OPTIMISING EATING QUALITY OF STEAKS BY USING TRI-GAS MAP

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1.0 EXECUTIVE SUMMARY

Modified atmosphere packaging (MAP) is widely used for packaging of fresh and processed foods. Traditionally, red meat is packed in 70-80% oxygen (O_2) to obtain an attractive bloom colour and in 20-30% carbon dioxide (CO_2) to extend shelf life (Singh, Wani & Saengerlaub, 2011). Unfortunately, it appears that meat retail packed in high oxygen MAP is less tender and less juicy with a more rancid flavour and increased premature browning (PMB), for pork (Lund, Lametsch, Hviid, Jensen & Skibsted, 2007; Tørngren, Darré & Kristensen, 2013) as well as for beef (Tørngren, 2003; Kim et al., 2010; Lagerstedt, Lundstrøm & Lindahl, 2011). The reduced eating quality is caused by oxidative changes of lipids and structural proteins, initiated by the high oxygen level in the headspace (Lund, Lametsch, Hviid, Jensen & Skibsted, 2007; Estévez, 2011; Bao, Poulanne & Ertbjerg, 2016).

Previous studies have recommended non-oxygen retail packing (Tørngren, 2003; Cayuela et al., 2004; Clausen et al., 2009) for optimizing eating quality, but an obvious disadvantage of the method is the less attractive non-bloomed colour. In recent years, several studies have shown that tri-gas MAP could be a useful alternative to traditional high oxygen MAP (Esmer et al., 2011; Tørngren, Darré & Kristensen, 2013; Tørngren, 2014; Muhlisin et al., 2014; Gammariello et al., 2015; Tørngren & Darré 2015; Xiaoyin et al., 2016; Tørngren et al., 2016) whereas others found no benefits of using tri-gas MAP compared with high Ox MAP (Resconi et al., 2012; Owczarek-Fendor et al., 2014).

The objective of the present study is to document how the tri-gas MAP solution (30% O_2 + 30% CO_2 + 40% N_2), recently developed and recommended for Danish beef steaks, affects the shelf life and organoleptic quality of Australian beef exported to Europe in comparison with the traditional high oxygen MAP.

The experiments were designed as two separate trials, one trial for documentation of the shelf life and one trial for documentation of the eating quality. For both trials, striploins were selected at an Australian slaughterhouse, then exported to Denmark, sliced and repacked for retail display using four packaging methods (1) tri-gas MAP (30% O_2 + 30% CO_2 + 40% N_2), (2) high Ox MAP (70% O_2 + 30% CO_2), (3) vacuum/skin and (4) wrap. During retail display (5°C, 1200 lux, up to 35 days), samples were analysed to document effects on organoleptic quality, microbial growth of the raw meat and changes in sensory attributes of cooked meat for documentation of eating quality.

Shelf life was determined by organoleptic evaluation of raw meat and psychrotrophic plate count (PCA, 6.5°C, 10 days). Furthermore, the gas composition was measured in the headspace of MAP samples. The organoleptic evaluation of raw meat odour and appearance was performed by a panel of 3-5 assessors, on a 4-point scale using 1 and 2 for acceptable samples and 3 and 4 for unacceptable samples.

Samples for sensory evaluation were cooked on a frying pan at 170°C to a core temperature of 63-64°C. The samples (3.5 x 2.5 cm) were served on pre-heated plates and evaluated by 8 trained assessors using a 15-point unstructured line scale anchored at the extremes (0 = low intensity and 15 = high intensity). The descriptive attributes were determined and tested during two training sessions focussing on odour, appearance, flavour, taste and texture.

Shelf life of beef steaks from aged (0.2°C, 75 days) striploins depended on the packaging methods used for retail display. Wrap packed steaks had a shelf life of approx. 6-7 days, MA packed steaks had a shelf life of approx. 8-9 days, and vacuum/skin packed steaks had a shelf life of approx. 17 days.

The colour stability of the fresh meat was three times as long when steaks were packed in non-oxygen vacuum/skin packaging (approx. 27 days) compared to MAP and wrap packed meat (7-9 days).

The eating quality was impaired by the oxygen level in the traditional high Ox MAP compared to the other packaging methods. To optimize odour and flavour, tri-gas MAP can be used without compromising the shelf life. For optimizing tenderness and juiciness, steaks must be wrap packed and stored for only 3 days, because MAP and vacuum/skin packing results in decreased texture characteristics then reaching the end of the shelf life.

Recommendations: To obtain minimal quality changes of the eating quality of long time distribution of exported Australian beef, it is recommended to pack steaks in wrap and store the meat for up to 3 days at 5°C. These packaging conditions will preserve the fresh meat characteristics of odour and flavour (roasted meat, metallic and acid) and minimise development of off-flavours due to oxidation and spoilage. If longer shelf life is needed, modified atmosphere packaging in low oxygen tri-gas MAP (30% O_2, 30% CO_2 + 40% N_2) is recommended, because development of rancid flavour and odour is significantly reduced compared to the use of traditional high Ox MAP.

2.0 INTRODUCTION

Packaging in modified atmosphere (MAP) is becoming more and more common for retail display of meat. For MAP of red meat, the industry often uses a gas mixture with a high content of oxygen (high Ox MAP) usually containing 70-80% O_2 in the headspace, because it results in an attractive red colour and increased shelf life. However, high Ox MAP reduces the organoleptic quality of the cooked meat, caused by oxidative degradation of fat and proteins. A well-known alternative to high Ox MAP is vacuum/skin packing or MAP in a non-oxygen MAP. The main drawback of these methods is the raw meat colour, which becomes purple instead of red. DMRI has worked on developing a tri-gas solution to overcome the dilemmas observed with the traditional two-gas solution. The developed tri-gas MAP contains 30% O_2, 30% CO_2 and 40% N_2 and shows a positive effect on the organoleptic quality compared with meat packed in high Ox MAP. Thus, the tri-gas MAP solution combines the positive effect of CO_2 on shelf life with the positive effect of oxygen on colour without compromising the shelf life or the eating quality. Due to the time consuming transport to Europe, the storage history of Australian beef is very different from domestically produced beef in Europe. It is therefore unclear how the newly developed tri-gas solution will influence shelf life and quality of this meat.

3.0 PROJECT OBJECTIVES

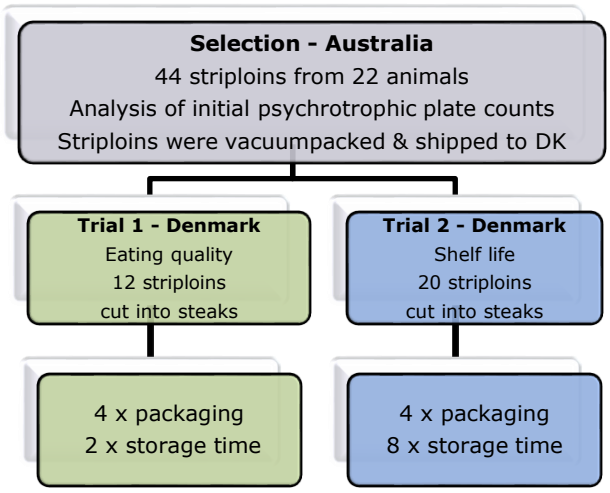
The objective is to document how the new retail tri-gas MAP solution (30% O_2 + 30% CO_2 + 40% N_2) affects the shelf life and organoleptic quality of Australian beef exported to Europe in comparison with the traditional solutions used for retail packaging in Europe.

4.0 METHODOLOGY

The experiments were designed as two separate trials, one trial for documentation of the shelf life and one trial for documentation of the eating quality. For both trials, striploins were selected at an Australian slaughterhouse, exported to Denmark, sliced and repacked for retail display using the following four packaging methods:

1. Tri-gas MAP (30% O_2 + 30% CO_2 + 40% N_2)
2. High Ox MAP (70% O_2 + 30% CO_2)
3. Vacuum/skin packing
4. Wrap

During retail display, samples were analysed at DMRI to document effects on microbial growth and organoleptic quality of the raw meat and changes in sensory attributes for documentation of eating quality.



The pictures below show some of the important steps made in Australia before shipping the striploins to Denmark. In co-operation with AMPC, an Australian slaughterhouse was chosen for selection of the carcasses (Picture 1). Selection criteria were chosen according to EU specification 7771, however, modified from a 1-rib striploin to a 4-rib striploin in order to obtain steaks enough for the analyses. After selection of the carcasses, samples for initial psychrotrophic plate counts were collected at the slaughterhouse (Picture 2) and analysed at a local laboratory.

Picture 1. Selection of carcasses.

Picture 2. Sampling for initial psychrotrophic plate counts.

The striploins were then vacuum packed individually and placed in cardboard boxes along with temperature loggers for measuring temperature profiles in all boxes during shipping.

For cooling the meat to approx. 0-2°C, the boxes were placed in a chiller for at least 24 hours (Picture 3) before they were closed and moved to a warehouse until shipping. Samples for initial psychrotrophic plate counts were diluted, spread on agar, and incubated at a local laboratory (Picture 4).

Picture 3. Open cardboard boxes in the chiller for at least 24 hours.

Picture 4. Samples with DMRI temperature loggers in the incubator at the microbial lab.

4.1 Selection of carcasses

44 beef striploins from 22 carcasses were selected in corporation with an Australian slaughterhouse. DMRl participated in the selection of striploins, sampling and analysis of initial psychrotrophic plate counts and ensured that time and temperature information was logged during the transport to Denmark.

Striploins from the right and left side of carcass 1-20 were alternately included in the trials for eating quality and shelf life. However, four extra striploins from carcass 21 and 22 were included in case of leakers during transport to Denmark or other unforeseen circumstances:

- Carcass 1-10 were used for eating quality and shelf life
- Carcass 11-12 were used for training of the sensory panel and for shelf life
- Carcass 13-20 were included in the trial for shelf life and for extra samples
- Carcass 21-22 were extra striploins in case of leakers

Table 4.1 outlines how the left and right sides of the 22 carcasses were distributed between the two trials.

Table 4.1. Distribution of carcasses and carcass sides between studies.

Carcass	Left side or right side
1-10	Eating quality or shelf life
11-12	Training eating quality or shelf life
13-20	Shelf life/extra samples
21-22	Extra striploins in case of leakers

4.1.1 Selection criteria

Selection of the carcasses was performed after pH measurement and grading. The carcasses were selected according to breed, feeding type, category, age/dentition, carcass weight, fat colour and meat colour.

The criteria for selecting the animals were specified in order to minimize animal variation as well as process variation and to obtain as uniform loins as practically possible from a group of homogenous carcasses, from the same day of slaughter.

For selection of the carcasses, the EU specification 7771 was used. However, modified from a 1-rib striploin to a 4-rib striploin to make sure that all striploins had a sufficient length for the necessary amount of steaks.

Table 4.2. Selection criteria for the 22 carcasses

7771 boneless beef *YP* striploin 4-rib (fat off)	Min	Max
Breed	Cross, British	
Feed type	Grass	
Category	YP (EU)	
Dentition	0	4
Weight of carcass [kg]	250	350
Fat colour	1	3
Meat colour	1C	4

During the selection, right and left sides of the forerib/loin were labelled with ID-tags indicating carcass number 1-22, side L/R and test 1-2. After selection, carcasses were sorted into the chilling room on a separate rail and equalized for approx. 18 hours.

4.2 Initial psychrotrophic plate counts

To obtain knowledge about microbial growth during shipping and facilitate prediction of organoleptic shelf life (www.DMRIpredict.dk), a sample was taken from each carcass and analysed quantitatively for initial psychrotrophic plate count.

The samples were taken before vacuum packing the striploins, by removing a 10 cm^2 sample from the striploin surface, including fat, lean meat, connective tissue or combinations. The samples were taken from striploins belonging to the shelf life trial only (carcass 1-20). The samples were removed and transferred to stomacher bags. The samples were placed in a cooling box with cooler bricks. Paper towels were used to separate the samples in the box. The analyses of the samples were performed by a local laboratory less than 24 hours after sampling, and the samples were kept at a max temperature of 4°C ± 2°C in accordance with the NMKL procedure No 86, 5^{th} edition (Appendix 9.3).

4.3 Packaging & Shipping

All striploins were vacuum-packed and chilled in cardboard boxes without lids for at least 24 hours. Temperature loggers were placed in each cardboard box before chilling.

The striploins were vacuum-packed for shipping on the 6th of July 2016, shipped to Denmark on the 21st of July 2016 and arrived at a warehouse in Denmark on the 3rd of September 2016. Retail cutting and packaging were performed on the 19th of September 2016, after training of the sensory panel was performed. The striploins were stored at -1.5°C to 0°C until re-packing at DMRI.

Consequently, the duration of the shipping and ageing of the striploins was:

- 44 days of container shipping (from AU warehouse to DK warehouse)
- 59 days of AU shipping (from AU slaughterhouse to DK warehouse)
- 75 days for total shipping/ageing (from AU slaughterhouse to DK retail packaging)

4.4 Retail packaging

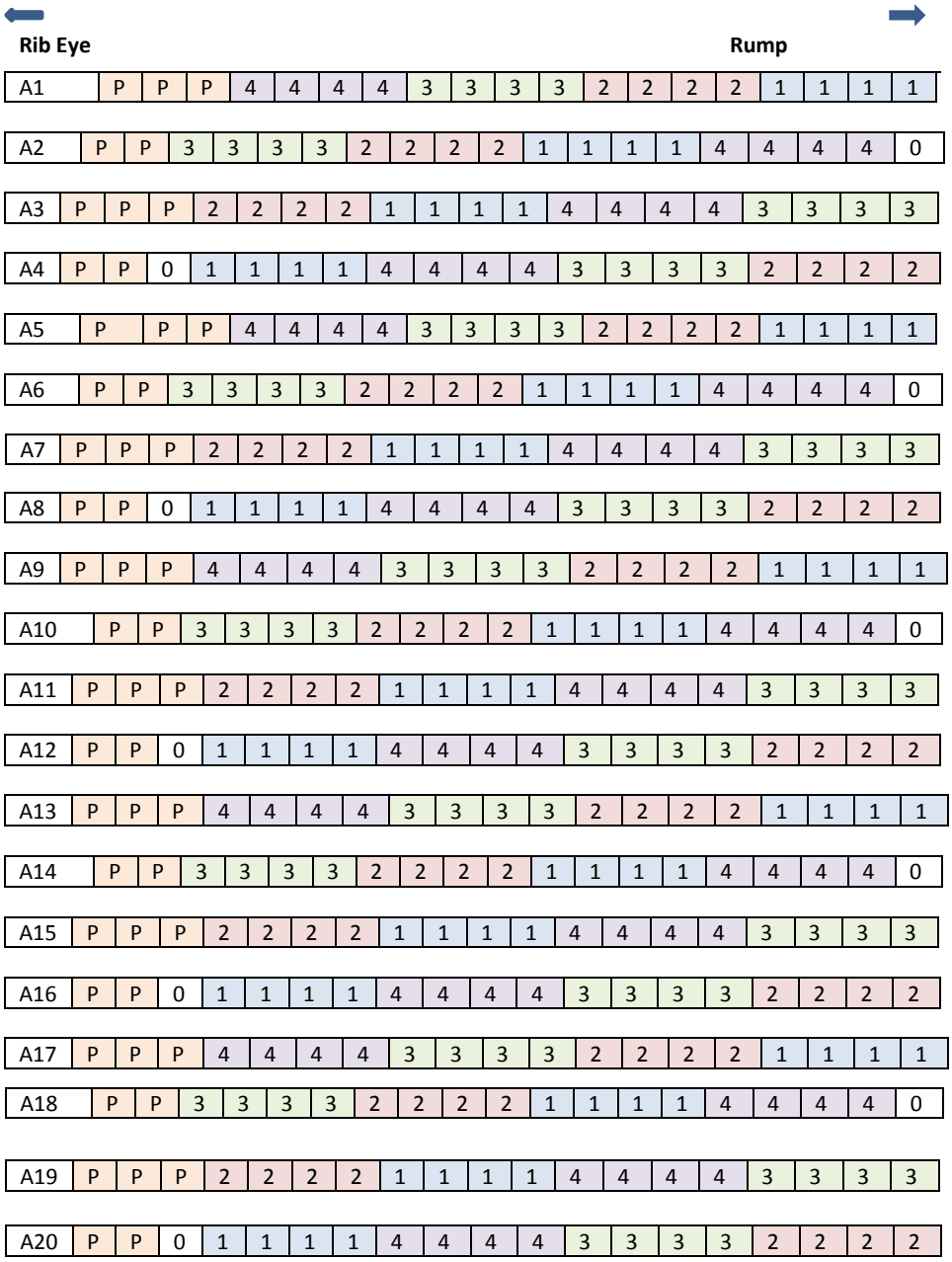
After arrival at DMRl, pH was measured in one striploin per animal (Appendix 1), and the striploins were sliced into steaks and retail packed using the four packaging methods shown in Table 4.3 and stored at retail display conditions at 5°C. For sensory analysis, the samples were evaluated on the day of packaging (day 0) and close to the estimated 'end of shelf life' date.

Table 4.3. The five different treatments.

No	Method	Gas mixture	Steaks per pack	Steaks per sample	
				Shelf life	Eating quality
0	Retail packaging, day 0	Air	-	2	3
1	Tri-gas MAP	30% O_2 + 30% CO_2 + 40% N_2	2	2	4
2	High Ox MAP	70% O_2 + 30% CO_2	2	2	4
3	Vacuum/skin	None	1	2	3
4	Wrap	Air	2	2	4

4.4.1 Cutting samples for shelf life trial

For the shelf life trial, the 20 striploins were hand cut into 19 steaks of 1.5 cm thickness starting at the rump end of the striploin. The steaks were distributed for evaluation on the day of retail packaging ('0') and for eight shelf life evaluations during the storage period. In the drawing below, the four types of retail packaging are marked as '1', '2', '3' and '4', and samples for photographing the surface appearance during storage are marked as 'P'.



4.4.2 Cutting samples for eating quality trial

To minimize influence of animal variation, all four packaging methods were tested within each animal using 10 replicates per treatment. Because of muscle variation, samples were taken from five different parts of the loins.

For the eating quality trial, the 10 striploins were hand cut into 19 steaks of 2 cm thickness starting at the rump end of each striploin. To get a fresh cut, the first steak was discarded (X) and the next 18 steaks were distributed between treatments as shown below for animal # 1-10.

Rib Eye **Rump**

4	4	4	4	3	3	3	2	2	2	2	1	1	1	1	0	0	0	X
3	3	3	2	2	2	2	1	1	1	1	0	0	0	4	4	4	4	X
2	2	2	2	1	1	1	1	0	0	0	4	4	4	4	3	3	3	X
1	1	1	1	0	0	0	4	4	4	4	3	3	3	2	2	2	2	X
0	0	0	4	4	4	4	3	3	3	2	2	2	2	1	1	1	1	X
4	4	4	4	3	3	3	2	2	2	2	1	1	1	1	0	0	0	X
3	3	3	2	2	2	2	1	1	1	1	0	0	0	4	4	4	4	X
2	2	2	2	1	1	1	1	0	0	0	4	4	4	4	3	3	3	X
1	1	1	1	0	0	0	4	4	4	4	3	3	3	2	2	2	2	X
0	0	0	4	4	4	4	3	3	3	2	2	2	2	1	1	1	1	X

4.4.3 Packaging materials

MAP samples were packed with two steaks per tray and vacuum/skin packed samples were packed with one steak per pack.

Picture 5. pH measurement and cutting of steaks

Picture 6. DMRI display room

The MAP samples were packed on a tray sealer (Multivac T200) using a PBI gas mixer (Dansensor, DK) for adjusting the initial gas compositions in the two MAP atmospheres.

The initial deviation of each gas concentration was maximum 5%, and the settings for the tray sealer was: 20 millibar vacuum, 980 gas insufflation, 1.5 second of welding, 130°C of welding and 450 film feeding.

The materials used for retail packaging were:

MAP	Trays: Færch Plast K2190-53H Clear MAPETII, code number 2190116007 Film: Færch Plast TOPLEX HB B-PE 45 AF
Vacuum/skin	Bags: LogiCon 200x270x0.090 PA/PE/90
Wrapped	Trays: Færch Plast K2190-53H Clear MAPETII, code number 2190116007 Film: Wrap, ABENA PVC sprocketed 300mX45cm Cater. Line code number 15571

After packaging, the trays were placed in retail display (Picture 6) at approx. 5°C (Appendix 9.2) and display light (1200 lux from 07:00 a.m. to 07:00 p.m.) until the day of assessments, which was up to 35 days dependent on the packaging method. The temperature was logged during retail display on four Testo 174 temperature loggers placed among the trays in the chilling room.

4.5 Shelf life analysis

During the retail storage, five packages per packaging method were randomly selected for shelf life analysis. For determination of shelf life, an organoleptic evaluation of raw meat and sampling for psychrotrophic plate count were conducted. Furthermore, the gas composition was measured in the headspace of MAP samples.

4.5.1 Organoleptic evaluation of raw meat

The organoleptic evaluation was performed by a trained panel of 3-5 assessors. For evaluation of the raw meat odour and visual appearance during storage, the following 4-point scale was used:

Raw meat odour:

1. Fresh
2. Slightly diverging, but acceptable
3. Diverging to an unacceptable degree
4. Putrid/Rotten

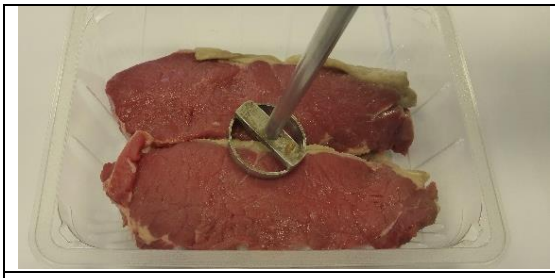
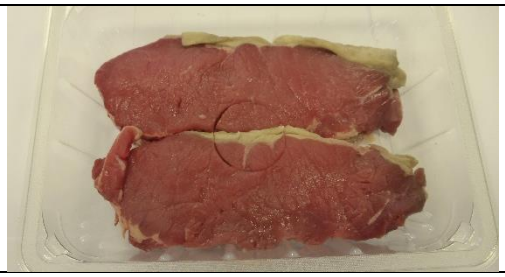
Visual appearance:

1. No discolouration
2. Slightly discoloured, but acceptable
3. Discoloured to an unacceptable degree
4. Very discoloured

In the project outcome (5.1.4), shelf life is defined as 'time until the odour or appearance is inacceptable in 50% of the packs', and is reached when the average score is 2.5.

4.5.2 Psychrotrophic plate count

When sampling for the psychrotrophic plate count, all surface types were included (lean meat, fat and connective tissue). 10 cm^2 of the surface was marked (Picture 7), removed and transferred to a stomacher bag. To each bag, 100 ml of Buffered Peptone Water was added, and the bag was stomached for 1 min. The samples were then diluted, plated on PCA and incubated at 6.5°C for 10 days. For further details, see Appendix 9.3 for NMKL No. 86, 5th Ed., 2013.

Picture 7. Sampling for the psychrotrophic plate count

Table 4.4 illustrates the sampling period for each packaging method. On each of the eight sampling days, five samples were randomly selected from the display room.

Table 4.4. Distribution of samples during storage for the four types of retail packaging.

DAY	Tri-gas MAP	High Ox MAP	Vacuum/skin	Wrap
0	x			
2				
3				x
4	x	x	x	x
7	x	x		x
8				x
9	x	x		x
10	x	x	x	x
11				x
14	x	x	x	x
15	x	x		
18	x	x	x	
21	x	x		
25			x	
28			x	
32			x	
35			x	

4.6 Eating Quality

Eating quality was determined twice during the storage period for all four packaging methods. The first evaluation was performed on the day of packaging, and the second evaluation was performed close to the end of the estimated shelf life. A certified, trained sensory panel was used to profile sensory attributes related to eating quality.

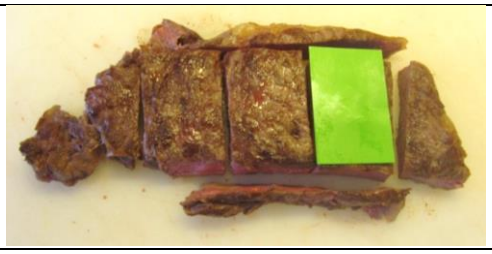
4.6.1 Cooking and serving of samples

Before cooking, the steaks were tempered at room temperature to 10-15°C. For calculation of cooking loss, the weight of the steaks was measured before and after cooking.

Cooking of the steaks was performed in a standardized manner on a frying pan at 170°C to a core temperature of 63-64°C (Picture 8). Each steak was cut into three samples using a template of 3.5 x 2.5 cm (Picture 9). The samples were served on pre-heated plates and were identified by a 3-digit code.

In order to keep the samples warm after cooking (Picture 10), an aluminium lid was used to cover the samples until evaluation.

Picture 8. Cooking on a pan.



Picture 9. Cutting of samples.

Picture 10. Serving.

4.6.2 Training of sensory panel

Prior to the sensory analysis, the panel was trained on the expected extremes in relation to oxidative degradation of fat and proteins. The treatments chosen for training were:

- High Ox MAP, stored for 6-7 days
- Vacuum/skin, stored for 6-7 days

Striploins from animal 11 and 12 were used for training of the sensory panel. They were hand cut into 36 steaks of 2 cm thickness, packed individually and placed on retail display (at 5°C in 1200 lux).

Animal #11-12:

Rib Eye → Rump

X	3	3	3	2	2	2	3	3	3	2	2	2	3	3	3	2	2	2	X
	Serving 3			Serving 3			Serving 2			Serving 2			Serving 1			Serving 1			

X	3	3	3	2	2	2	3	3	3	2	2	2	3	3	3	2	2	2	X
	Serving 6			Serving 6			Serving 5			Serving 5			Serving 4			Serving 4			

The training was performed within two days. On day one, the assessors had three servings and evaluated the two samples based on the sensory attributes used in previous studies on beef.

- Serving #1 – a common terminology was defined
- Serving #2 – attributes were evaluated
- Serving #3 – the consensus profile was tested

Afterwards, the assessors were asked if they agreed or had any further attributes to add to the list:

- Taste/flavour (roasted meat, rancid, stale, WOF)
- Texture (hardness, juiciness, tenderness)
- Appearance (internal colour of the meat, pinholes)

On day two, the consensus profile was tested again with three repetitions.

4.6.3 Sensory evaluation

For sensory evaluation, the samples were cooked as described in section 4.6.1 and evaluated by 8 trained assessors using a 15-point unstructured line scale anchored at the extremes (0 = low intensity and 15 = high intensity). The descriptive attributes were:

Odour	Appearance	Flavour	Taste	Texture
Roasted meat	Well-done	Roasted meat	Acid	Hardness
Metallic	Pinholes	Metallic	Sour	Juiciness
Acid		WOF	Bitter	Tenderness
Sour		Rancid		Chewing time
WOF				
Rancid				

For more details about the descriptive attributes determined and tested during the training sessions, see Appendix 9.7.

4.6.4 Statistical analysis

Sensory data were analysed using mixed models (SAS, 9.2, 2002-2008). The model included packaging method and storage time as fixed effects, and assessors and animal as random effects. Non-significant interactions were deleted from the model. Least squares (LSmeans) were calculated and separated using probability of difference. Levels of significance: $p > 0.05$ = non-significant (ns), $0.05 > p > 0.01$ = *, $0.01 > p > 0.001$ = **, $p < 0.001$ = ***

(See Appendix 9.8 for LSMEANS and significances).

5.0 PROJECT OUTCOMES

Results concerning shelf life and eating quality of retail packed steaks from exported Australian striploins are shown in the following section.

5.1 Shelf life analysis

Shelf life of retail packed steaks was determined from measurements of psychrotrophic plate count and organoleptic evaluations of the raw meat during storage.

5.1.1 Microbial growth during shipping

As shown in Figure 5.1, psychrotrophic plate count, on the surface of the loins, increased during the 75 days of shipping (days from selection to re-packaging at DMRI). Microbial growth was observed on striploins from each of the 20 carcasses with an average growth of 4.0 log cfu/cm^2 (1.8-6.0). Average initial psychrotrophic plate counts were 2.8 log cfu/cm^2 (0.7-4.0) increasing to an average of 7.0 log cfu/cm^2 (4.8-8.2) after shipping.

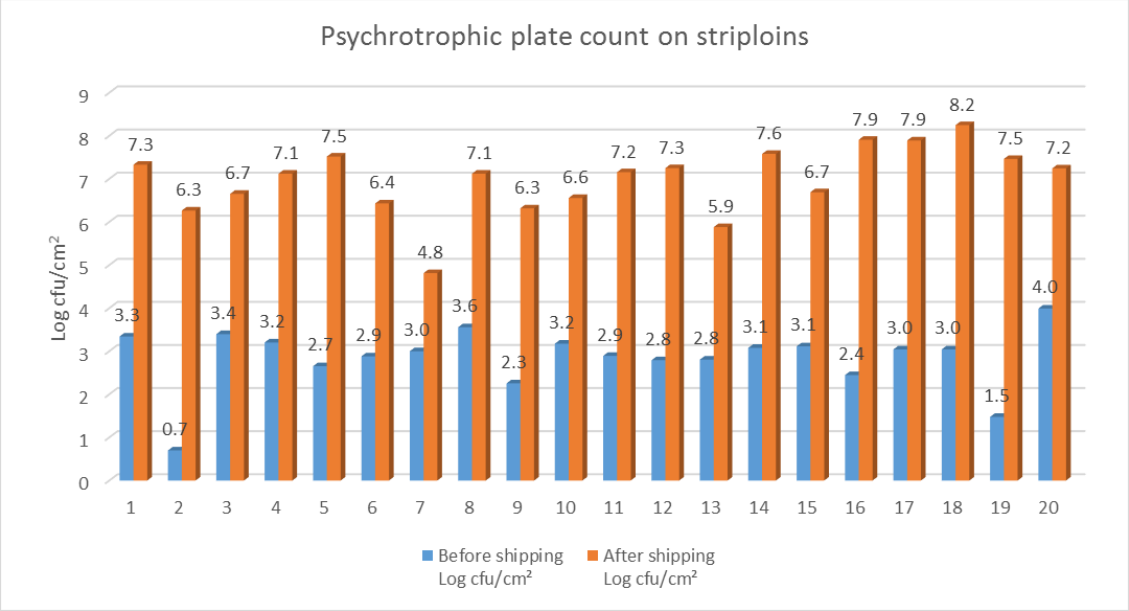


Figure 5.1. Psychrotrophic plate count (log cfu/cm^2) from the surface of the striploins from 20 carcasses. Blue bars showing the counts after selection at the slaughterhouse and red bars showing the counts after shipping at the day of retail packaging.

Temperature logging during the 75 days of total shipping is shown in Appendix 9.2. The average temperature during shipping was approx. 0.2°C. Nevertheless, it is important to note that that there was a substantial variation between the lowest and the highest temperatures. During the 44 days of shipping in the container, the difference between the highest/lowest measured temperatures was approx. 2°C. This means that the shelf life of the meat can vary with as much as 13 days depending on the position in the container during transport (www.DMRIpredict.dk).

5.1.2 Microbial growth during retail storage

Microbial growth on the retail packed steaks was measured during storage at 5°C. As shown in Figure 5.2, growth is faster in wrap packed steaks compared to MAP and vacuum/skin packed steaks. These results were expected as similar growth patterns were observed in other studies (Muhlisin et al., 2014; Xiaoyin et al., 2016).

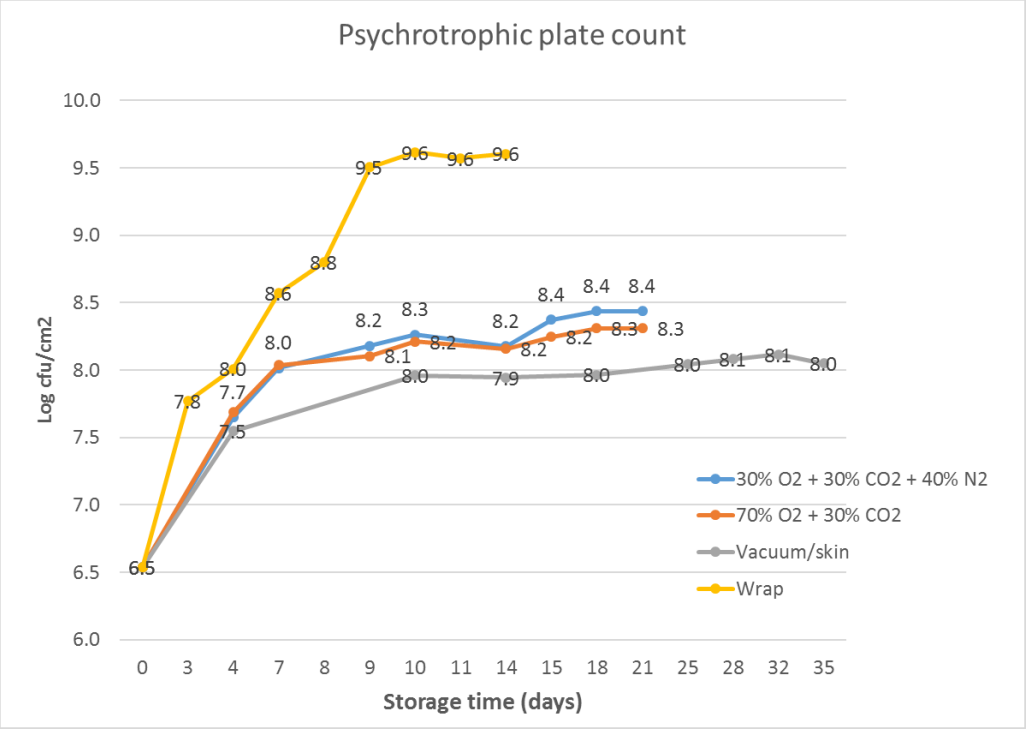


Figure 5.2. Psychrotrophic plate count (log cfu/cm^2) from retail packed steaks during retail storage at 5°C depending of packaging method (n=5).

In accordance with Muhlisin et al., 2014, there was no difference in growth between high Ox MAP (70% O_2 + 30% CO_2) and tri-gas MAP (30% O_2 + 30% CO_2 + 40% N_2). The growth in vacuum/skin packed steaks was to be expected with the initial psychrotrophic plate counts and temperatures found during transport and display (www.DMRIpredict.dk).

5.1.3 Gas composition in MAP during retail storage

Measured gas composition in tri-gas MAP (30% O_2 + 30% CO_2 + 40% N_2) and high Ox MAP (70% O_2 + 30% CO_2) during retail storage is shown in Figure 5.3. The gas composition changes during storage due to O_2 binding with myoglobin and microbial respiration. Therefore, it is expected that oxygen decreases and carbon dioxide increases during storage. For tri-gas MAP, the initial oxygen concentration in the headspace was 28% and decreased to only 4% during the sampling period, whereas carbon dioxide increased from 29% to 51%.

For high Ox MAP, the initial oxygen concentration in the headspace was 67% and decreased to 45%, whereas carbon dioxide increased from 29% to 48% during the same storage period.

The higher oxygen level throughout the storage period in high Ox MAP may contribute to a more stable colour, which will be discussed further in section 5.1.4.

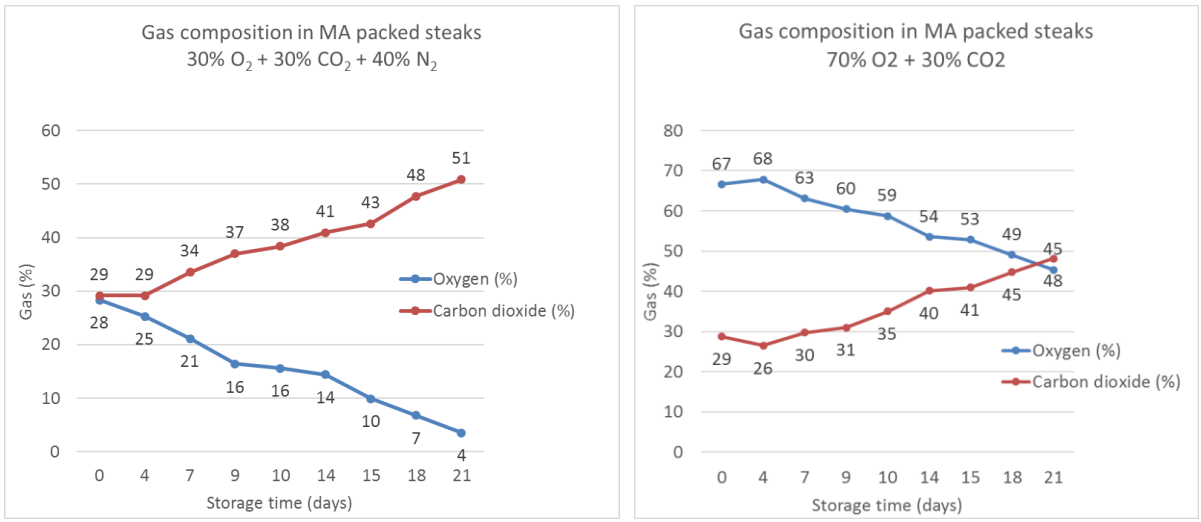


Figure 5.3. Gas composition development for oxygen and carbon dioxide in MAP headspace of fresh beef steaks during retail storage at 5°C, 1200 lux. Left tri-gas MAP and right high Ox MAP (n=5).

5.1.4 Shelf life of retail packed beef steaks

In this section, shelf life is defined as 'time until the odour is inacceptable in 50% of the cuts/packs'. Shelf life or acceptability was measured on bloomed and degassed meat (30 minutes after opening of the package) using a 4-point scale from 1 to 4 as described in section 4.5.1. The maximum shelf life is reached when the average score reaches 2.5, shown as a green line in Figure 5.4-5.7. Photos of the surface colour after 30 minutes of degassing and blooming are shown in Table 5.1-5.4 and for all sampling days in Appendix 9.6.

As shown in Figure 5.4, appearance of steaks packed in tri-gas MAP will reach an unacceptable level before the odour. The limit is reached after approx. 8 days of storage while odour reached the limit after 9.5 days.

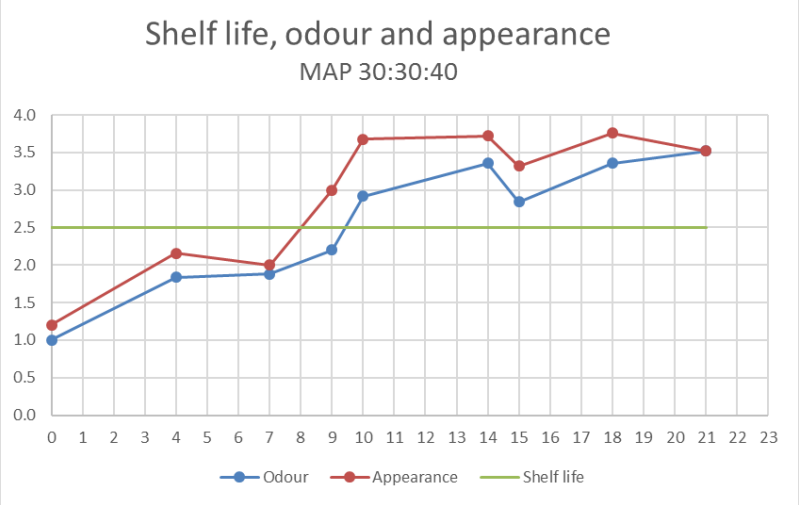


Figure 5.4. Development in odour and appearance of tri-gas MA packed (30% O_2 + 30 CO_2 + 40 N_2) fresh beef steaks (ageing period 75 days at 0.2°C) during retail storage at 5°C, 1200 lux. The green line indicates maximum shelf life.

However, it is important to mention that the reason for the high appearance scores was caused by grey shading of the fat, and not meat discolouration. Normally, odour gets unacceptable before appearance, and this opposite behaviour might be caused by the lower O_2 level in the headspace.

Table 5.1. Visual appearance of MA packed (30% O_2 + 30 CO_2 + 40 N_2) beef steaks close to maximum shelf life.

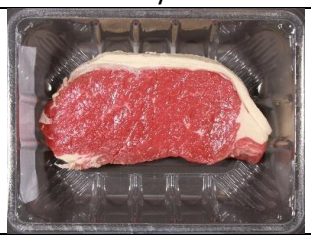
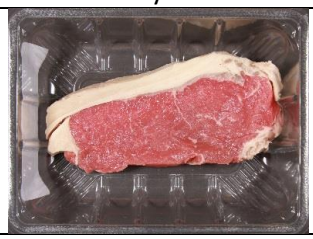
	Day 8	Day 11	Day 15
			

Figure 5.5 shows the development in odour and appearance during retail storage of steaks packed in high Ox MAP. The off-odour develops faster than discolouration of fat and meat and reaches an unacceptable level after 9 days.

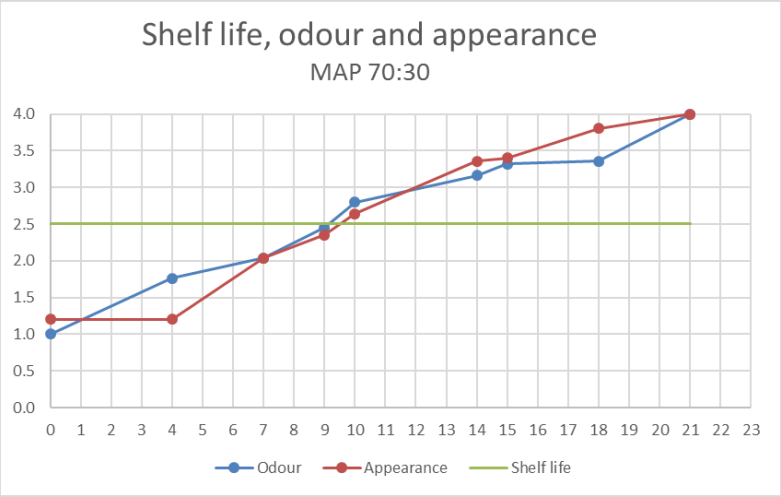


Figure 5.5. Development in odour and appearance of high Ox MA packed (70% O_2 + 30 CO_2) fresh beef steaks (ageing period 75 days at 0.2°C) during retail storage at 5°C, 1200 lux. The green line indicates maximum shelf life.

Visual appearance reaches an unacceptable level after 9-10 days of storage and is characterized by brown spots and a slimy surface. Visual appearance of samples close to shelf life is shown in Table 5.2.

Table 5.2. Visual appearance of MA packed (70% O_2 + 30 CO_2) beef steaks close to maximum shelf life.

	Day 10	Day 11	Day 14
			

Development in odour and appearance of vacuum/skin packed fresh beef steaks is shown in Figure 5.6. Odour reached an unacceptable level long before the limit for visual appearance was reached. Therefore, shelf life of vacuum/skin packed steaks should be specified in relation to off-odour, which gives the product 17 days in shelf life retail display.

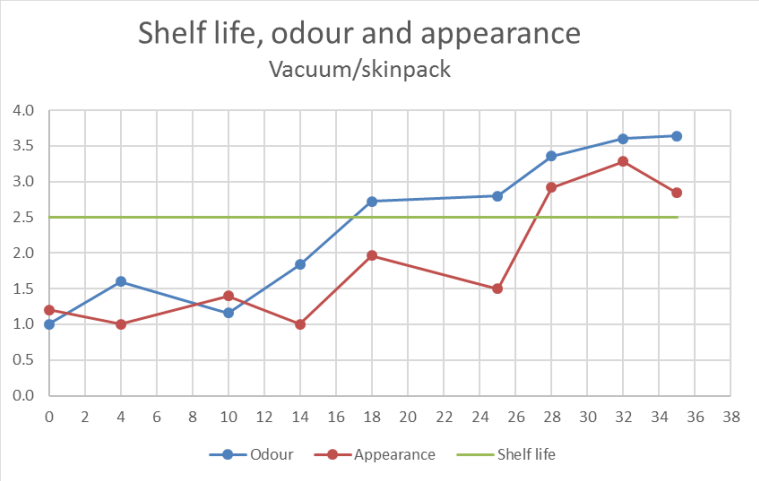
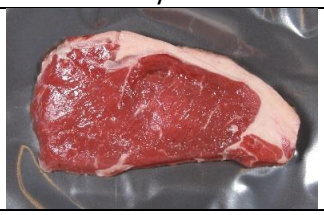
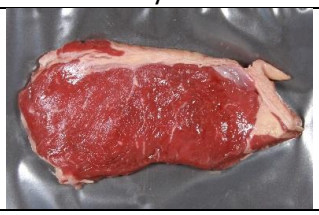
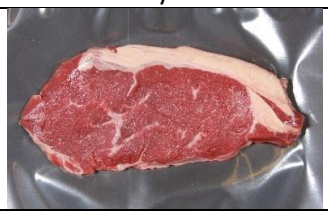


Figure 5.6. Development in odour and appearance of vacuum/skin packed fresh beef steaks (ageing period 75 days at 0.2°C) during retail storage at 5°C, 1200 lux. The green line indicates maximum shelf life.

Visual appearance reaches an unacceptable level after approx. 27 days of storage, which confirms that non-oxygen packaging methods are to be chosen for maximum colour stability of red meat.

Table 5.3. Visual appearance of vacuum/skin packed beef steaks close to maximum shelf life.

	Day 28	Day 32	Day 35
			

In Figure 5.7, shelf life of wrap packed steaks of aged striploins are shown. The off-odour develops faster than discolouration and reaches an unacceptable level after 6-7 days of retail storage.

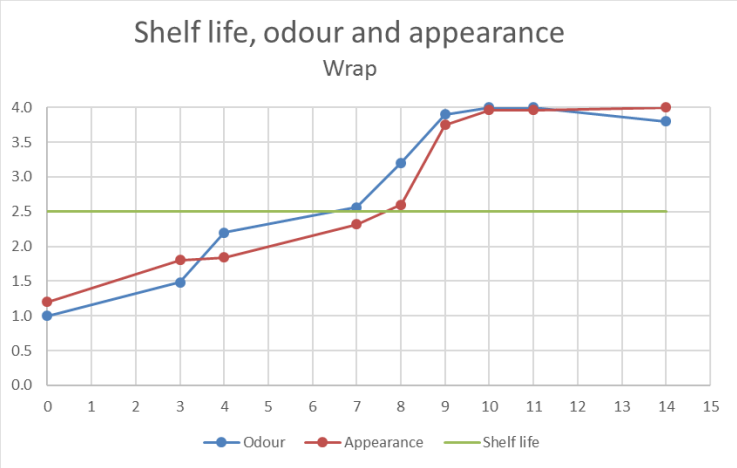
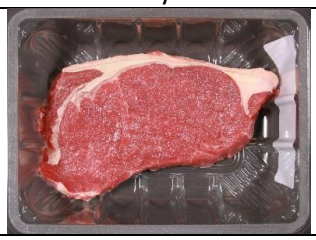
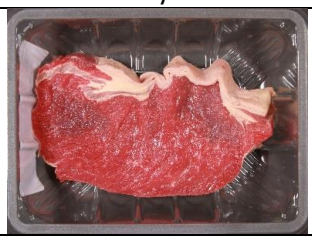


Figure 5.7. Development in odour and appearance of wrap packed fresh beef steaks (ageing period 75 days at 0.2°C) during retail storage at 5°C, 1200 lux. The green line indicates maximum shelf life.

Visual appearance reached an unacceptable level after 7-8 days of storage and was characterized by brown spots and a slimy surface. Visual appearance of samples close to the end of shelf life is shown in Table 5.4.

Table 5.4. Visual appearance of wrap packed beef steaks close to maximum shelf life.

	Day 4	Day 7	Day 8
			

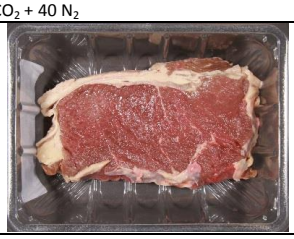
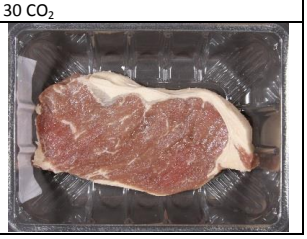
Shelf life of beef steaks from aged (0.2°C, 75 days) striploins is shown in Table 5.5 and depends on the packaging method used for retail display. Wrap packed steaks have a shelf life of approx. 6-7 days, MA packed steaks have a shelf life of approx. 8-9 days, and vacuum/skin packed steaks have a shelf life of approx. 17 days.

Table 5.5. Shelf life (time until the odour or appearance is inacceptable in 50% of the packs) of retail packed steaks evaluated on odour and appearance.

	MAP 30% O_2 + 30 CO_2 + 40 N_2	MAP 70% O_2 + 30 CO_2	Vacuum/skin	Wrap
Odour	9.5 days	9 days	17 days	6.5 days
Appearance	8 days	9.5 days	27 days	7.5 days

It is important to mention that a large variation in initial psychrotrophic plate counts and storage conditions during shipping will affect the subsequent shelf life in retail. Therefore, it is expected that even though steaks are stored under the same conditions, shelf life will be reached at different times. This is illustrated in Table 5.6 and shows the common variation in appearance for replicates.

Table 5.6. Difference in appearance for MA packed steaks stored in retail display for 18 days.

Day	MAP 30% O_2 + 30 CO_2 + 40 N_2		MAP 70% O_2 + 30 CO_2	
18				

5.2 Eating Quality

Eating quality was determined twice during the storage period for all four packaging methods. The first evaluation was performed at the day of packaging, and the second evaluation close to the use by date. This means that MA packed samples were evaluated at day 0 and day 7, vacuum/skin packed samples were evaluated at day 0 and day 18, and wrap-packed samples were evaluated at day 0 and day 3.

5.2.1 Odour of cooked beef steaks

Odour attributes of the cooked steaks are affected by the packaging method and storage time for: roasted meat odour, metallic odour, acid odour, sour odour, WOF odour and to some extent rancid flavour (see Appendix 9.8).

Figure 5.8 shows the intensity of roasted meat flavour on day 0 and close to the end of shelf life for the respective packaging methods. For all types of packaging, the intensity of roasted meat flavour decreases during storage. For steaks packed in high Ox MAP, the effect is more pronounced than for steaks packed in tri-gas MAP, vacuum/skin pack or wrap. For optimum preservation of the meat odour throughout storage, steaks should be packed in wrap or tri-gas MAP before retail display.

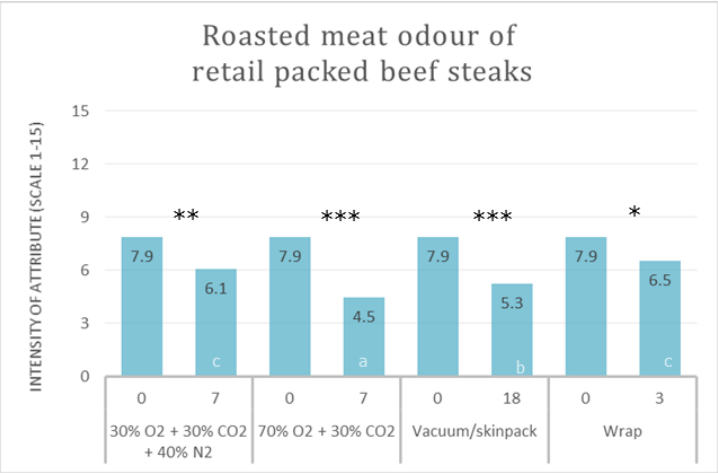


Figure 5.8. Roasted meat odour of retail packed fresh beef steaks (ageing period 75 days at 0.2°C) evaluated on the day of cutting and retail packaging (day 0) and close to the end of shelf life (n=80).

For the off-odours WOF and rancid odour, storage time results in a clear increase for all packaging methods. The lowest intensities were found for samples packed in wrap and tri-gas MAP after 3 days and 7 days of storage, respectively (Figure 5.9). This indicates that loss of meat odour might be caused by development of off-odours, related to lipid oxidation. Furthermore, these results confirm that using a lower oxygen concentration in the headspace will contribute to an enhanced eating quality of the meat.

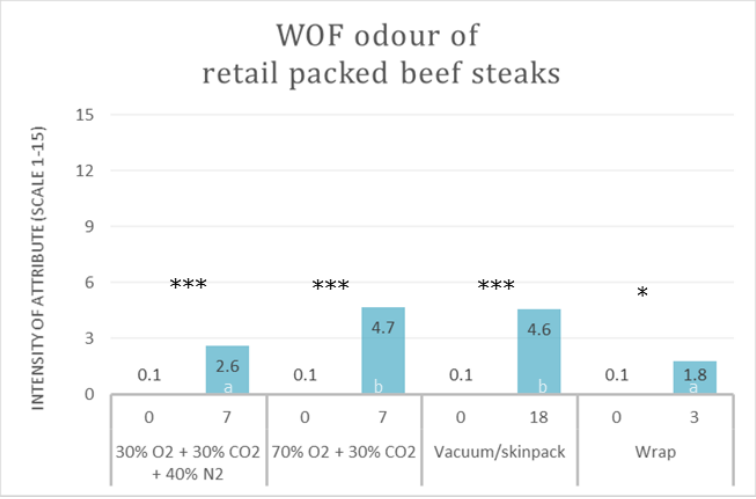


Figure 5.9. WOF odour of retail packed fresh beef steaks (ageing period 75 days at 0.2°C) evaluated on the day of cutting and retail packaging (day 0) and close to the end of shelf life (n=80).

For metallic odour, acid odour and sour odour, tri-gas MAP is more comparable with the vacuum/skin packed steaks. Metallic and acid odour are common fresh meat attributes and are expected to decrease during storage due to microbial spoilage and oxidative degradation. As shown in Figure 5.10, wrap packed meat results in the smallest changes probably due to the short storage time, whereas high Ox MAP results in the largest. Tri-gas MAP and vacuum/skin packed steaks are comparable when evaluated after 7 and 18 days of storage, respectively.

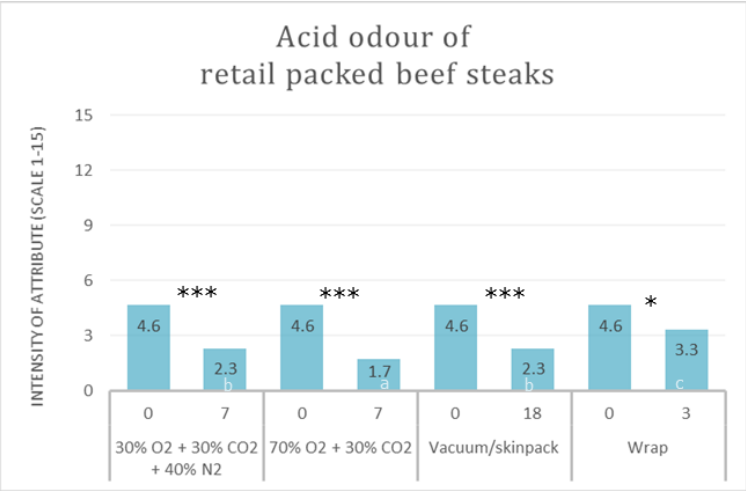


Figure 5.10. Acid odour of retail packed fresh beef steaks (ageing period 75 days at 0.2°C) evaluated on the day of cutting and retail packaging (day 0) and close to the end of shelf life (n=80).

Sour odour is often related to microbial spoilage of the meat, and from Figure 5.11, it is clear that storage in MAP and in vacuum/skin pack will increase this attribute over time, whereas wrap packed meat stored for 3 days is comparable with fresh meat. When comparing these results with the results from the shelf life trial (Table 5.5), the low intensity of sour odour, for wrap packed meat, is well explained by the surprisingly long shelf life of 6-7 days. Vacuum/skin packed and MA packed steaks were evaluated closer to the end of shelf life, and therefore an increase in sour odour is expected. The intensity of sour odour of vacuum/skin packed steaks was expected to be higher than for steaks packed in MAP, because the shelf life of 17 days is exceeded, and spoilage and lactic acid production are expected to be significant.

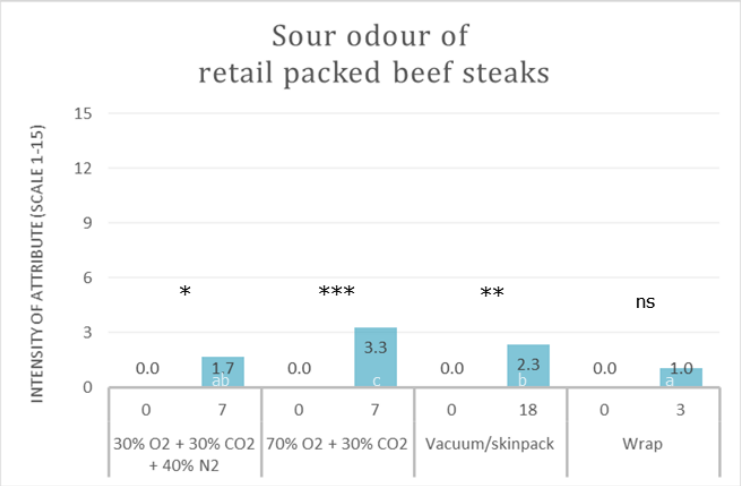


Figure 5.11. Sour odour of retail packed fresh beef steaks (ageing period 75 days at 0.2°C) evaluated on the day of cutting and retail packaging (day 0) and close to the end of shelf life (n=80).

5.2.2 Appearance of cooked beef steaks

Appearance of the meat was evaluated from a cross-sectional view (Appendix 9.8). Well-done appearance and pinhole development were influenced by the packaging method as well as the storage time. In Figure 5.12, the effect on well-done appearance is shown. When using a controlled cooking method, differences in well-done appearance are often related to premature browning (PMB).

PMB increased during storage for MA packed and vacuum/skin packed steaks, most explicit for steaks packed in high Ox MAP followed by tri-gas MAP as expected.

Unexpectedly, PMB increased during storage for vacuum/skin packed steaks, even though there was no oxygen in the headspace. It is well-known that PMB formation is related to the chemical state of the pigment (myoglobin) (Warren et al., 1996) and is explained by a low denaturation temperature for oxymyoglobin and metmyoglobin (Machlik, 1965). Therefore, the increase in PMB for vacuum/skin packed samples might be due to metmyoglobin formation, whereas PMB in MAP samples can be explained by oxymyoglobin formation (John et al., 2005).

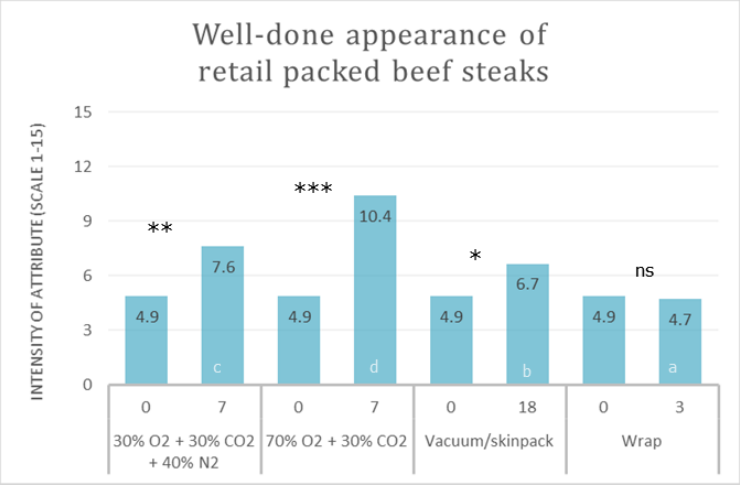


Figure 5.12. Well-done appearance of retail packed fresh beef steaks (ageing period 75 days at 0.2°C) evaluated on the day of cutting and retail packaging (day 0) and close to the end of shelf life (n=80).

Pinholes is a phenomenon seen when using elevated carbon dioxide (CO_2) concentrations for MAP (Tørngren et al., 2013). In this case, intensity of pinholes was low in general, but increased for MAP and vacuum/skin packed steaks during storage. For vacuum/skin packed steaks, this was unexpected, but could be explained by the formation of CO_2 due to anaerobic growth on the surface of the meat.

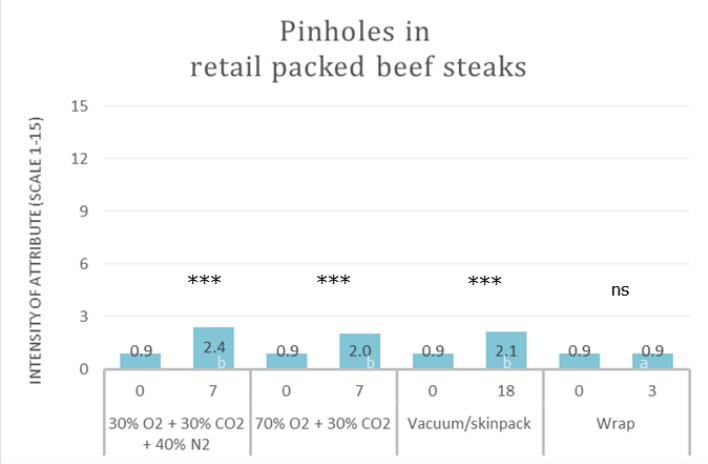


Figure 5.13. Pinholes in retail packed fresh beef steaks (ageing period 75 days at 0.2°C) evaluated on the day of cutting and retail packaging (day 0) and close to the end of shelf life (n=80).

Picture 11 illustrates pinholes in a cross section of a vacuum/skin packed steak. The steaks were vacuum packed and stored for 18 days before sensory evaluation.

Picture 11. Pinholes in vacuum/skin packed steaks day 18.

5.2.3 Flavour of cooked beef steaks

The flavour profile of cooked beef steaks was affected by the packaging method and the storage time (Appendix 9.8). As shown in Figure 5.14, roasted meat flavour decreased during storage for all packaging methods, most significantly for high Ox MAP and vacuum/skin packed steaks. For optimum preservation of the meat flavour, steaks should be packed in wrap or tri-gas MAP for retail display.

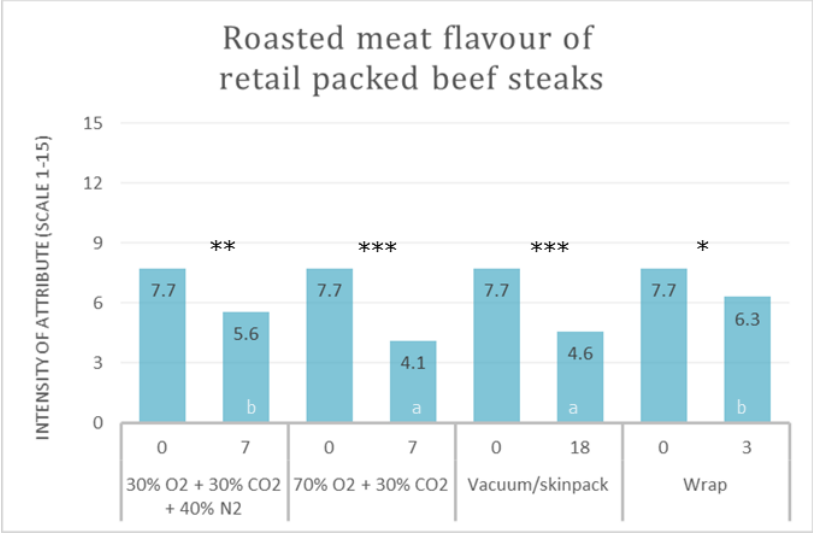


Figure 5.14. Roasted meat flavour in retail packed fresh beef steaks (ageing period 75 days at 0.2°C) evaluated on the day of cutting and retail packaging (day 0) and close to the end of shelf life (n=80).

Metallic flavour is a common fresh meat attribute for red meat. Like the roasted meat flavour, the intensity of metallic flavour decreased during storage for MA packed and vacuum/skin packed steaks (Figure 5.15). Whereas wrap packed steaks stored for 3 days showed a non-significant decrease.

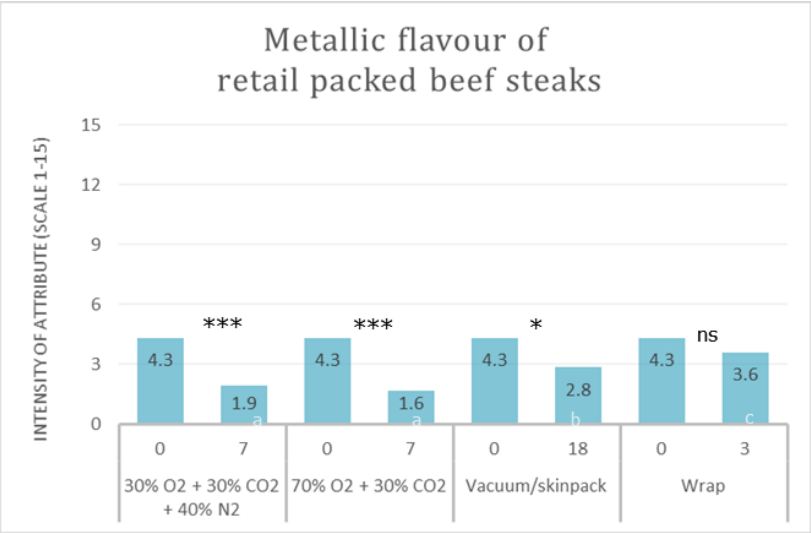


Figure 5.15. Metallic flavour in retail packed fresh beef steaks (ageing period 75 days at 0.2°C) evaluated on the day of cutting and retail packaging (day 0) and close to the end of shelf life (n=80).

The decrease in roasted beef flavour and metallic flavour might be explained by an increase in warmed over flavour (Figure 5.16), which was pronounced in both vacuum/skin packed steaks and steaks packed in high Ox MAP. For high Ox MAP, the increase was most probably due to lipid oxidation facilitated by the high oxygen concentration in the headspace (Muhlisin et al., 2014; Grobbel et al., 2008; Kim et al., 2010).

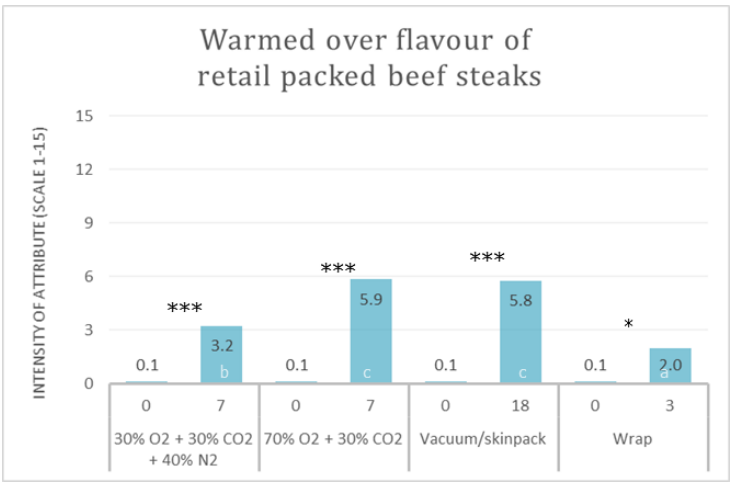


Figure 5.16. Warmed over flavour in retail packed fresh beef steaks (ageing period 75 days at 0.2°C) evaluated on the day of cutting and retail packaging (day 0) and close to the end of shelf life (n=80).

However, the increase in warmed over flavour and in rancid flavour (Figure 5.17) for vacuum/skin packed steaks was unexpected, because the non-oxygen containing environment prevented oxidation. Nevertheless, the evaluation shows an increased intensity, which might be due to some kind of non-oxygen generated degradation in the meat, facilitated by e.g. enzymes and/or the long storage period.

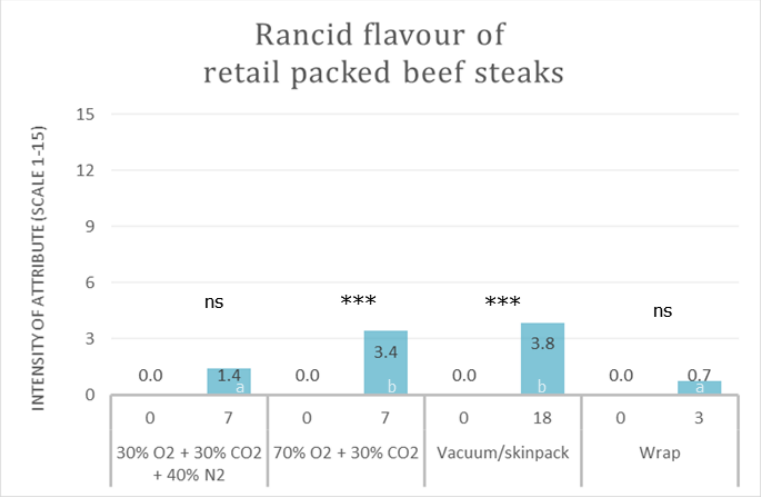


Figure 5.17. Rancid flavour in retail packed fresh beef steaks (ageing period 75 days at 0.2°C) evaluated on the day of cutting and retail packaging (day 0) and close to the end of shelf life (n=80).

5.2.4 Texture of cooked beef steaks

Oxidative changes in structural proteins can result in decreased tenderness of fresh meat, (Lund et al., 2007) and is a known argument for recommending non-oxygen packaging before high Ox MAP.

Tenderness of retail packed beef steaks decreased during storage for all types of packaging (Figure 5.18), but the influence of the packaging method was small, probably due to the long distribution time. No significant differences in tenderness were found between high Ox MAP and tri-gas MAP, which is in accordance with recent studies (Zakrys-Waliwander et al., 2011 and Tørngren & Darré, 2015), and the fact that tenderization of the beef due to proteolytic activities is limited after approx. 3 weeks post mortem.

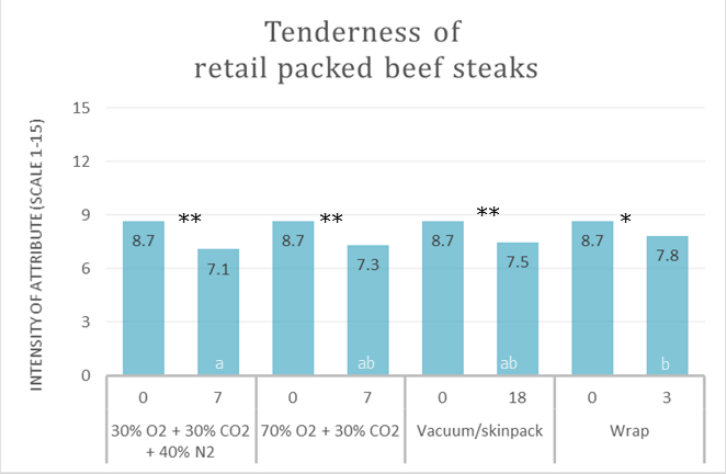


Figure 5.18. Tenderness of retail packed fresh beef steaks (ageing period 75 days at 0.2°C) evaluated on the day of cutting and retail packaging (day 0) and close to the end of shelf life (n=80).

The same tendency was observed for juiciness, with decreasing juiciness of MA packed steaks and vacuum/skin packed steaks during storage, and no difference between packaging in MAP and non-oxygen vacuum/skin pack was found. Nevertheless, wrap packed steaks stored for only 3 days were significantly juicier than other packaging methods and did not decrease during storage.

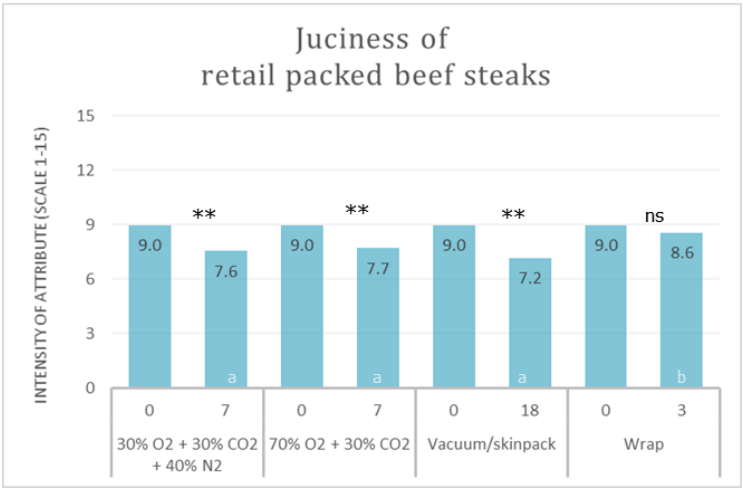


Figure 5.19. Juiciness of retail packed fresh beef steaks (ageing period 75 days at 0.2°C) evaluated on the day of cutting and retail packaging (day 0) and close to the end of shelf life (n=80).

5.2.5 Cooking loss

Comparing the results for perceived juiciness (Figure 5.19) and the calculated cooking losses (Table 5.7) of the cooked steaks, the same pattern was observed. No differences were found between MAP (day 7) and vacuum/skin packed steaks (day 18), and between fresh steaks (day 0) and wrap packed steaks (day 3).

Table 5.7. Cooking loss of retail packed fresh beef steaks (ageing period 75 days at 0.2°C) calculated on day 0 and close to the end of shelf life (n=10).

	Day 0	Day 3	Day 7	Day 18
MAP 30% O_2 + 30 CO_2 + 40 N_2	14.3		16.8	
MAP 70% O_2 + 30 CO_2			16.0	
Vacuum/skin pack				17.0
Wrap		14.1		

6.0 DISCUSSION

The results obtained in this study can be used as guidelines for new retail packaging strategies for exported beef to the European market, and depending on shelf life demands, different packaging methods can be recommended for retail display. For maximum quality, steaks must be consumed shortly after retail packaging, because quality will decline during storage regardless of the choice of packaging methods. As expected, shelf life declines in the following order: Vacuum/skin pack > MAP > wrap, but taking the levels of initial psychrotrophic plate counts into account, the shelf life of wrapped and vacuum/skin packed steaks was surprisingly long, whereas shelf life of MA packed steaks was as expected.

The average temperature during shipping was 0.2°C, which gives a predicted shelf life of 79 days for beef with similar initial psychrotrophic plate counts (2.8 cfu/cm^2) as obtained in this study (Figure 6.1, left).

For beef shipped for 44 days and subsequently stored in a warehouse at 2°C, the predicted shelf life is only 69 days, which results in 25 days of residual shelf life. In this study, total shipping was 75 days, which means that most of the residual shelf life was used in warehouse storage.

Shelf life in retail storage could easily be increased, if warehouse storage was minimized, and especially for products with higher initial psychrotrophic plate counts.

For long distance distribution of Australian beef, it is significant to keep the temperature as low as possible throughout the distribution chain (Figure 6.1, right). In this study, a substantial variation between the lowest and the highest temperatures was observed in the container. During the shipping period of 44 days, the difference between the highest/lowest measured temperatures was approx. 2°C. This means that the shelf life of the meat can vary with as much as 13 days depending on the position in the container during transport (www.DMRIpredict.dk). Therefore, lowering the temperature is the most effective way to optimize shelf life of meat, and if the striploin had been stored at -0,5°C from the slaughterhouse to retail packaging, shelf life could be increased by 18 days.

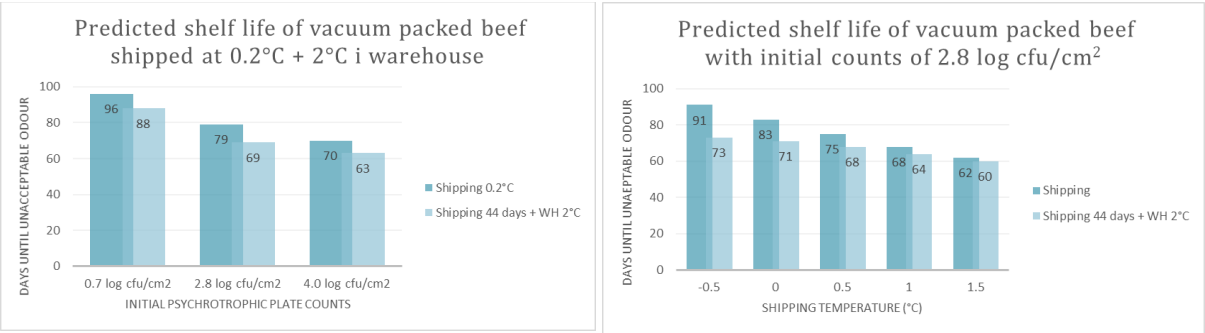


Figure 6.1. Predicted shelf life (days) of vacuum-packed beef. Left: Stored at 0.2°C with varying initial psychrotrophic plate counts. Right: With initial psychrotrophic plate counts of 2.8 log cfu/cm^2 stored at different temperatures (according to www.DMRIPredict.dk).

To optimize eating quality of MA packed beef steaks, these results suggest packaging in a low oxygen tri-gas MAP (30% O_2 + 30% CO_2 + 40% N_2) as a useful alternative to high Ox MAP. But, besides gas composition, eating quality will also depend on intrinsic factors such as microbial load, pH, fatty acid composition, antioxidant capacity, metmyoglobin reducing activity (MRA) and extrinsic factors such as packaging materials and storage conditions (time, temperature, light and headspace).

Before using these results for new retail packaging strategies, it is important to notice the limitations behind the study: For example:

- Only one set of selection criteria was used
- Only one trial was conducted
- Only 20 carcasses were used from one slaughterhouse
- Only one set of ageing/shipping (time/temperature condition) was examined
- The experimental design for evaluating eating quality made it impossible to compare treatments directly during storage, as storage times differed between packaging methods

Furthermore, it is yet to be documented if European consumers can differentiate between beef re-packed in different retail packaging systems, and if non-oxygen packaging is still preferred over MAP (Aaslyng, 2010), if tri-gas MAP is an option Nevertheless, these results are an important contribution to the growing knowledge pool of how tri-gas MAP can be used as a successful preservation strategy for fresh meat.

7.0 CONCLUSIONS/RECOMMENDATIONS

Traditional wrap packing of steaks is the optimal way to preserve the organoleptic characteristics of exported Australian beef in retail display. For longer shelf life, modified atmosphere packaging is an option, and this study has shown that the gas composition in MA packed beef steaks can be modified from high Ox MAP to tri-gas MAP, thereby optimizing the eating quality without compromising the shelf life. The longest shelf life is obtained if steaks are packed in vacuum/skin pack, but significant quality changes will occur during storage, regardless of the packaging method used.

Organoleptic evaluation of the raw meat shows that shelf life of retail packed beef steaks is approx. 6-7 days in wrap, 8-9 days in MAP (regardless of gas composition) and 17 days in vacuum/skin pack. In general, shelf life is determined by the raw meat odour because this parameter reaches an unacceptable level before surface discolouration.

The colour stability of the fresh meat is shown to be three times as long when steaks are packed in non-oxygen vacuum/skin packaging (approx. 27 days) compared to MAP and wrap packed meat (7-9).

Eating quality is impaired by the oxygen levels in the traditional high Ox MAP. To optimize odour, flavour and appearance (PMB), tri-gas MAP of wrap packed meat can be used. For optimizing tenderness and juiciness, steaks must be wrap packed, because MAP and vacuum/skin packing results in decreased texture characteristics at the end of the shelf life.

Recommendations: To obtain minimal quality changes of the eating quality of long time distribution of exported Australian beef, it is recommended to pack steaks in wrap and store the meat for up to 3 days at 5°C. These packaging conditions will preserve the fresh meat characteristics of odour and flavour (roasted meat, metallic and acid) and minimise development of off-flavours due to oxidation and spoilage. If longer shelf life is needed, modified atmosphere packaging in low oxygen tri-gas MAP (30% O_2, 30% CO_2 + 40% N_2) is recommended, because development of rancid flavour and odour is significantly reduced compared to the use of traditional high Ox MAP.

8.0 BIBLIOGRAPHY

BAO, Y., PUOLANNE, E. & ERTBJERG, P. 2016. Effect of oxygen concentration in modified atmosphere packaging on color and texture of beef patties cooked to different temperatures. Meat Science, 121, 189-195.

CAYUELA, J. M., GIL, M. D., BANON, S. & 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.

CLAUSEN, I., JAKOBSEN, M., ERTBJERG, P. & MADSEN, N. 2009. Modified atmosphere packaging affects lipid oxidation, myofibrillar fragmentation index and eating quality of beef. Packaging Technology and Science, 22, 85-96.

ESMER, O., IRKIN, R., DEGIRMENCIOGLU, N. & DEGIRMENCIOGLU, A. 2011. The effects of modified atmosphere gas composition on microbiological criteria, color and oxidation values of minced beef meat. Meat Science, 88, 221-226.

ESTÉVEZ, M. 2011. Protein carbonyls in meat systems: A review. Meat Science, 89, 259-279.

GAMMARIELLO, D., INCORONATO, A. L., CONTE, A. & NOBILE, M. A. D. 2015. Use of sodium lactate and modified atmosphere packaging for extending the shelf life of ready-to-cook fresh meal. Packaging Technology and Science, 28, 101-112.

GROBBEL, J. P., DIKEMAN, M. E., HUNT, M. C. & MILLIKEN, G. A. 2008. Effects of packaging atmospheres on beef instrumental tenderness, fresh color stability, and internal cooked color. Journal of Animal Science. 2008, 86(5), 1191-1199.

JOHN, L., CORNFORTH, D., CARPENTER, C. E., SØRHEIM, O., PETTEE, B. C. & WHITTIER, D. 2005. Color and thiobarbituric acid values of cooked top sirloin steaks packaged in modified atmospheres of 80% oxygen, or 0,4% carbon monoxide, or vacuum. Meat Science, 69, 441-449.

KIM, Y. H., HUFF-LONERGAN, E., SEBRANEK, J. G. & LONERGAN, S. M. 2010. High-oxygen modified atmosphere packaging system induces lipid and myoglobin oxidation and protein polymerization. Meat Science, 85, 759-767.

LAGERSTEDT, S., LUNDSTRÖM, K. & LINDAHL, G. 2011. Influence of vacuum or high-oxygen modified atmosphere packaging on quality of beef M. longissimus dorsi steaks after different ageing times. Meat Science, 87, 101-106.

LUND, M. N., LAMETSCH, R., HVIID, M. S., JENSEN, O. N., & 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.

MACHLIK, S. M. 1965. The effect of heat on bovine myoglobin derivatives in model systems and in beef semitendinosus muscle. Ph.D. dissertation, Purdue Univ., West Lafayette, IN

MUHLISIN, PANJONO, DONG SOO, K., YEONG RAE, S., SUNG-JIN, L., JEONG KOO, L. & SUNG KI, L. 2014. Effects of gas composition in the modified atmosphere packaging on the shelf-life of Longissimus dorsi of Korean native black pigs – duroc crossbred during refrigerated storage. Asian-Australasian Journal of Animal Sciences, 27, 1157-1163.

OWCZAREK-FENDOR, A., VERMEULEN, A., BREE, I. V., ERIKSSON, M., LESCOUHIER, S., SMET, S. D., MEULENAER, B. D. & DEVLIEGHERE, F. 2014. Effect of muscle, ageing time and modified atmosphere packaging conditions on the colour, oxidative and microbiological stability of packed beef. International Journal of Food Science & Technology, 49, 1090-1098.

RESCONI, V. C., ESCUDERO, A., BELTRAN, J. A. & ET AL. 2012. Color, lipid oxidation, sensory quality, and aroma compounds of beef steaks displayed under different levels of oxygen in a modified atmosphere package. Journal of Food Science, 71, pp. 10.

SINGH, P., WANI, A. A., & SAENGERLAUB S. 2011. Understanding critical factors for the quality and shelf-life of MAP fresh meat: a review. Critical Reviews in Food Science and Nutrition, 51(2), 146-177.

TOERNGREN, M. A. 2003. Effect of packing method on colour and eating quality of beef loin steaks. 49th International Congress of Meat Science and Technology. Brazil, September. 495-496.

TØRNGREN, M. A., DARRÉ, M. T. & KRISTENSEN, L. (2013). Optimising the eating quality and shelf-life of retail pork cuts using muscle-specific three-gas MA-packaging. 59th International Congress of Meat Science and Technology (ICoMST), Izmir, Turkey.

TØRNGREN, M.A. (2014) Optimising eating quality and shelf life of enhanced and marinated pork chops using three-gas MAP. 60th International Congress of Meat Science and Technology, 17-22nd August 2014, Punta Del Este, Uruguay.

TØRNGREN, MA & DARRÉ, M, 2015. Three-gas MAP is optimizing quality and shelf life of retail packed beef steaks. 61st International Congress of Meat Science and Technology, 23-28th August 2015, Clermont-Ferrand, France.

TØRNGREN, MA, SPANOS, D, BARON, CP, CHRISTENSEN, M & HEDEDAL HOFER, L 2016, 'Enhancing Crispiness of Pork Belly by Tri-gas MAP'. 62nd International Congress of Meat Science and Technology (ICoMST), 14-19th August 2016, Bangkok, Thailand.

WARREN, K. E., HUNT, M. C. & KROPF, D. H. 1996. Myoglobin oxidative state affects internal cooked color development in ground beef patties. Journal of Food Science, 61, 513-515.

XIAOYIN, Y., LEBAO, N., LIXIAN, Z., RONGRONG, L., YIMIN, Z. & XIN, L. 2016. Shelf-life extension of chill-stored beef longissimus steaks packaged under modified atmospheres with 50% O_2 and 40% CO_2. Journal of Food Science, 81, C1692-C1698.

ZAKRYS-WALIWANDER, P. I., O'SULLIVAN, M. G., WALSH, H., ALLEN, P. & KERRY, J. P. 2011. Sensory comparison of commercial low and high oxygen modified atmosphere packed sirloin beef steaks. Meat Science, 88, 198-202.

AASLYNG, M. D., TØRNGREN, M. A. & MADSEN, N. T. 2010. Scandinavian consumer preference for beef steaks packed with or without oxygen. Meat Science, 85, 519-524.

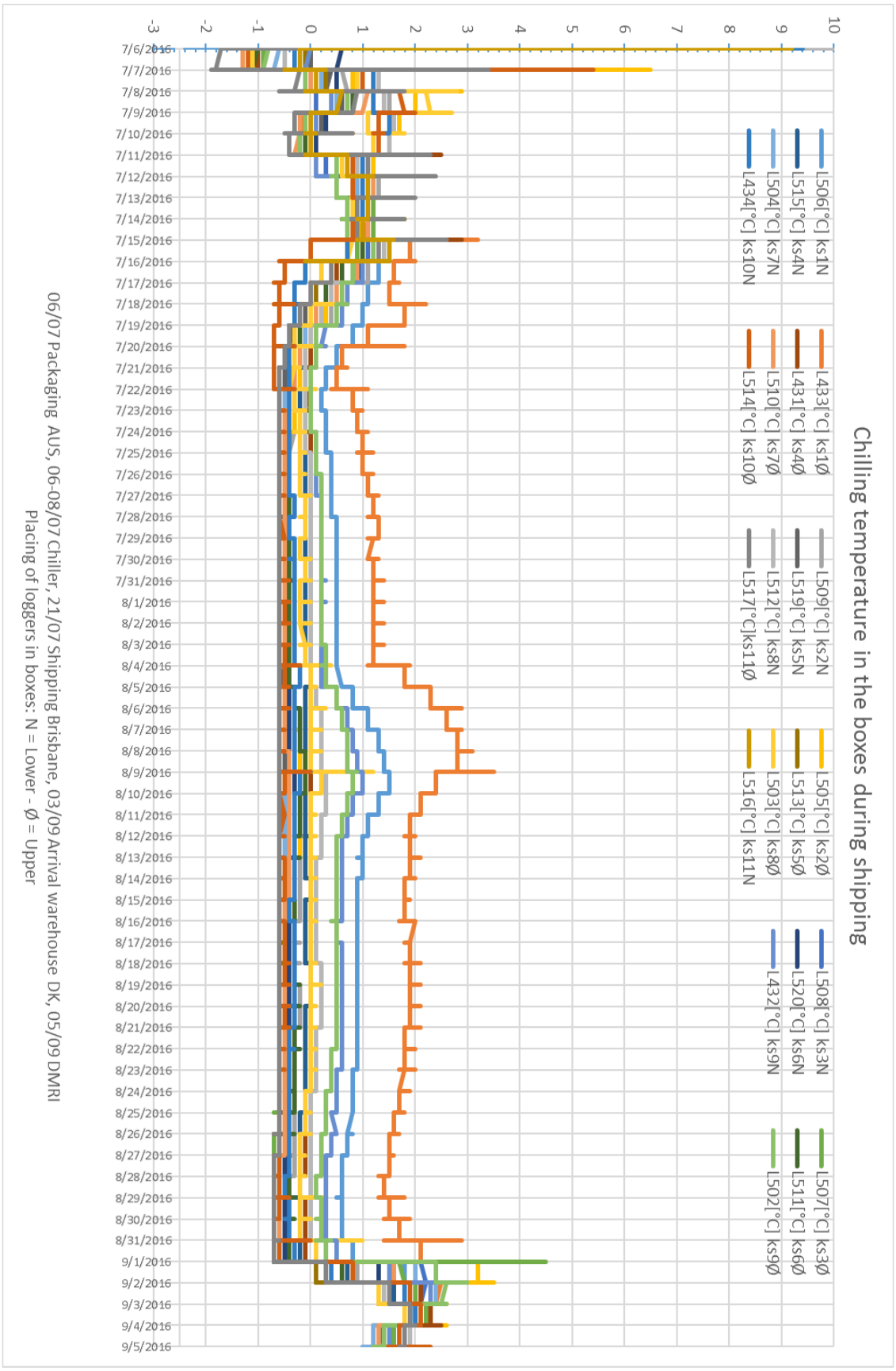
9.0 APPENDICES

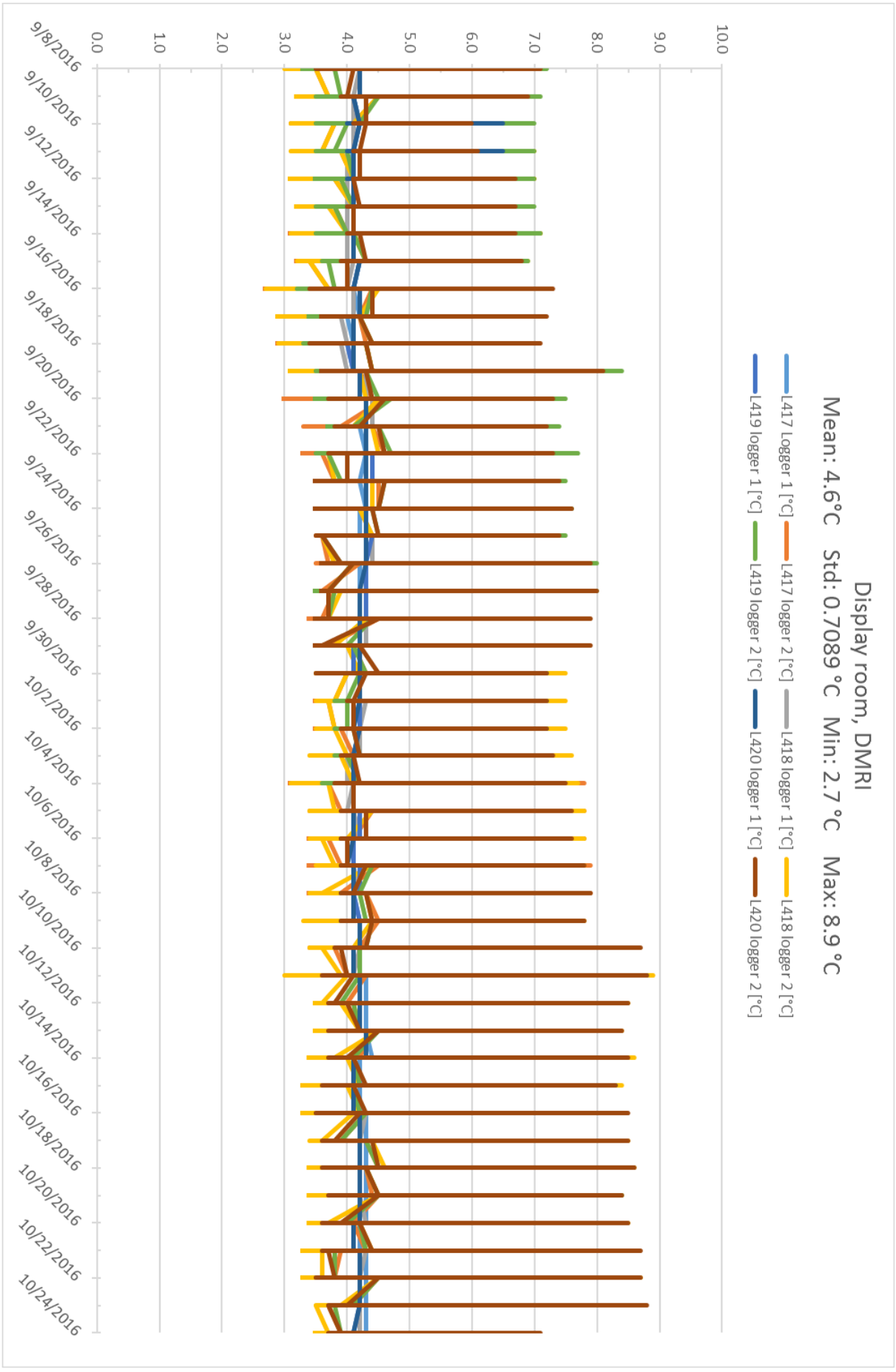
This section includes supporting documentation which has been referenced in the report.

9.1 Appendix 1 – Selected carcasses

DMRI No	Slaughter date	Body No	Side	Sex	Cattle type	Feed	Carcass weight	Graded weight	Category statutory	Category company	Denti-tion	Fat colour	Ausmeat marbling	Meat colour	MSA marbling	AUS pH	DMRI pH
1	20160705	143	1	M	Ox/Str	GRASS FED		162.6	YGS	YGS	2	2	0	2	230	5.5	5.69
1	20160705	143	2	M	Ox/Str	GRASS FED	322.2	159.6	YGS	YGS	2	2	0	2	230	5.5	5.69
2	20160705	78	1	M	Ox/Str	GRASS FED		158.6	YS	YGS	0	3	1	3	370	5.52	5.75
2	20160705	78	2	M	Ox/Str	GRASS FED	315.6	157	YS	YGS	0	3	1	3	370	5.52	5.75
3	20160705	145	1	M	Ox/Str	GRASS FED		153.4	YPS	YPS	4	2	1	2	330	5.55	5.67
3	20160705	145	2	M	Ox/Str	GRASS FED	307.4	154	YPS	YPS	4	2	1	2	330	5.55	5.67
4	20160705	147	1	M	Ox/Str	GRASS FED		159.4	YPS	YPS	4	2	0	3	270	5.59	5.75
4	20160705	147	2	M	Ox/Str	GRASS FED	318.2	158.8	YPS	YPS	4	2	0	3	270	5.59	5.75
5	20160705	158	1	M	Ox/Str	GRASS FED		158.6	YGS	YGS	2	2	0	2	230	5.6	5.75
5	20160705	158	2	M	Ox/Str	GRASS FED	315.2	156.6	YGS	YGS	2	2	0	2	230	5.6	5.75
6	20160705	139	1	M	Ox/Str	GRASS FED		169.4	YPS	YPS	4	3	1	3	310	5.64	5.72
6	20160705	139	2	M	Ox/Str	GRASS FED	336.2	166.8	YPS	YPS	4	3	1	3	310	5.64	5.72
7	20160705	138	1	M	Ox/Str	GRASS FED		164.6	YGS	YGS	2	3	1	3	380	5.66	5.81
7	20160705	138	2	M	Ox/Str	GRASS FED	329.6	165	YGS	YGS	2	3	1	3	380	5.66	5.81
8	20160705	148	1	M	Ox/Str	GRASS FED		165.8	YPS	YPS	4	3	0	4	200	5.72	5.8
8	20160705	148	2	M	Ox/Str	GRASS FED	327.2	161.4	YPS	YPS	4	3	0	4	200	5.72	5.8
9	20160705	121	1	M	Ox/Str	GRASS FED		163.8	YGS	YGS	2	3	2	4	400	5.74	5.83
9	20160705	121	2	M	Ox/Str	GRASS FED	327	163.2	YGS	YGS	2	3	2	4	400	5.74	5.83
10	20160705	133	1	M	Ox/Str	GRASS FED		157.6	YPS	YPS	4	3	1	4	380	5.78	5.76
10	20160705	133	2	M	Ox/Str	GRASS FED	315.8	158.2	YPS	YPS	4	3	1	4	380	5.78	5.76
11	20160705	137	1	M	Ox/Str	GRASS FED		160.8	YGS	YGS	2	3	0	4	180	5.79	5.75
11	20160705	137	2	M	Ox/Str	GRASS FED	321.2	160.4	YGS	YGS	2	3	0	4	180	5.79	5.75
12	20160705	152	1	M	Ox/Str	GRASS FED		162	YPS	YPS	3	2	1	3	330	5.55	5.81
12	20160705	152	2	M	Ox/Str	GRASS FED	322.8	160.8	YPS	YPS	3	2	1	3	330	5.55	5.81
13	20160705	153	1	M	Ox/Str	GRASS FED		155.2	YPS	YPS	4	3	1	4	340	5.73	5.77
13	20160705	153	2	M	Ox/Str	GRASS FED	312.2	157	YPS	YPS	4	3	1	4	340	5.73	5.77
14	20160705	155	1	M	Ox/Str	GRASS FED		157	YGS	YGS	2	2	1	3	320	5.68	5.8
14	20160705	155	2	M	Ox/Str	GRASS FED	317.8	160.8	YGS	YGS	2	2	1	3	320	5.68	5.8
15	20160705	123	1	M	Ox/Str	GRASS FED		163.8	YGS	YGS	2	3	0	3	200	5.64	5.7
15	20160705	123	2	M	Ox/Str	GRASS FED	327.8	164	YGS	YGS	2	3	0	3	200	5.64	5.7
16	20160705	126	1	M	Ox/Str	GRASS FED		150.2	YPS	YPS	4	3	1	4	320	5.76	6.01
16	20160705	126	2	M	Ox/Str	GRASS FED	299.6	149.4	YPS	YPS	4	3	1	4	320	5.76	6.01
17	20160705	128	1	M	Ox/Str	GRASS FED		162.4	YPS	YPS	4	2	1	3	300	5.6	5.7
17	20160705	128	2	M	Ox/Str	GRASS FED	323.8	161.4	YPS	YPS	4	2	1	3	300	5.6	5.7
18	20160705	130	1	M	Ox/Str	GRASS FED		157.6	YGS	YGS	2	3	1	2	390	5.51	5.65
18	20160705	130	2	M	Ox/Str	GRASS FED	312.4	154.8	YGS	YGS	2	3	1	2	390	5.51	5.65
19	20160705	132	1	M	Ox/Str	GRASS FED		164.8	YPS	YPS	4	3	1	3	370	5.55	5.75
19	20160705	132	2	M	Ox/Str	GRASS FED	325.6	160.8	YPS	YPS	4	3	1	3	370	5.55	5.75
20	20160705	142	1	M	Ox/Str	GRASS FED		158.4	YPS	YPS	4	3	1	3	320	5.6	5.8
20	20160705	142	2	M	Ox/Str	GRASS FED	316.4	158	YPS	YPS	4	3	1	3	320	5.6	5.8
21	20160705	157	1	M	Ox/Str	GRASS FED		159.8	YGS	YGS	2	3	1	4	310	5.79	
21	20160705	157	2	M	Ox/Str	GRASS FED	320.2	160.4	YGS	YGS	2	3	1	4	310	5.79	
22	20160705	156	1	M	Ox/Str	GRASS FED		163.8	YGS	YGS	2	2	0	4	200	5.81	
22	20160705	156	2	M	Ox/Str	GRASS FED	327.8	164	YGS	YGS	2	2	0	4	200	5.81	

9.2 Appendix 2 – Temperature logging during shipping and retail storage




Display room, DMRI
Mean: 4.6°C Std: 0.7089 °C Min: 2.7 °C Max: 8.9 °C
L417 Logger 1 [°C]
L417 logger 2 [°C]
L418 logger 1 [°C]
L418 logger 2 [°C]
L419 logger 1 [°C]
L419 logger 2 [°C]
L420 logger 1 [°C]
L420 logger 2 [°C]
10.0
9.0
8.0
7.0
6.0
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9/8/2016
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10/20/2016
10/22/2016
10/24/2016

9.3 Appendix 3 – Psychrotrophic plate count (NMKL procedure)

Nr. 86 5. utgave 2013	NORDISK METODIKKOMITÉ FOR NÆRINGSMIDLER NORDIC COMMITTEE ON FOOD ANALYSIS	No 86 5th Ed. 2013

Aerobe mikroorgansimer. Bestemmelse i næringsmidler ved 37 °C, 30 °C, 25 °C, 20 °C, 17/7 °C eller 6,5 °C efter kolonitalsmetoden.

1. INTRODUKSJON

Denne NMKL-metoden erstatter følgende NMKL-metoder:

- Nr. 86, 4 utg., 2006: Aeroba mikroorganismer. Bestämning i livsmedel.
- Nr. 74, 3. utg., 2000: Psykrotrofe mikro-organismer. Bestemmelse ved kolonitals-metoden.

2. ENDRINGER

I denne udgave af NMKL 86 er tilføjet flg. Dyrkningstemperaturer i forhold til udgave 4: 37 °C, 25°C og 17/7 °C.

3. FORMÅL OG ANVENDELSESOMRÅDE

Med metoden bestemmes antallet af levende aerobe mikroorganismer i næringsmidler.

Det aerobe kimtal kan nogle gange indikere kvalitet og fordærvelsesgrad af et produkt.

Metoden kan anvendes for alle typer næringsmidler. For fisk og fiskvarer anbefales dog NMKL 184.

Kimtal benyttes ofte til overvågning af hygiejne ved

Aerobic microorganisms. Determination in foods at 37 °C, 30 °C, 25 °C, 20 °C, 17/7 °C or 6.5 °C by the colony count method.

1. INTRODUCTION

This NMKL method replaces the following NMKL methods:

- No 86, 4rd Ed., 2006: Aerobic microorganisms. Determination in foods.
- No 74, 3rd Ed., 2000: Psychrotrophic micro-organisms. Determination by the colony count method.

2. ADDITIONS

To this edition of NMKL 86, the following incubation temperatures have been added: 37°C, 25°C and 17/7 °C.

3. SCOPE AND FIELD OF APPLICATION

The method is suitable for determining the number of viable aerobic microorganisms in foods.

The aerobic count can sometimes be used to indicate the quality and spoilage level of the product.

The method can be applied to all kinds of foods. For aerobic count in fish and fish products, see NMKL 184.

Aerobic count is often used as a surveillance of

levnedsmiddelproduktion. Kimtallet i en fødevare kan hjælpe til at bedømme hygiejnen under produktion.

Til overvågning er det vigtigt at benytte samme metode hver gang analysen udføres.

Andre inkubationstider og -temperaturer end de i denne standard nævnte kan dog benyttes, hvis temperaturen er den samme i en overvågning og især hvis virksomheden har en erfaring med en anden tid/temperaturkombination.

hygiene in production of food. The number of microorganisms in a food product will aid in evaluating sanitary practices during processing and handling.

When doing surveillance it is important to use exactly the same conditions every time the analysis is performed.

It might though be possible to use other incubation temperatures and incubation times than the ones standardized in this standard as long as the temperature is kept the same in one surveillance and especially if a business has an experience with another time/temperature combination.

4. DEFINISJONER

4.1 Aerobe mikroorganismer:
Mikroorganismer som vokser under aerobe forhold når analysen utføres etter den beskrevne metoden.

4.2 Psykrotrofe mikroorganismer:
Aerobe mikroorganismer som har evnen til relativt hurtig vekst ved temperaturer mellom 0 og 10 °C. Psykrotrofe bakterier har ofte betydelig enzymatisk aktivitet.

4.3 Antal aerobe (eller psykrotrofe) mikroorganismer:
Antalet levende aerobe mikroorganismer som finns per gram (eller ml) i næringsmiddel når analysen utføres etter den beskrevne metoden.

4. DEFINITIONS

4.1 Aerobic microorganisms:
Microorganisms, growing under aerobic conditions when the test is carried out according to the method described.

4.2 Psychrotrophic microorganisms
Aerobic microorganisms capable of relatively rapid growth at temperatures between 0 and 10 °C. Psychrotrophic microorganisms usually have considerable enzymatic activity.

4.3 Aerobic (or psychrotrophic) colony count:
The number of viable aerobic microorganisms found per gram (or ml) of food providing that the test is carried out in accordance with the method described.

5. REFERANSER

5.1 NMKL-procedure nr. 23 2008: Handledning i kvalitetssäkring för mikrobiologiska laboratorier.

5.2 NMKL-metode nr. 91, 5. udgave 2010: Prøvetagning og forbehandling af levnedsmidler og foderstoffer til kvantitativ mikrobiologisk undersøgelse.

5.3 NMKL-protokol nr. 2: 2006: Harmonisering af mikrobiologiske metoder. Model for udarbejdelse af mikrobiologiske metoder i NMKL.

5.4 NMKL-prosedyre nr. 12, 2002: Håndbok i prøvetaking av næringsmidler.

5. REFERENCES

5.1 NMKL Procedure No. 23, 2008: Quality Assurance Guidelines for Microbiological Laboratories.

5.2 NMKL Method No. 91, 5th ed. 2010: Sampling and pre-treatment of foods and animal feedstuffs for quantitative microbiological examination.

5.3 NMKL Protocol No. 2, 2006: Harmonization of microbiological methods. Template for the preparation of microbiological methods in NMKL.

5.4 NMKL Procedure No. 12, 2002: Guide on sampling for analysis of foods.

5.5 ISO 4833, 2003: Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of microorganisms -- Colony-count technique at 30 °C.

5.6 ISO 6730, 2005 (IDF 101:2005) Milk -- Enumeration of colony-forming units of psychrotrophic micro-organisms -- Colony-count technique at 6,5 °C.

6. PRINSIPP

Aerobe mikroorganismer bestemmes ved å gjøre i stand en fortynningsrekke av prøvematerialet etter vanlige mikrobiologiske prinsipper hvorpå det foretas innstøpning i et agarmedium i petriskåler.

Inkubering foretas under aerobe forhold ved en av de følgende temperaturer: 37 °C for 3 døgn, 30 °C i 3 døgn, 25 °C i 3 døgn, 20 °C i 3 døgn, 17°C i 1 døgn efterfulgt af 7°C i 3 døgn eller 6,5 °C i 10 døgn.

Antallet levende aerobe mikroorganismer per milliliter eller gram prøve beregnes fra telte kolonier på utvalgte petriskåler.

7. SIKKERHETSANVISNINGER

Der er ingen sikkerhetsanvisninger for denne metode.

8. FORTYNNINGSVÆSKE OG SUBSTRATER

8.1 Fortyndingsvæske

Fortynningsvæske tillages i henhold til prosedyrer beskrevet i NMKL 91 (5.2).

8.2 Substrater

8.2.1 Plate Count Agar [1)]

Trypsinhydrolysert kasein	5,0 g
Gjærekstrakt	2,5 g
Glukose	1,0 g
Agar	15,0 g
Destillert eller likeverdig vann	1000 ml

5.5 ISO 4833, 2003: Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of microorganisms -- Colony-count technique at 30 °C.

5.6 ISO 6730, 2005 (IDF 101:2005) Milk -- Enumeration of colony-forming units of psychrotrophic micro-organisms -- Colony-count technique at 6.5 °C.

6. PRINCIPLE

The aerobic plate count is determined by preparing a dilution series of the sample material according to general microbiological principles, followed by pour-plating into an agar medium in Petri dishes.

Incubate sample(s) under aerobic conditions at either 37 °C for 3 days, 30 °C for 3 days, 25 °C for 3 days, 20 °C for 3 days, 17°C for 1 day followed by 7°C for 3 days or 6.5 °C for 10 days.

The number of viable aerobic microorganisms per millilitre or gram of sample is calculated from the number of colonies counted on selected plates.

7. SAFETY PRECAUTION

There are no safety instructions concerning this method.

8. DILUTENT AND SUBSTRATES

8.1 Diluent

Diluent is prepared according to procedures described in NMKL 91 (5.2).

8.2 Substrates

8.2.1 Plate Count Agar [1)]

Tryptic hydrolysate of casein	5.0 g
Yeast extract, powder	2.5 g
Glucose	1.0 g
Agar	15.0 g
Distilled or equivalent water	1000 ml

[1] Tilsett 1,0 g skummetmelkpulver per liter dyrkningsmedium når melk og melkeprodukter skal analyseres. Skummetmelkpulveret skal være fritt for hemmende stoffer.

Løs enkeltkomponentene, eller det komplette dehydrerte mediet, i vannet ved koking. Juster pH slik at mediet etter autoklavering har pH 7,0 ± 0,2 målt ved 20 – 25 °C.

Fordel dyrkningsmediet på flasker. Autoklaver ved 121 ± 2,0 °C i 15 min.

[1] When dairy products are examined, add 1.0 g of skimmed milk powder per litre of the culture medium. The skimmed milk powder shall be free from inhibitory substances.

Dissolve the ingredients, or the dehydrated complete medium, in the water by bringing to boiling point. Adjust the pH to 7.0 ± 0.2 at 20 – 25 °C after sterilization.

Transfer the culture medium to flasks. Sterilize in an autoclave for 15 min at 121 ± 2.0 °C.

9. APPARATUR / UTSTYR

Se også NMKL 91 (5.2) og NMKL procedure nr. 23 (5.1).

9.1 Inkubatorer: 37,0 ± 1,0 °C, 30,0 ± 1,0 °C; 25,0 ± 1,0 °C; 20,0 ± 1,0 °C; 17,0 ± 1,0 °C; 7,0 ± 1,0 °C og 6,5 ± 1,0 °C

9.2 Vannbad, 45,0 ± 1,0 °C

9.3 Koloniteller, med belysning, mørk bakgrunn, forstørrelseslinse og mekanisk eller elektronisk telleverk.

9. APPARATUS / EQUIPMENT

See also NMKL 91 (5.2) and NMKL procedure no 23 (5.1).

9.1 Incubators, 37,0 ± 1,0 °C; 30.0 ± 1.0 °C; 25,0 ± 1,0 °C; 20.0 ± 1.0 °C; 17,0 ± 1.0 °C; 7.0 ± 1.0 °C and 6.5 ± 1.0 °C

9.2 Water bath, 45.0 ± 1.0 °C

9.3 Colony counter, with light, dark background, magnifying lens and mechanical or electronic digital counter.

10. PRØVETAKING

Prøvetaking bør foretas i henhold til etablerte mikrobiologiske prosedyrer som beskrevet i NMKL 91 (5.2) og NMKL prosedyre nr 12 (5.4).

10. SAMPLING

Sampling should be carried out according to established microbiological procedures as described in NMKL 91 (5.2) and NMKL Procedure no 12 (5.4).

11. PROSEDYRE

11.1 Forbehandling og fortynning

Forbehandling og fortynning bør utføres i henhold til etablerte mikrobiologiske prosedyrer som beskrevet i NMKL 91 (5.2), NMKL-prosedyre nr. 12 (5.4) og NMKL-procedure nr. 23 (5.1).

11. PROCEDURE

11.1 Pretreatment and dilution

Pretreatment and dilution should be carried out according to established microbiological procedures as described in NMKL 91 (5.2), NMKL Procedure no. 12 (5.4) and NMKL Procedure no. 23 (5.1).

11.2 Utsæd

Overfør 1 ml av en ufortynnet prøve, og/eller

11.2 Inoculation

Transfer 1 ml of the homogenate and/or suitable

passende fortynninger av denne, i en tom steril petriskål. Tilsett straks 15-20 ml smeltet, temperert (45,0 ± 1,0 °C) agar (8.2) i hver petriskål. Bland agar og prøve som beskrevet i NMKL procedure nr 23 (5.1). For analyse for psykrotrofe kim ved 6.5 °C kan der benyttes overfladeudsæd på en i forvejen støbt Plate Count Agar.

Plasser petriskålene horisontalt inntil innholdet har stivnet.

11.3 Inkubering

Inkuber inokulerte petriskåler med bunnen opp i en inkubator ved én av de angitte temperaturer:

- 37,0 ± 1,0 °C i 72 ± 6 timer
- 30,0 ± 1,0 °C i 72 ± 6 timer
- 25,0 ± 1,0 °C i 72 ± 6 timer
- 20,0 ± 1,0 °C i 72 ± 6 timer
- 17,0 ± 1,0 °C i 20 ± 3 timer, efterfulgt af inkubation ved 7,0 ± 1,0 °C i 72 ± 6 timer
- 6,5 ± 1,0 °C i 10 døgn

Skålene kan gemmes 2 dage på køl efter endt inkubation og før aflæsning.

11.4 Avlesning

Velg om mulig skåler med 25-250 kolonier og tell disse. Hvis der kun er skåle med under 25 kolonier tælles disse. Bruk koloniteller (9.3) eller lupe ved tellingen. Vær opmærksom på, at der kan forekomme produktpartikler, som kan ligne kolonier.

12. BEREGNING AV RESULTATER

12.1 Antall aerobe mikroorganismer

Dersom kun en skål inneholder 25-250 kolonier beregnes antallet mikroorganismer per gram (eller milliliter) prøve ved å multiplisere kolonitallet med fortynningsfaktoren (10, 10^2, 10^3, 10^4, 10^5 osv).

Dersom to skåler fra to påfølgende fortynninger inneholder 25-250 kolonier, beregnes en vektet middelverdi av antallet telte kolonier som beskrevet i NMKL procedure nr 23, afsnit 11 (5.1).

13. RAPPORTERING AV RESULTAT

Angi antallet aerobe mikroorganismer per 1 gram

dilutions of the homogenate into empty sterile Petri dishes. Immediately after, pour into each dish 15 to 20 ml of the melted culture medium (8.2) cooled to 45.0 ± 1.0 °C. Mix the contents of the Petri dishes as described in NMKL procedure no 23 (5.1). When analysing for aerobic count with incubation at 6.5 °C surface inoculation may be used.

Make sure that the dishes are in a horizontal position while the mixture is solidifying.

11.3 Incubation

Incubate the inverted Petri dishes at one of the mentioned temperatures:

- 37.0 ± 1.0 °C for 72 ± 6 hours
- 30.0 ± 1.0 °C for 72 ± 6 hours
- 25.0 ± 1.0 °C for 72 ± 6 hours
- 20.0 ± 1.0 °C for 72 ± 6 hours
- 17.0 ± 1.0 °C for 20 ± 3 hours, followed by 7.0 ± 1.0 °C for 72 ± 6 hours
- 6.5 ± 1.0 °C for 10 days

The plates can be kept refrigerated for 2 days after incubation and before reading.

11.4 Reading

Choose, if possible, plates with 25-250 colonies and count these. If there are only plates with less than 25 colonies, these are counted. Use a colony counter (9.3) or a pocket lens for the counting. Pay attention to the fact that product particles can look similar to colonies.

12. CALCULATION OF THE RESULTS

12.1 Number of aerobic microorganisms

If only one plate contains 25-250 colonies, calculate the number of microorganisms per gram (or millilitre) of the sample by multiplying the number of colonies by the dilution factor (10, 10^2, 10^3, 10^4, 10^5 etc.).

If plates from two successive dilutions contain 25-250 colonies, calculate the weighted mean value of the bacterial count according to NMKL procedure no 23, section 11 (5.1).

13. EXPRESSION OF THE RESULT

Report the counted number of aerobic

(eller 1 milliliter) prøve med to signifikante siffer, f.eks. 7,9 x 10^4 cfu/g.

Alternativt kan aerobe mikroorganismer dyrket ved 6,5 ± 1,0 °C og 17/7 °C angis som psykrotrofe mikroorganismer.

Angi valgt tid og inkuberingstemperatur for analysen.

microorganisms per 1 gram (or 1 millilitre) of the sample, using two significant figures, e.g. 7.9 x 10^4 cfu/g.

Alternatively aerobic microorganisms incubated at 6.5 ± 1.0 °C and 17/7 °C can be reported as psychotrophic microorganisms.

Specify the time and temperature of incubation used in the analysis.

14. REFERENT

Denne reviderte NMKL-metode er utarbeidet av: Majbritt Moos, Eurofins Steins, Hjaltesvej 8, DK-7500 Holstebro. E-postadresse: dmm4@eurofins.dk

14. REFEREE

This revised NMKL-method has been elaborated by: Majbritt Moos, Eurofins Steins, Hjaltesvej 8, DK-7500 Holstebro. E-mail: dmm4@eurofins.dk.

9.4 Appendix 4 – Form for organoleptic shelf life evaluation

Date:_____ Day:_____ Assessor (Initials):_____

Sample ID+ Carcass no.	Raw meat odour		Raw meat visual appearance		Acceptable	
	MARK	Note	MARK	Note	YES	NO

Raw meat odour:

5. **Fresh**
6. **Slightly diverging, but acceptable**
7. **Diverging to an unacceptable degree**
8. **Putrid/Rotten**

Visual appearance:

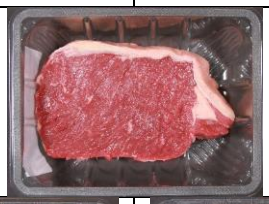
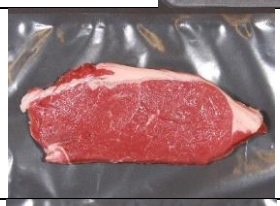
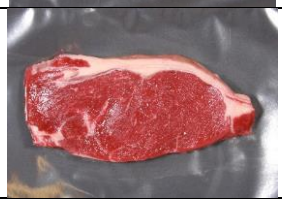
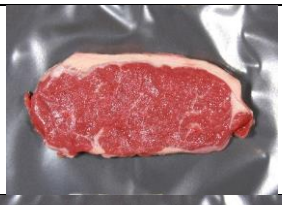
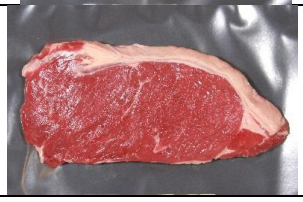
5. **No discolouration**
6. **Slightly discoloured, but acceptable**
7. **Discoloured to an unacceptable degree**
8. **Very discoloured**

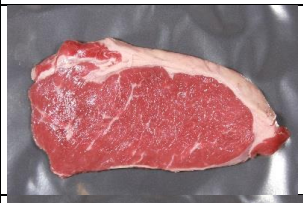
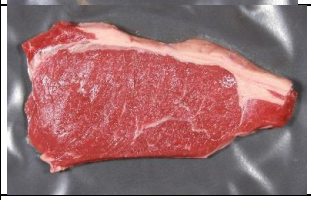
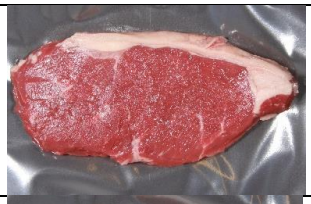
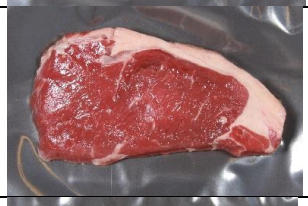
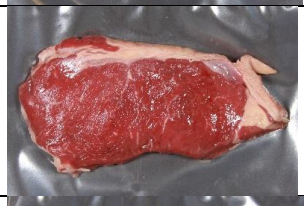
9.5 Appendix 5 – Overview of data from organoleptic shelf life evaluation

ODOUR 30 min		1	2	3	4		ODOUR 30 min		1	2	3	4	Accept	Unaccept
Day 0	0	25	0	0	0	25	Day 0	0	100	0	0	0	100	0
O2-30%, CO2-30%, N2-40%	4	10	9	6	0	25	O2-30%, CO2-30%, N2-40%	4	40	36	24	0	76	24
	7	4	20	1	0	25		7	16	80	4	0	96	4
	9	0	16	4	0	20		9	0	80	20	0	80	20
	10	0	5	17	3	25		10	0	20	68	12	20	80
	14	0	0	16	9	25		14	0	0	64	36	0	100
	15	0	6	17	2	25		15	0	24	68	8	24	76
	18	0	0	16	9	25		18	0	0	64	36	0	100
	21	0	0	12	13	25		21	0	0	48	52	0	100
O2-70%, CO2-30%	4	10	11	4	0	25	O2-70%, CO2-30%	4	40	44	16	0	84	16
	7	5	14	6	0	25		7	20	56	24	0	76	24
	9	0	11	9	0	20		9	0	55	45	0	55	45
	10	0	5	20	0	25		10	0	20	80	0	20	80
	14	0	2	17	6	25		14	0	8	68	24	8	92
	15	0	3	11	11	25		15	0	12	44	44	12	88
	18	0	0	16	9	25		18	0	0	64	36	0	100
	21	0	0	0	25	25		21	0	0	0	100	0	100
Vacuum/ skinpack	4	16	3	6	0	25	Vacuum/ skinpack	4	64	12	24	0	76	24
	10	21	4	0	0	25		10	84	16	0	0	100	0
	14	5	19	1	0	25		14	20	76	4	0	96	4
	18	0	10	12	3	25		18	0	40	48	12	40	60
	25	0	7	10	3	20		25	0	35	50	15	35	65
	28	0	3	10	12	25		28	0	12	40	48	12	88
	32	0	3	4	18	25		32	0	12	16	72	12	88
	35	0	1	7	17	25		35	0	4	28	68	4	96
Wrap	3	13	12	0	0	25	Wrap	3	52	48	0	0	100	0
	4	0	20	5	0	25		4	0	80	20	0	80	20
	7	0	12	12	1	25		7	0	48	48	4	48	52
	8	0	1	18	6	25		8	0	4	72	24	4	96
	9	0	0	2	18	20		9	0	0	10	90	0	100
	10	0	0	0	25	25		10	0	0	0	100	0	100
	11	0	0	0	25	25		11	0	0	0	100	0	100
	14	0	0	5	20	25		14	0	0	20	80	0	100

APPEARANCE 30		1	2	3	4		APPEARANCE 30 min		1	2	3	4	Accept	Unaccept
Day 0	0	20	5	0	0	25	Day 0	0	80	20	0	0	100	0
O2-30%, CO2-30%, N2-40%	4	5	11	9	0	25	O2-30%, CO2-30%, N2-40%	4	20	44	36	0	64	36
	7	5	15	5	0	25		7	20	60	20	0	80	20
	9	0	0	20	0	20		9	0	0	100	0	0	100
	10	0	2	4	19	25		10	0	8	16	76	8	92
	14	0	0	7	18	25		14	0	0	28	72	0	100
	15	0	2	13	10	25		15	0	8	52	40	8	92
	18	0	0	6	19	25		18	0	0	24	76	0	100
	21	0	0	12	13	25		21	0	0	48	52	0	100
O2-70%, CO2-30%	4	20	5	0	0	25	O2-70%, CO2-30%	4	80	20	0	0	100	0
	7	1	22	2	0	25		7	4	88	8	0	92	8
	9	0	13	7	0	20		9	0	65	35	0	65	35
	10	1	8	15	1	25		10	4	32	60	4	36	64
	14	0	2	12	11	25		14	0	8	48	44	8	92
	15	0	3	9	13	25		15	0	12	36	52	12	88
	18	0	0	5	20	25		18	0	0	20	80	0	100
	21	0	0	0	25	25		21	0	0	0	100	0	100
Vacuum/ skinpack	4	25	0	0	0	25	Vacuum/ skinpack	4	100	0	0	0	100	0
	10	15	10	0	0	25		10	60	40	0	0	100	0
	14	25	0	0	0	25		14	100	0	0	0	100	0
	18	5	16	4	0	25		18	20	64	16	0	84	16
	25	10	10	0	0	20		25	50	50	0	0	100	0
	28	1	5	14	5	25		28	4	20	56	20	24	76
	32	2	3	6	14	25		32	8	12	24	56	20	80
	35	0	9	11	5	25		35	0	36	44	20	36	64
Wrap	3	5	20	0	0	25	Wrap	3	20	80	0	0	100	0
	4	5	19	1	0	25		4	20	76	4	0	96	4
	7	2	15	6	2	25		7	8	60	24	8	68	32
	8	1	8	16	0	25		8	4	32	64	0	36	64
	9	0	0	5	15	20		9	0	0	25	75	0	100
	10	0	0	1	24	25		10	0	0	4	96	0	100
	11	0	0	1	24	25		11	0	0	4	96	0	100
	14	0	0	0	25	25		14	0	0	0	100	0	100

9.6 Appendix 6 – Photos of visual appearance during retail storage

Time 30 min.				
Day	30% O_2 + 30% CO_2 + 40% N_2	70% O_2 + 30% CO_2	Vacuum/skin	Wrapped
0				
3				
4				
7				
8				
9				
10				
11				

Time 30 min.				
Day	30% O_2 + 30% CO_2 + 40% N_2	70% O_2 + 30% CO_2	Vacuum/skin	Wrapped
14				
15				
18				
21				
25				
28				
32				
35				

9.7 Appendix 7 – Sensory attributes for profiling of cooked steaks

Sensory attribute	Sense	Scale	Description
Please evaluate the roasted meat odour of the whole meat sample. Then please cut the sample along the meat fibres and evaluate the other odours			
Roasted meat	Odour	weak → strong	Intensity of roasted meat odour
Metallic	Odour	weak → strong	Intensity of metallic odour
Acid	Odour	weak → strong	Intensity of acid odour
Sour	Odour	weak → strong	Intensity of sour odour, not fresh
Warmed Over/stale	Odour	weak → strong	Intensity of warmed over odour and stale
Rancid	Odour	weak → strong	Intensity of rancid odour
Please look at the internal surface of the sample			
Internal colour of the meat	Appearance	Pink --> Well done	Intensity of colour (from pink to well done)
Holes	Appearance	Few-->many	Amount of holes in the sample
Please take one of the samples into the mouth and evaluate the flavour/taste attributes			
Roasted meat	Flavour	weak → strong	Intensity of roasted meat flavour
Metallic	Taste	weak → strong	Intensity of metallic taste
Acid taste	Flavour	weak → strong	Intensity of acid taste
Sour taste	Flavour	weak → strong	Intensity of sour taste (not fresh) fresh
Bitter taste	Taste	weak → strong	Intensity of bitter taste
Warmed Over Flavour/stale	Flavour	weak → strong	Intensity of warmed over flavour/stale flavour
Rancid	Flavour	weak → strong	Intensity of rancid flavour
Please take the last sample into the mouth and evaluate the texture attributes			
Hardness at first bite	Texture	weak → strong	Hardness at first bite with the molar teeth
Juiciness	Texture	weak → strong	Amount of juice after five chews
Tenderness	Texture	weak → strong	Perception of sample disintegration during chewing
Chewing time	Texture	short → long	Time needed before the sample is ready for swallowing

9.8 Appendix 8 – Sensory profile of cooked beef steaks

	Packaging	None	MAP 30% O_2 + 30% CO_2 + 40% N_2	MAP 70% O_2 + 30% CO_2	Vacuum /skin pack	Wrap	p-values	
	Time	Day 0	Day 7	Day 7	Day 18	Day 3	Packaging	Time
Odour	ROASTED_MEAT	7.9	6.1c	4.5a	5.3b	6.5c	0.0022	0.0014
	METALLIC	4.1	2.4b	1.3a	2.9bc	3.4c	0.0006	0.0179
	ACID	4.6	2.3b	1.7a	2.3b	3.3c	0.002	0.0025
	SOUR	0.0	1.7ab	3.3c	2.3b	1.0a	0.0121	0.0003
	WOF	0.1	2.6a	4.7b	4.6b	1.8a	<0.0001	0.0186
	RANCID	0.1	1.1a	2.4b	2.2b	0.8a	0.0859	0.0037
Appea-rance	WELL_DONE	4.9	7.6c	10.4d	6.7b	4.7a	<0.0001	0.0168
	PINHOLES	0.9	2.4b	2.0b	2.1b	0.9a	0.0002	0.0007
Flavour	ROASTED_MEAT	7.7	5.6b	4.1a	4.6a	6.3b	0.0015	0.0021
	METALLIC	4.3	1.9a	1.6a	2.8b	3.6c	<0.0001	0.0168
	WOF	0.1	3.2b	5.9c	5.8c	2.0a	0.0003	<0.0001
	RANCID	0.0	1.4a	3.4b	3.8b	0.7a	0.0092	0.0036
Taste	ACID	5.1	2.7b	1.9a	2.3ab	3.6c	0.0029	0.0011
	SOUR	0.0	2.3a	4.6b	5.1b	1.2a	0.0004	0.0013
	Bitter	4.0	4.1ab	4.5b	5.5c	3.8a	0.0004	0.2252
Texture	HARDNESS	3.9	4.9bc	4.7ab	5.4c	4.3a	0.0506	0.0175
	JUCINESS	9.0	7.6a	7.7a	7.2a	8.6b	0.0985	0.0025
	TENDERNESS	8.7	7.1a	7.3ab	7.5ab	7.8b	0.4011	0.001
	CHEWING_TIME	4.9	6.2a	6.3a	6.3a	5.7a	0.434	0.0336