b}	**Cut**	<0.001
	TOP	47.8^{a}	50.7^{a}	57.5^{b}	43.2^{c}	50.3^{a}	56.8^{b}	**Packaging**	<0.001
								Days Aged	0.07
								PCK.DA	0.03

		5 Days Hi-Ox-MAP	5 Days Hi-Ox-DB	5 Days VSP	10 days Hi-Ox-MAP	10 days Hi-Ox-DB	10 days VSP	Effect	P value
Liking of Smell	**BK**	63.3^{a}	61.4a	65.0^{ab}	59.4^{c}	63.0^{a}	64.6^{a}	**Cut**	<0.001
	TOP	56.3^{c}	59.7^{b}	62.3^{a}	58.1^{b}	58.5^{b}	59.9^{b}	**Packaging**	0.011

		5 Days Hi-Ox-MAP	5 Days Hi-Ox-DB	5 Days VSP	10 days Hi-Ox-MAP	10 days Hi-Ox-DB	10 days VSP	Effect	P value
Overall Liking	**BK**	66.6^{a}	64.5^{a}	70.8^{b}	60.3^{c}	65.3^{a}	72.6^{b}	**Cut**	<0.001
	TOP	45.5^{a}	49.1^{b}	56.9^{c}	41.6^{d}	49.1^{b}	55.3^{c}	**Packaging**	<0.001
								Days Aged	0.09
								PCK.DA	0.07

		5 Days Hi-Ox-MAP	5 Days Hi-Ox-DB	5 Days VSP	10 days Hi-Ox-MAP	10 days Hi-Ox-DB	10 days VSP	Effect	P value
Overall	**BK**	3.6^{a}	3.6^{a}	3.8^{b}	3.3^{c}	3.6^{a}	3.8^{b}	**Cut**	<0.001
Satisfaction	**TOP**	2.8^{a}	2.9^{a}	3.2^{b}	2.7^{c}	2.9^{a}	3.1^{b}	**Packaging**	<0.001
								Days Aged	0.2

		5 Days Hi-Ox-MAP	5 Days Hi-Ox-DB	5 Days VSP	10 days Hi-Ox-MAP	10 days Hi-Ox-DB	10 days VSP	Effect	P value
CMQ-4	**BK**	65.2^{a}	63.8^{a}	69.9^{b}	59.8^{c}	64.7^{a}	71.4^{b}	**Cut**	<0.001
	TOP	44.7^{a}	47.7^{a}	55.2^{b}	40.8^{c}	48.0^{a}	54.4^{b}	**Packaging**	<0.001
								Days Aged	0.24
								PCK.DA	0.06

Table 4: Mean colour characteristics after storage at 1 °C for 5 and 10 days. Statistical significance given for main effects and significant interactions only ($P < 0.05$). BK = backstrap, TOP = topside. Samples in the same row sharing different superscripts are significantly different. All differences between BK and TOP were significant; hence, no superscripts used to denote differences between muscle types. PCK= packaging, DA= days aged.

		Day 5 Hi-Ox-MAP	Day 5 Hi-Ox-DB	Day 5 VSP	Day 10 Hi-Ox-MAP	Day 10 Hi-Ox-DB	Day 10 VSP	Effect	P value
Chroma	**BK**	20.6^{a}	18.7^{b}	18.7^{b}	18.4^{b}	17.2^{c}	19.1^{b}	**Cut**	0.31
	TOP	19.8^{a}	17.5^{c}	20.1^{a}	17.9^{c}	16.3^{d}	20.3^{e}	**Packaging**	<0.001
								Days Aged	<0.001
								CUT.PCK	<0.001
								PCK.DA	<0.001

		Day 5 Hi-Ox-MAP	Day 5 Hi-Ox-DB	Day 5 VSP	Day 10 Hi-Ox-MAP	Day 10 Hi-Ox-DB	Day 10 VSP	Effect	P value
Hue angle	**BK**	18.5^{a}	17.5^{a}	-2.5^{b}	20.8^{c}	22.1^{c}	-1.2^{d}	**Cut**	0.001
	TOP	18.8^{a}	20.6^{c}	-2.5^{b}	22.3^{c}	24.9^{e}	-1.1^{d}	**Packaging**	<0.001
								Days Aged	<0.001
								CUT.PCK	0.027
								PCK.DA	0.028

		Day 5 Hi-Ox-MAP	Day 5 Hi-Ox-DB	Day 5 VSP	Day 10 Hi-Ox-MAP	Day 10 Hi-Ox-DB	Day 10 VSP	Effect	P value
L* Lightness	**BK**	36.9^{a}	36.2^{a}	37.5^{a}	40.3^{b}	40.1^{b}	37.4^{a}	**Cut**	0.138
	TOP	36.9^{a}	36.9^{a}	34.7^{c}	39.4^{b}	39.4^{b}	35.2^{c}	**Packaging**	0.01
								Days Aged	<0.001
								PCK.DA	0.1

		Day 5 Hi-Ox-MAP	Day 5 Hi-Ox-DB	Day 5 VSP	Day 10 Hi-Ox-MAP	Day 10 Hi-Ox-DB	Day 10 VSP	Effect	P value
a* redness	**BK**	19.5^{a}	17.6^{c}	18.7^{b}	17.2^{c}	16.0^{d}	19.0^{b}	**Cut**	0.11
	TOP	18.7^{a}	16.3	20.1	16.6	14.8	20.3	**Packaging**	<0.001
								Days Aged	<0.001
								CUT.PCK	<0.001
								PCK.DA	<0.001

		Day 5 Hi-Ox-MAP	Day 5 Hi-Ox-DB	Day 5 VSP	Day 10 Hi-Ox-MAP	Day 10 Hi-Ox-DB	Day 10 VSP	Effect	P value
b* yellowness	**BK**	6.6^{a}	5.6^{b}	-0.8^{c}	6.5^{a}	6.5^{a}	-0.4^{d}	**Cut**	0.11
	TOP	6.3^{a}	6.2^{a}	-0.8^{c}	6.7^{b}	6.8^{b}	-0.4^{c}	**Packaging**	<0.001
								Days Aged	0.002

4.1.5 Fresh meat odour

The MSA consumer panels assessed the odour of grilled lamb samples only. No formal sensory assessment of the raw lamb samples was performed. An informal sensory assessment of the odour of raw lamb backstrap samples after 10–days storage was made by two experienced flavour scientists. Six random backstrap samples were selected from HI-OX-DB, VSP and Hi-Ox-MAP packaging after 10-days. The odour of the samples was assessed "blind" without knowledge of the packaging treatment. The informal odour descriptors given for the raw odour are summarised in **Table 5.** None of the samples had obvious signs of spoilage or any distinctive off-odours. For each packaging type, the intensity of the odour ranged from very mild to strong. Although not a formal assessment, the data suggests that the odour was quite variable within packaging type, with no characteristic packaging related odours detected or presence of a discernible "off" odours.

Table 5: Informal sensory descriptors for selected HI-OX-DB, VSP and Hi-Ox-MAP samples

Replicate	Packaging Type	Odour description
1	**HI-OX-DB**	*Mild fishy, mild hay, pleasant*
2	**HI-OX-DB**	*Very mild raw meat*
3	**HI-OX-DB**	*Mild sweet, lamb, fatty*
4	**HI-OX-DB**	*Sweet lamb, hay, strong fishy*
5	**HI-OX-DB**	*Strong fatty, fishy, lamb*
6	**HI-OX-DB**	*Lamb fatty, slight fishy*
1	**Hi-Ox-MAP**	*Mild lamb, metallic, blood, hay*
2	**Hi-Ox-MAP**	*Mild lamb*
3	**Hi-Ox-MAP**	*Strong lamb, sweet, fatty*
4	**Hi-Ox-MAP**	*Lamb, fatty, slight metallic*
5	**Hi-Ox-MAP**	*Mild lamb, mild fatty*
6	**Hi-Ox-MAP**	*Very mild, slight hay*
1	**VSP**	*Very mild lamb, slightly fishy, bit fatty*
2	**VSP**	*Very mild lamb*
3	**VSP**	*Mild lamb, fishy, hay*
4	**VSP**	*Fatty, strong lamb*
5	**VSP**	*Mild lamb, fatty*
6	**VSP**	*Fatty, metallic, hay*

4.1.6 Volatile composition of the raw meat headspace

Few volatile differences were measured in the raw lamb backstrap samples according to packaging format (**Table 6**), in agreement with the informal sensory assessment. The main volatile difference was a significantly higher amount of selected methyl ester compounds in the VSP samples. The formation of methyl esters is consistent with anaerobic bacterial metabolism (Casaburi *et al.* 2015). Overall, these data indicated that after 10-days storage, packaging related odour changes were minimal from both a sensory and volatile perspective. The data did not suggest that significant oxidation of fat had occurred in the Hi-Ox-MAP samples. Significant fat oxidation would be expected to be reflected in a higher concentration of certain known lipid breakdown volatiles; e.g. hexanal and nonanal.

Table 6: List of main volatile compounds identified in the headspace above raw lamb backstrap samples after 10-days storage. Mean values for each packaging treatment (n=6). Semi-quantitative data only – relative concentration units. Quan *m/z* = quantitative ion. RT = retention time

Volatile	Quan *m/z*	RT	HI-OX-DB	Hi-Ox MAP	VSP	P value
methyl butanoate	74	7.65	7.99	7.42	13.3	**0.005**
2,3-pentanedione	57	10.38	4.27	1.96	2.41	0.154
hexanal	56	10.96	43.4	23	48.6	0.47
1-penten-3-ol	57	13.81	19.5	11	12.7	0.231
methyl pentanoate	74	14.39	25.1	17.8	37.6	**<0.001**
heptanal	70	14.82	1.53	0.84	0.86	0.394
2 pentyl furan	81	15.78	4.03	3.42	3.75	0.922
(*Z*)-4-heptenal	68	16.08	0.526	0.293	0.247	0.167
1-pentanol	55	16.5	20.3	9.6	10.9	0.056
2-penten-1-ol	57	18.61	3.47	2.33	2.61	0.391
1-hexanol	56	19.5	22	15	13.8	0.407
2 butanol	45	19.85	1.24	0.86	0.53	0.057
methyl octanoate	74	20.54	18.6	13.8	22.3	**0.01**
nonanal	57	20.70	4.96	4.64	3.96	0.831
(E)-2-octenal	55	21.71	0.63	0.555	0.444	0.59
1-octen-3-ol	57	22.17	29.6	21.9	33.3	0.406
1-heptanol	70	22.3	7.81	4.12	4.13	**0.037**
(E,E)-2,4-heptadienal	81	22.72	1.75	0.92	1.32	0.42
2-ethylhexanol	57	23.21	12.5	18.3	11.3	0.108
methyl nonanoate	74	23.30	12.42	7.28	10.17	**0.028**
(E,E)-3,5-octadien-2-one	95	24.13	1.38	0.8	0.92	0.259
benzaldehyde	106	24.41	3.24	2	2.18	0.291
(E)-2-nonenal	70	24.52	1.08	0.53	0.61	0.127
1-octanol	69	24.99	2.95	1.54	2.92	0.35
methyl decanoate	74	25.95	23.2	18	23	0.307
2-octen-1-ol	57	26.3	1.71	1.23	1.9	0.364
2-methylbenzaldehyde	91	26.77	0.44	0.257	0.229	0.401
(E,E)-2,4-nonadienal	81	28.63	0.96	0.5	0.54	0.178
2-ethylbenzaldehyde	134	28.8	0.509	0.373	0.424	0.538
3-methylphenol	107	36.72	1.125	0.861	0.957	0.521
2-methylphenol	107	36.88	3.26	2.51	2.76	0.486

4.1.7 Free amino acids after 10-days storage

A lower concentration of free amino acids and peptides (e.g. carnosine) in the Hi-Ox-MAP samples compared to the VSP samples would be expected after storage (10 days) if oxidation and inhibition of proteolysis enzymes were significant factors leading to lack of tenderisation during high oxygen packaging. Average concentrations of selected free amino acids and other non-volatile metabolites in raw meat after 10-days storage are shown in **Table 7**. In general, there was little evidence of inhibition of proteolysis in the Hi-Ox-MAP packaging after 10-days. Few differences in the concentration of free-amino acids were measured, except that the concentration of aspartic acid was lower in the VSP (approached significance at P< 0.05). Of interest, the concentration of succinic acid was significantly higher in the VSP samples compared to the other packaging types. Succinic acid is known to be formed through anaerobic fermentation by lactobacillus bacteria (Kaneuchi *et al.* 1988). Succinic acid is a natural flavour enhancer shown to be important in meat flavour (Schlichtherle-Cerny and Grosch 1998). The higher succinic acid present in the VP samples may partially explain the higher consumer flavour scores given for these samples, notwithstanding aforementioned "halo" effects.

Table 7: Semi-quantitative data for free-amino acids and other non-volatile compounds in raw meat extracts from lamb backstraps after 10-days storage under HI-OX-DB, Hi-Ox-MAP and VSP

Volatile	Retention time	Quan Ion (*m/z*)	HI-OX-DB	Hi-Ox-MAP	VSP	P value
fumaric acid	10.84	115	0.36	0.28	0.24	**0.003**
acetic acid	11.05	43	7.8	9.07	9.65	0.44
glycine	12.00	88	0.16	0.16	0.15	0.587
succinic acid	12.74	115	1.61	1.39	3.95	**<.001**
lactic acid	12.95	59	154.54	156.3	159.5	0.866
alanine	16.03	102	183	180	158	0.528
ascorbic acid	16.54	116	2.01	1.62	1.39	0.053
leucine	17.60	144	50.33	44.9	47.43	0.391
L-carnosine	18.67	88	12.41	10.65	10.1	0.429
proline	19.63	128	58.39	58.13	42.27	0.139
palmitic acid	21.48	74	2.94	2.88	2.19	0.436
creatine	22.39	102	0.96	0.76	0.6	0.106
valine	23.95	130	95.36	86.94	90.81	0.495
aspartic acid	24.28	160	15.61	13.67	7.38	0.054
oleic acid	24.57	55	1.35	1.09	0.86	0.47
a-ketoglutaric acid	24.80	115	20.09	18.75	19.72	0.73
isoleucine	24.86	88	44.36	41.27	46.29	0.534
tryptophan	24.90	130	43.46	47.16	44.98	0.373
linoleic acid	25.20	67	0.44	0.57	0.31	0.349
methionine	26.22	61	20.48	18.2	19.7	0.345
glutamic acid	26.46	114	4.18	2.29	4.19	0.113
phenylalanine	28.31	162	43.65	38.19	43.36	0.264
lysine	30.21	142	25.25	18.83	37.87	0.064
cysteine	33.80	59	1.77	1.48	1.92	0.188

5 Discussion

5.1.1 Case ready MAP for Lamb?

Tender and juicy lamb is essential for a good eating experience and is clearly important to consumers (Russell *et al.* 2005). Packaging formats that compromise the texture of lamb may be detrimental to consumer repeat purchase decisions. The data clearly demonstrated that when lamb backstrap and topside is directly packed (without a vacuum pre-aging period) into high O_2 packaging systems (either high-Ox-MAP or Hi-Ox-DB) at 1 day post-slaughter, there is a failure to age and reduced tenderisation. VSP case ready lamb steaks had the best eating quality compared to both Hi-Ox-MAP and Hi-Ox-DB after both 5- and 10-days storage. After 10-days Hi-Ox-DB was significantly better than Hi-Ox-MAP. The findings confirm that direct packaging of lamb steaks into Hi-Ox-MAP at 1 day post-slaughter leads to a reduction in eating quality – especially reduced flavour, tenderness and juiciness - compared to direct packaging into VSP. Direct packaging of lamb into Hi-Ox-DB offers only modest improvement in eating quality at 10-days compared to Hi-Ox-MAP.

A recent study (Lagerstedt *et al.* 2011) on effects of packaging (Hi-Ox-MAP, VP and VSP) on beef eating quality concluded that;

"Hi-Ox-MAP cannot [generally] be recommended for either the meat industry or for retail displays".

In their study, the beef primal (*M. Longissimus dorsi*) was first pre-aged and tenderised for 7 days in VP, then opened and repackaged in Hi-Ox-MAP, VP or VSP. They found that VP and VSP beef had similar tenderness and juiciness after 14 and 21 days; both were significantly better than Hi-Ox-MAP. Their data also indicated that any tenderisation that was achieved in the VP pre-ageing period was reversed by subsequent Hi-Ox-MAP. We were unable to test the effect of high O_2 MAP on samples which had initially been aged for 5 or more days, due to the loss of samples.

Because VSP packaged beef has lower purge loss and better visual appearance compared to VP, VSP is regarded a better packaging option, on these grounds alone. In an Australian study (Geesink *et al.* 2015) it was concluded that packaging of beef under Hi-Ox-MAP was detrimental to the eating quality. Our results from lamb support the conclusions reached in these previous studies. Our data also showed that the Darfresh® Bloom system did not provide a packaging solution able to deliver both optimal red colour and tenderness, although it provided modest improvements in eating quality compared to Hi-Ox-MAP.

Current sheep meat eating quality (SMEQ) processing parameters for MSA lamb list a minimum of 5 days ageing in vacuum at 1°C, prior to freezing or cooking (and longer if carcasses enter rigor (pH 6.0) outside the 18-25°C target range) to achieve desired tenderness and eating quality (Channon and Warner 2011). Direct packaging of lamb steaks into Darfresh® VSP for either 5 or 10 days allowed significant increases in lamb eating quality. The increased presence of VSP on supermarket shelves is an indicator that the purple colour of the fresh meat is no longer a barrier to consumer acceptance. The Darfresh® Bloom system retained a better fresh meat red colour; although the eating quality was significantly lower than VSP. Based on the current study, direct packaging of lamb steaks into Hi-Ox-MAP, without prior ageing, would not be recommended assuming current SMEQ criteria and not withstanding other commercial production considerations, such as cost, inventory and merchandising benefits for the various packaging treatments. SIRE as a random effect was shown to have no significant effects on the meat eating quality in this experiment, possibly because differences in the % IMF were minimal. Both the Hi-Ox-MAP and Hi-Ox-DB treatments had the same modified gas headspace composition (80 % O_2, 20% CO_2), indicating that the high oxygen was the main factor responsible for the lower sensory scores (lack of tenderisation) relative to the VSP treatment. However the sensory scores for the Hi-Ox-DB samples were generally better than Hi-Ox-MAP after 10 days of storage.

Meat tenderness has been linked to a number of factors: meat collagen content, the average sarcomere length and the degree of postmortem protein degradation by protease enzymes and the amount of IMF (Jacob and Pethick 2014; Purslow 2014; Starkey *et al.* 2015). While the two former variables are determined mainly by animal genetics and pre-slaughter factors, the packaging format could have an effect on proteolysis. Two main theories have been proposed to explain the failure in tenderisation or the increase in toughening during ageing in Hi-Ox-MAP:

- Oxidation damages the calcium activated proteases, especially μ – calpain. μ – Calpain induced proteolysis is thought to be a key factor involved in the tenderisation process (Lund *et al.* 2008; Fu *et al.* 2015);

- Intermolecular cross links between myofibrillar proteins (myosin and desmin) form in an oxidative environment, leading to meat toughening (Geesink *et al.* 2015)

There is published evidence to show that inhibition of caplain degradation is a major cause of failure of tenderisation in Hi-Ox-MAP (Rowe *et al.* 2004b, 2004a; Koohmaraie and Geesink 2006). There is also new evidence showing that oxidative cross-linking of proteins may be the main pathway to toughening (Lund *et al.* 2007a; Lund *et al.* 2008; Kim *et al.* 2010b, 2010a; Geesink *et al.* 2015). The lack of difference in free amino acid concentrations/ peptides between the packaging treatments found in this study support the idea that differences in the degree of proteolysis were unlikely to be responsible for differences in tenderness.

6 Conclusions/recommendations

6.1.1 Next steps for MAP packaging of lamb

The key unresolved question remains; is it possible to get both optimal tenderisation and an acceptable red colour with an extended shelf-life through a combined MAP packaging approach? If all samples had been completed as per the original design (**Figure 1**), the consequences of "pre-aging" or "pre-tenderisation" of the primal in VP, retail packaging of cut steaks under Hi-Ox-MAP or Hi-Ox-DB would have been understood. It is possible that the pre-aging in VP may have allowed an initial "irreversible" tenderisation to occur, which may have subsequently been maintained together with a superior red colour with subsequent Hi-Ox-MAP or HI-OX-DB.

This outcome seems unlikely, because it was shown previously that VP pre-aged beef primals did not maintain tenderness with subsequent Hi-Ox-MAP (Lagerstedt *et al.* 2011) compared to VP or VSP. A desirable packaging system would be able to optimise tenderness and retain good fresh meat colour. The Darfresh® Bloom system offered some advantages in eating quality compared to Hi-Ox-MAP, suggesting that this combined technology has potential for further development. After 24 hours storage, the colour of the Hi-Ox-DB packaged meat was already bright red, indicating that the oxygen transmission rates were quite high across the barrier film. Sealed Air has been made aware of the results of this packaging trial. In recent times, Sealed Air has been developing alternative very low oxygen transmission barrier multi-layer films for use in new packaging formats.

The next generation of Darfresh® Bloom type systems will incorporate barrier materials with much lower oxygen transmission rates. These new systems may offer better meat tenderisation with an acceptable retail pack bloom, although this remains to be demonstrated. Alternatively a smart packaging system, where the oxygen concentration can be increased "on demand" in the retail pack prior to purchase may also be a solution. It is unknown whether late exposure to oxygen would reverse any in pack gains in tenderisation. Further research into the nature of oxidative cross linking processes is recommended to better understand in pack toughening. Finally, based on the data generated in designed experiments such as this, a concerted advertising campaign to better educate lamb consumers that a purple meat colour is a sign of "guaranteed good eating quality" may be effective. The purple colour is quite distinctive and different from the brown hue of oxidised meat. There may be potential to increase consumer engagement in their lamb consumption and preparation, for example by demonstrating the superior eating quality of VSP packaged lamb.

Recommendation for MSA SMEQ pathways committee:

- The data in this project has clearly shown that packaging of backstrap or topside in a high O_2 MAP system (Hi-Ox-DB or Hi-Ox-MAP) at 1 day post-slaughter results in inferior eating quality, due to a failure to exhibit normal ageing. Thus in order for sheep meat to be eligible for MSA grading, it is essential that the recommended ageing period for the particular carcass/cut is undertaken in anaerobic (vacuum packaged, VSP) environment.

- There was no data collected on whether there is deterioration in the quality of sheep meat which has undergone ageing before high O_2 packaging. Thus it is recommended that research is undertaken to collect this data.

- Industry will require flexible options for packaging in the future. Thus it is recommended that methods to ameliorate the deterioration in eating quality during MAP should be included in the research in point 2 above. Possible strategies could include, nitrite/antioxidants embedded in the packaging material or using CO in the gas mixture.

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Appendix 1

List of features, benefits and specifications for the VSP and HI-OX-DBpackaging systems. Information taken directly from the Sealed-Air website in April 2015 (http://www.cryovac.com/NA/EN/food-packaging-products/darfresh-barrier-packaging.aspx)

Packaging Format	**Cryovac Darfresh ® Barrier vacuum skin packaging (VSP)**	**Cryovac Darfresh ® Bloom (HI-OX-DB)**
Features	• High-performance package that provides a unique combination of longer shelf life and dramatic product presentation. • Utilizes two films to create a vacuum package which has a second skin appearance (VSP). • Oxygen-barrier properties • Easy-open features available	• Same prolonged freshness and shelf life as standard tray-lid MAP packs • Vacuum skin technology ensures leak-proof, secure pack for vertical display • Oxygen-barrier properties and optional easy-open features • Compartment enables insertion of coupons, leaflets and other promotional materials • Reduced headspace offers optimal package size • Flat lid stock surface for printing • Permeable flexible top film with rigid bottom web
Benefits	• Fresh red meat cuts have a higher quality taste and tenderness because no MAP gases are used and the product "wet ages" in the package • Extended shelf life in refrigerator • Easy to open, never have to touch the product • Freezer ready, no need to re-wrap • Product appearance differentiates the package in the case • Opportunity to leverage consumers' association of vacuum packaging with freshness by using point of sale materials • Improved product quality • Extended shelf life means reduced shrink and better in-stock position, overall cost savings • Allows vertical display of product, reduced packaging allows better utilization of case space • Total seal maintains purge to the meat, keeps a tight, clean package • Leak-proof Darfresh® packaging = clean meat case • Extended shelf-life • Reduced packaging means better shipping efficiencies • Increased sales from higher product turnover • Better product consistency (taste & color) from package to package • Versatile; meets requirements for a wide product range	Prolonged shelf life Preserves the color, flavor and integrity of the product Reduces purge and eliminates the need for an absorbent pad; encourages meat maturation More profitable logistics, reduced out-of-stocks, optimized retail shelf utilization and less waste
Specifications	Printing capabilities for lidstock Wide range of choices in both top and bottom forming webs	A wide range of choices in both top and bottom forming webs as well as pre-made trays (foam or rigid)

Appendix 2: Example paper ballot for consumer assessment of grilled lamb samples

TPL TPL

PLEASE PICK UP THE PLATE, **SMELL THE SAMPLE**
AND ANSWER THE NEXT QUESTION

Liking of smell

Dislike extremely — Like extremely

NOW PLEASE EAT AT LEAST 3 MOUTHFULS AND ANSWER THE FOLLOWING QUESTIONS

Tenderness

Not Tender — Very Tender

Juiciness

Not Juicy — Very Juicy

Liking of Flavour

Dislike Extremely — Like Extremely

Overall Liking

Dislike Extremely — Like Extremely

Please mark **X** in one of the following boxes to rate the quality of the lamb sample you have just eaten
Choose **one** only (you must make a choice)

2 ☐ Unsatisfactory
3 ☐ Good everyday quality
4 ☐ Better than everyday quality
5 ☐ Premium quality

TPL Version 14.6 TPL

8 Literature cited

Brooks, JC, Alvarado, M, Stephens, TP, Kellermeier, JD, Tittor, AW, Miller, MF, Brashears, MM (2008) Spoilage and safety characteristics of ground beef packaged in traditional and modified atmosphere packages. *Journal of Food Protection* **71**, 293-301.

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

Channon, HA, Warner, RD (2011) Delivering consistent quality australian pork to consumers - a systems approach. (Ed. RJ Barneveld.) pp. 262-293. (Australasian Pig Science Association (Inc.): Werribee, Victoria, Australia)

Corbin, CH, O'Quinn, TG, Garmyn, AJ, Legako, JF, Hunt, MR, Dinh, TTN, Rathmann, RJ, Brooks, JC, Miller, MF (2015) Sensory evaluation of tender beef strip loin steaks of varying marbling levels and quality treatments. *Meat Science* **100**, 24-31.

Delmore, RJ, 2009. Beef Shelf-life. Beef Facts. National Cattlemen's Beef Association Colorado, USA.

Dransfield, E, Zamora, F, Bayle, MC (1998) Consumer selection of steaks as influenced by information and price index. *Food Quality and Preference* **9**, 321-326.

Eilert, SJ (2005) New packaging technologies for the 21st century. *Meat Science* **71**, 122-127.

Ertbjerg, P, Mielche, MM, Larsen, LM, ¸ller, AJ (1999) Relationship between proteolytic changes and tenderness in prerigor lactic acid marinated beef. *Journal of the Science of Food and Agriculture* **79**, 970-978.

Estevez, M (2011) Protein carbonyls in meat systems: A review. *Meat Science* **89**, 259-279.

Fu, QQ, Liu, R, Zhang, WG, Li, YP, Wang, J, Zhou, GH (2015) Effects of Different Packaging Systems on Beef Tenderness Through Protein Modifications. *Food and Bioprocess Technology* **8**, 580-588.

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

Grebitus, C, Jensen, HH, Roosen, J, Sebranek, JG (2013) Fresh Meat Packaging: Consumer Acceptance of Modified Atmosphere Packaging including Carbon Monoxide. *Journal of Food Protection* **76**, 99-107.

Hughes, JM, McPhail, NG, Kearney, G, Clarke, F, Warner, RD (2015) Beef longissimus eating quality increases up to 20 weeks of storage and is unrelated to meat colour at carcass grading. *Animal Production Science* **55**, 174-179.

Hunt, MR, Garmyn, AJ, O'Quinn, TG, Corbin, CH, Legako, JF, Rathmann, RJ, Brooks, JC, Miller, MF (2014) Consumer assessment of beef palatability from four beef muscles from USDA Choice and Select graded carcasses. *Meat Science* **98**, 1-8.

Jacob, RH, Pethick, DW (2014) Animal factors affecting the meat quality of Australian lamb meat. *Meat Science* **96**, 1120-1123.

Kaneuchi, C, Seki, M, Komagata, K (1988) PRODUCTION OF SUCCINIC ACID FROM CITRIC-ACID AND RELATED ACIDS BY LACTOBACILLUS STRAINS. *Applied and Environmental Microbiology* **54**, 3053-3056.

Kiermeier, A, Tamplin, M, May, D, Holds, G, Williams, M, Dann, A (2013) Microbial growth, communities and sensory characteristics of vacuum and modified atmosphere packaged lamb shoulders. *Food Microbiology* **36**, 305-315.

Kim, YH, Huff-Lonergan, E, Sebranek, JG, Lonergan, SM (2010a) Effects of lactate/phosphate injection enhancement on oxidation stability and protein degradation in early postmortem beef cuts packaged in high oxygen modified atmosphere. *Meat Science* **86**, 852-858.

Kim, YH, Huff-Lonergan, E, Sebranek, JG, Lonergan, SM (2010b) High-oxygen modified atmosphere packaging system induces lipid and myoglobin oxidation and protein polymerization. *Meat Science* **85**, 759-767.

Kim, YHB, ¸dker, S, Rosenvold, K (2012) Influence of lamb age and high-oxygen modified atmosphere packaging on protein polymerization of long-term aged lamb loins. *Food Chemistry* **135**, 122-126.

Koohmaraie, M (1994) Muscle proteinases and meat ageing. *Meat Science* **36**, 93-104.

Koohmaraie, M, Geesink, GH (2006) Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Science* **74**, 34-43.

Lagerstedt, A, Ahnstrom, ML, Lundstrom, K (2011) Vacuum skin pack of beef - A consumer friendly alternative. *Meat Science* **88**, 391-396.

Lawless, HT, Heymann, H. (1999) 'Sensory Evaluation of Food, Principles and Practices.' (Aspen Publishers, Inc.: Gaithersburg, Maryland, USA)

Leggio, A, Belsito, EL, De Marco, R, Liguori, A, Siciliano, C, Spinella, M (2012) Simultaneous extraction and derivatization of amino acids and free fatty acids in meat products. *Journal of Chromatography A* **1241**, 96-102.

Li, X, Lindahl, G, Zamaratskaia, G, Lundstrom, K (2012) Influence of vacuum skin packaging on color stability of beef longissimus lumborum compared with vacuum and high-oxygen modified atmosphere packaging. *Meat Science* **92**, 604-609.

Lund, MN, Christensen, M, Fregil, L, Hviid, MS, Skibsted, LH (2008) Effect of high-oxygen atmosphere packaging on mechanical properties of single muscle fibres from bovine and porcine longissimus dorsi. *European Food Research and Technology* **227**, 1323-1328.

Lund, MN, Hviid, MS, Skibsted, LH (2007a) The combined effect of antioxidants and modified atmosphere packaging on protein and lipid oxidation in beef patties during chill storage. *Meat Science* **76**, 226-233.

Lund, MN, Lametsch, R, Hviid, MS, Jensen, ON, Skibsted, LH (2007b) High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine longissimus dorsi during chill storage. *Meat Science* **77**, 295-303.

Mancini, RA, Hunt, MC (2005) Current research in meat color. *Meat Science* **71**, 100-121.

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

McMillin, KW, Huang, NY, Ho, CP, Smith, BS (1998) Quality and shelf-life of meat in dynamic modified atmosphere packaging. *Abstracts of Papers of the American Chemical Society* **215**, U16-U16.

McPhail, NG, Barlow, R, Stark, JL, Warner, RD (2014a) Greening of vacuum-packaged lamb – causative factors. Project A.MQA.0003. Final report to MLA.

McPhail, NG, Stark, JL, Ball, AJ, Warner, RD (2014d) Factors influencing the occurrence of high ultimate pH in three muscles of lamb carcasses in Australia. *Animal Production Science* **54**, 1853-1859.

Mills, J, Donnison, A, Brightwell, G (2014) Factors affecting microbial spoilage and shelf-life of chilled vacuum-packed lamb transported to distant markets: A review. *Meat Science* **98**, 71-80.

Moya, VJ, Flores, M, Aristoy, MC, Toldra, F (2001) Pork meat quality affects peptide and amino acid profiles during the ageing process. *Meat Science* **58**, 197-206.

O'Quinn, TG, Brooks, JC, Polkinghorne, RJ, Garmyn, AJ, Johnson, BJ, Starkey, JD, Rathmann, RJ, Miller, MF (2012) Consumer assessment of beef strip loin steaks of varying fat levels. *Journal of Animal Science* **90**, 626-634.

Polkinghorne, R, Watson, R, Thompson, JM, Pethick, DW (2008) Current usage and future development of the Meat Standards Australia (MSA) grading system. *Australian Journal of Experimental Agriculture* **48**, 1459-1464.

Purslow, PP (2014) New Developments on the Role of Intramuscular Connective Tissue in Meat Toughness. *Annual Review of Food Science and Technology, Vol 5* **5**, 133-153.

Rowe, LJ, Maddock, KR, Lonergan, SM, Huff-Lonergan, EJ (2004a) Influence of early postmortem protein oxidation on beef quality. *Journal of Animal Science* **82**, 785-793.

Rowe, LJ, Maddock, KR, Lonergan, SM, Huff-Lonergan, EJ (2004b) Oxidative environments decrease tenderization of beef steaks through inactivation of {micro}-calpain. *Journal of Animal Science* **82**, 3254-3266.

Russell, BC, McAlister, G, Ross, IS, Pethick, DW (2005) Lamb and sheep meat eating quality - industry and scientific issues and the need for integrated research. *Australian Journal of Experimental Agriculture* **45**, 465-467.

Schlichtherle-Cerny, H, Grosch, W (1998) Evaluation of taste compounds of stewed beef juice. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung a-Food Research and Technology* **207**, 369-376.

Smart, KF, Aggio, RBM, Van Houtte, JR, Villas-Boas, SG (2010) Analytical platform for metabolome analysis of microbial cells using methyl chloroformate derivatization followed by gas chromatography-mass spectrometry. *Nature Protocols* **5**, 1709-1729.

Starkey, CP, Geesink, GH, Oddy, VH, Hopkins, DL (2015) Explaining the variation in lamb longissimus shear force across and within ageing periods using protein degradation, sarcomere length and collagen characteristics. *Meat Science* **105**, 32-37.

Warner, RD (2014) Measurements of water-holding capacity and color - objective and subjective. In 'Encyclopedia of Meat Sciences.' (Eds ME Dikeman, CE Devine.) Vol. 2 pp. 164-171. (Elsevier Publishing: London)

Warner, RD, Dunshea, FR, Ponnampalam, EN, Cottrell, JJ (2005a) Effects of nitric oxide and oxidation in vivo and postmortem on meat tenderness. *Meat Science* **71**, 205-217.

Warner, RD, Dunshea, FR, Ponnampalam, EN, Cottrell, JJ (2005b) Effects of nitric oxide and oxidation in vivo and postmortem on meat tenderness. *Meat Science* **71**, 205-217.

Watanabe, A, Ueda, Y, Higuchi, M (2004) Effects of slaughter age on the levels of free amino acids and dipeptides in fattening cattle. *Animal Science Journal* **75**, 361-367.

Watson, R, Gee, A, Polkinghorne, R, Porter, M (2008a) Consumer assessment of eating quality - development of protocols for Meat Standards Australia (MSA) testing. *Australian Journal of Experimental Agriculture* **48**, 1360-1367.

Watson, R, Polkinghorne, R, Thompson, JM (2008d) Development of the Meat Standards Australia (MSA) prediction model for beef palatability. *Australian Journal of Experimental Agriculture* **48**, 1368-1379.

Wilkinson, BHP, Janz, JAM, Morel, PCH, Purchas, RW, Hendriks, WH (2006) The effect of modified atmosphere packaging with carbon monoxide on the storage quality of master-packaged fresh pork. *Meat Science* **73**, 605-610.