final report

Project code:	A.TEC.0052
Prepared by:	Alison Small, Anita Sikes, Danielle O'Callaghan
	Food Science Australia
Date submitted:	June 2008
Date published:	April 2011

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Development of a deep chilling process for beef and sheepmeat

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

CONTENTS

EXECUTIVE SUMMARY

The limited storage life of vacuum packaged beef and lamb is often the major factor affecting the distribution chain and can limit the versatility and market penetration of these products. Storage temperature is usually limited to a minimum of -1.5°C to prevent the meat from freezing. Chilling has been identified by industry groups as a priority area for improvement. Customer requirements and anecdotal evidence from the industry suggests that storage lives of greater than the current expectations can be achieved, but there is no scientific evidence available for processors to use in validating the process. Previous legislation defined chilled meat as being stored between -1.5°C and 4°C, but the current legislation has no such prescription.

Deep chilling, super chilling or partial freezing are terms used to describe the process of cooling a product to one or two degrees below its freezing point. It has been utilised with some muscle foods, particularly seafood, to extend the storage life of products. In some applications crust freezing occurs without any measurable effect on final product quality. In other cases a portion of the freezable water has been converted to ice.

This report covers some preliminary investigations into the effect of storage of beef at temperatures just below 0°C (namely -1.5°C, -2.5°C or -5°C) on physical, microbiological and sensory aspects, as compared with beef stored at 0°C or -20°C. A small trial was also conducted to investigate the potential of various surface treatments (drying, salting, and coating with sodium lactate, glycerol or carrageenan) to inhibit freezing of beef primals.

Beef stored at -2.5°C or -5°C demonstrates characteristics of frozen meat in terms of microbiology and drip loss. In addition, unsightly small white spots form on the surface of the cuts. Neither temperature would appear to be suitable for a deep chilling process aimed at maintaining the attributes of fresh meat. Sodium lactate appeared to limit drip loss associated with storage at -5°C, as did salting. Salting however resulted in the meat becoming dark in appearance, firm to touch and salty-smelling.

When using deepchill temperatures, slow chilling helps to prevent freezing of beef cuts. This improves the visual quality of the product by reducing the incidence of white spots and reducing the drip loss, particularly during retail display. Slow chilling to -2.5°C resulted in only 12% of cuts being frozen solid, and thus displaying these undesirable attributes.

Deepchilling is feasible, but the product must be chilled slowly to its storage temperature, and strict control over the temperature of the product must be maintained. The temperature of -1.5°C has been confirmed to be the absolute minimum temperature that could be commercially viable for vacuum packed beef, unless it has been treated with a freezing inhibitor such as sodium lactate.

1.0 INTRODUCTION

The limited storage life of vacuum packaged beef and lamb is often the major factor affecting the distribution chain and can limit the versatility and market penetration of these products. Storage temperature is usually limited to a minimum of -1.5°C to prevent the meat from freezing.

Chilling has been identified by industry groups as a priority area for improvement. Customer requirements and anecdotal evidence from the industry suggests that storage lives of greater than the current expectations can be achieved, but there is no scientific evidence available for processors to use in validating the process. Previous legislation defined chilled meat as being stored between -1.5°C and 4°C, but the current legislation has no such prescription.

Deep chilling, super chilling or partial freezing are terms used to describe the process of cooling a product to one or two degrees below its freezing point. It has been utilised with some muscle foods, particularly seafood, to extend the storage life of products. In some applications crust freezing occurs without any measurable effect on final product quality. In other cases a portion of the freezable water has been converted to ice.

Ideally, formation of ice should be prevented or avoided with a muscle food in order to retain its inherent properties. Slow freezing can result in formation of large intracellular ice crystals that cause more damage to the muscle structure resulting in higher loss of drip on thawing and poorer water holding capacity.

There is some evidence that the formation of ice crystals in meat at sub-freezing temperatures can be inhibited by the application of agents that prevent nucleation of crystals. Vacuum packaging alone may be sufficient to prevent freezing at slight sub-freezing temperatures.

2.0 BACKGROUND

2.1 What is deep chilling?

Deep chilling, also referred to as superchilling or partial freezing, involves cooling a food to a temperature usually between 1°C and 2°C below the initial freezing point (Duun and Rustad 2007). This partial freezing with low ice content ensures the food stays tasting fresh and structural disintegration is less likely to occur.

If muscle is frozen slowly, large ice crystals form which damage muscle structure. Fast and controlled superchilling just below freezing point will only freeze the loosely bound water. The amount of ice crystals that form will depend on the product type and conditions.

There are many statements suggesting that constancy of temperature is important in maximising the temporal stability of supercooled meat and is thus critical for the development of an effective deep chilling process.

2.2 Ice formation

Ice crystal formation includes two stages, nucleation and growth, which are both dependent on prior supercooling of the tissue as its temperature falls. Nucleation is not well understood, but it is known that many particulate substances, including introduced ice crystals, are able to catalyse nucleation of ice. A degree of supercooling needs to occur in order for ice crystal formation to be initiated. The temperature of the solution

then rises back to the freezing temperature. This was discovered by the German, Gabriel Fahrenheit in the late 1700s and demonstrated to students by the Scottish scientist Joseph Black (1728-1799) (Guemez *et al*, 2002). Black showed that a small quantity of water could be cooled to 2 to 3°C below the freezing point as long as it was left undisturbed. As soon as the liquid was shaken, a portion of it instantly froze.

In food, heterogeneous nucleation occurs when water molecules aggregate in a crystalline arrangement on nucleating agents or nucleating activators such as suspended foreign particles, surface films or walls of containers (Sun and Zheng, 2006).

When cooled below 0°C, water starts to freeze. The effect of freezing on meat is to remove most of the water from the proteins to form ice but a significant amount will remain unfrozen in the tissues (bound water). The crystallisation of ice in meat begins at -1.1°C (Calvelo 1981) and the amount of ice increases rapidly as the temperature falls (Table 1). A temperature of around -1°C has been accepted as the minimum practicable storage temperature for chilled meat. If nucleation of ice crystals can be prevented, or partially at least, lower storage temperatures could be used.

Table 1: Amount of ice in beef muscles at various subfreezing temperatures, expressed as % (Reidel 1956).

Temperature (°C)	***Ice Content (%)***
-1	2
-2	48
-3	64
-5	71
-10	83
-20	88
-30	89

Freezing has been the only option available when meat has been required to be stored for extended periods. However the formation of ice crystals within the muscle structure can have adverse effects on some properties of the meat. When very rapid freezing occurs, ice crystals form *within* muscle cells whereas at slow freezing rates larger ice crystals form *between* cells.

The freezing rate is most often defined by the rate at which the freezing front moves through the product and is normally expressed as cm/h. The rate is therefore an average figure and will be higher at the surface and slower near the centre. Grujic *et al* (1993) investigated the effects of different freezing rates on beef muscle structure to determine the freezing rate at which ice crystals have the least damaging effect. It was found that at slow freezing rates (0.22 cm/h and 0.39 cm/h) ice crystals formed between cells and were of large diameter (43.62 µm and 30.00 µm respectively). At rapid freezing rates (>3.95 cm/h) crystals formed within the cells and were of smaller diameter - less that 20 µm. Microscopic study of the muscle ultrastructure after freezing showed that the greatest damage occurred during slow freezing (0.22 cm/h and 0.39 cm/h). Intercellular ice crystals separated the fibres into groups and myofibrils were compressed and deformed. The fastest freezing rate investigated (5.66 cm/h) also produced significant

myofibril damage. The least damage occurred at freezing rates between 3.33 and 3.95 cm/h.

The effects of these freezing rates on moisture losses during freezing, thawing and cooking were investigated by Petrovic *et al* (1993). The greatest losses were at slow freezing rates (0.22 and 0.39 cm/h) and the optimal conditions seemed to be when the average freezing rate was in the range 2 – 5 cm/h.

2.3 Deep chilling in the red meat industry

As mentioned in the introduction, the Export Meat Orders, 1985, stated that chilled carcases and carcase parts had to be held under conditions of refrigeration that ensured that the temperature of the goods was not more than 4°C and not less than -1.5°C at any point within the goods. Effectively therefore, to be exported chilled, the product could be no colder than -1.5°C. None of the current legislation, the Australian Meat Standard AS 4696:2007, the EC(MMP)Os:2005 or the AQIS Approved Arrangement Guidelines (Anon 2006; 2007a; b), specify temperatures for chilled and frozen products. Therefore, the opportunity exists within the meat industry to employ colder temperatures for chilled storage if a technical means is found to prevent the meat from freezing.

Research conducted at CSIRO in the 1980s involved supercooling where meat (beef and lamb primal cuts) was held in vacuum packs at -2.2°C to -2.6°C for several months without freezing occurring. This suggested that vacuum-packaging had an inhibitory effect on nucleation of ice crystals. The low storage temperature also slowed bacterial activity and extended storage life (Eustace and Bill 1988).

Researchers at the Foundation for Scientific and Industrial Research at the Norwegian Institute of Technology (Sintef) Energy Research found that superchilling pork chops between -1°C to -3°C extended the shelf life by up to 26 days (Reynolds 2007).

Lanari and Zaritzky (1991) studied the effect of packaging and frozen storage temperature on pigments (myoglobin) from beef *gluteus medius* muscle. They found that for vacuum-packaged beef, pigment concentration remained practically constant during frozen storage and partial freezing increased oxygenation capacity of the tissue compared with chilled frozen conditions.

2.4 Deep chilling in other industries

Much of the research work reported in the literature is focussed on the seafood and the poultry industries.

Seafood industry

Many studies on the effects of superchilling on different species of fish fillets have been reported in the literature. The results of these studies all indicate the extension of shelf life in terms of reduced bacterial growth, freshness (quality index, K value) and sensory acceptability (Chang, Chang, Shiau & Pan 1998, Sivertsvik, Rosnes & Kleiberg 2003; Olafsdottir, Lauzon, Martinsdottir, Oehlenschlager & Kristbergsson 2006; Rosnes, Kleiberg, Sivertsvik, Lunestad & Lorentzen 2006; Duun & Rustad 2007).

Superchilling methods for fish have been developed and continuously modified to extend the shelf life whilst reducing storage and transport costs. Sintef scientists found that superchilling of salmon fillets extended the freshness by up to 5 days, compared with conventional refrigeration (Reynolds 2007). It was expected that the first commercial Norwegian superchilling plant was to be open during 2007.

Duun and Rustad (2007) investigated superchilled storage at -2.2°C of Atlantic cod fillet portions and showed increased shelf life with respect to reduced growth of sulphide

producing bacteria compared to ice chilled. The drip loss was lower in the superchilled fillets, and the water holding capacity (measured by water loss during centrifugation) was higher in the superchilled fillets compared to ice chilled. A possible explanation was the freeze denaturation of muscle proteins which was supported by the lower extractability of salt soluble proteins.

Gallart-Jornet *et al*. (2007) evaluated the effect of superchilled storage against ice and frozen storage on the quality of Atlantic salmon fillets as the first step of smoked salmon production. They concluded that salmon superchilled for 9 days behaved as salmon stored on ice for 2 days with regard to hardness, protein solubility and free amino acids, with the superchilled product having a higher process yield.

A study on different cooling techniques (dry ice or ice packs) and storage temperature (chilling at 3°C or superchilling at -2°C) to prolong the shelf life of Arctic charr fillets was evaluated by sensory analysis, physical methods, chemical analysis and microbial analysis (Duy et al. 2007). These authors reported that the storage temperature had more influence than the type of cooling agent. Superchilling resulted in extended shelf life (by 6 days) compared to chilling and negative changes due to partial freezing such as excessive drip loss were not observed.

Whitemeat industries

A form of superchilling has been trialled in pork processing where hot-boned, vacuum-packed pork cuts were crust frozen while being rapidly chilled before being held at 2°C (Weakley *et al*, 1986). Rapid chilling did not have a beneficial effect on palatability or shelf life of hot-processed fresh pork.

The Australian poultry processing industry holds cuts of chicken at ‘deep chilling’ temperatures of -2°C or -8°C to extend their storage life. After thawing, they have a chilled storage life of 7 days (R. Chia, Personal communication).

2.5 Anti-freezing treatments

In order to initiate ice crystal formation, a solution will usually need to be supercooled to a certain extent. A very small volume of a pure solution may supercool tens of °C but most biological solutions contain ice nucleators which may initiate crystallisation at temperatures just below 0°C (Wolfe & Bryant, 2001). Some biological substances such as plant leaves often supercool a few degrees and can survive mild frosts without freezing damage. A number of materials are available which can depress the freezing point by inhibiting nucleation of ice or effectively lower the temperature at which nucleators become effective. Some of these are described below.

Salt

The freezing point of meat can be lowered by increasing the salt content. In experiments with a meat substitute, Tylose, James *et al* (2005) showed that the initial freezing temperature could be reduced to -3.1°C by the increasing the salt content to 2% and to -4.1°C at 3% salt.

Wolfe (2007) suggested that applying salt to the surface of meat may depress the freezing point.

Anti-freeze proteins

The best known inhibitors of nucleation are the antifreeze proteins (AFP) found in the blood of polar fish species (Eustace and Bill 1988). AFPs were first identified by De Vries and Wohlschlag (1969) in the blood of these fish where they serve to lower the

freezing point of the blood to below the freezing point of the seawater. They are also found in some invertebrates including insects and in plants, fungi and bacteria (Griffiths and Ewart, 1995).

Wen and Laursen (1993) proposed a model for the inhibition of ice crystal growth. AFP molecules are assumed to bind tightly to the ice surface so that the ice lattice is only allowed to grow in the spaces between AFP molecules, hence decreasing the stability of the surface at the ice water interface. Therefore the growth of the ice crystals is inhibited.

The commercial application of AFPs is currently limited by their high price. Chemical synthesis and genetic engineering may be a solution to the production of a cost-effective AFP which will result in application to food products (Sun & Zheng, 2006).

Dehydration

Many species of plants and animals survive freezing temperatures by achieving very low intracellular water contents (Wolfe & Bryant, 2001). Dehydration increases the osmotic pressure of the intracellular solution which depresses its freezing temperature. Rahman *et al* (2003) demonstrated that by dehydrating tuna from 26.7% solids to 44.1%, the freezing point could be lowered from -1.4°C to -3.8°C.

It is not feasible to dehydrate primal cuts to be vacuum packed, but Wolfe (2007) suggested that removing any surface moisture by passing the cut through warm air jets could remove any free water that could freeze and act as nucleators for freezing the meat.

2.6 Packaging possibilities

Plastic films

Eustace and Bill (1988) found that vacuum-packaged beef and lamb was more resistant to freezing than similar cuts wrapped in polyethylene. They attributed this partly to the absence of the opportunity for pure water ice to form on the surfaces of the meat or the packaging, as well as the possibility that components of the plastic film in the packaging may have inhibited nucleation.

Edible Films and Coatings

There is considerable interest in the development of bio-based food packaging materials, particularly from the point of view of environmental sustainability. The ideal is to produce a packaging material that is both biodegradable, and utilises a by-product of the food industry in its manufacture. A number of products have been investigated, and appear to reduce moisture loss and oxidation of meat (Gennadios, Hanna & Kurth, 1997). Edible films may be polysaccharide, lipid or protein based.

The polysaccharides include: starch; alginate; carrageenan; and cellulose. Starch-based films are commonly derived from plant products, and effectively reduce the a_w within the pack, resulting in less drip loss, and inhibition of microbial growth (Wong, Camirand & Pavlath, 1994, cited in Cutter, 2006). Alginates, produced from seaweed, have been the focus of much research. They require the addition of a gelling agent such as calcium or magnesium, and this can impart a bitter flavour (Allen, Nelson, Steinberg & McGill, 1963). However, research continues to find a more acceptable gelling agent, and alginate films have been used to extent the shelf-life of fish, shrimps and sausages (Earle & Snyder, 1966; Earle, 1968; Earle & McKee, 1976; Shetty, Bhaskar, Bhandary & Raghunath, 1996; Cutter 2006). Carrageenan has been used to coat fish or poultry,

resulting in an extended shelf-life (Meyer, Winter & Weister 1959; Pearce & Lavers 1949; Stoloff, Puncochar & Crowther 1948). Cellulose as an edible coating is well-recognised in the meat industry: cellulose sausage casings have been used for many years to contain the meat emulsion. Some research has shown that cellulose glazes used on poultry or seafood have reduced moisture loss (Cutter 2006), but there is little information on the potential for cellulose to provide protection during cold storage.

Lipids (fats) have been used in a number of applications to coat sausages, meat patties and poultry (Cutter 2006). Letney (1958), Anderson (1960, 1961a; b), Schneide (1972), and Stemmler & Stemmler 1976) all lodged patents claiming that coating meat with fats could extend the shelf-life and reduce moisture loss. Milk proteins have been investigated as potential, edible coatings for meat, and Khwaldia, Perez, Banon, Desobry & Hardy (2004) found that a casein-based coating reduced lipid oxidation and moisture loss in frozen salmon. There is little further information in the literature, and there is a concern that the addition of non-meat proteins to meat could result in health problems, such as allergic reactions, in consumers (Cutter 2006).

2.7 Summary

Deep chilling, super chilling or partial freezing are terms used to describe the process of cooling a product to one or two degrees below its freezing point. It has been utilised with some muscle foods, particularly seafood, to extend the storage life of products. In some applications crust freezing occurs without any measureable effect on final product quality. In other cases a portion of the freeze able water has been converted to ice.

Ideally, formation of ice should be prevented or avoided with a muscle food in order to retain its inherent properties. Slow freezing can result in formation of large intracellular ice crystals that cause more damage to the muscle structure resulting in higher loss of drip on thawing and poorer water holding capacity.

There is some evidence that the formation of ice crystals in meat at sub-freezing temperatures can be inhibited by the application of agents that prevent nucleation of crystals. Vacuum packaging alone may be sufficient to prevent freezing at slight sub-freezing temperatures.

3.0 PROJECT AIM

The aim of this project is to determine the effects of deep chilling on the physical, sensory and microbiological state of vacuum-packed beef, as compared with product that is stored chilled (0°C) or frozen (-20°C).

4.1 METHODS

Striploins, outsides and knuckles were sourced from a local commercial boning room, 24 hours post-slaughter. Each primal was quartered into cuts ranging 1-3kg, each piece vacuum packed individually, and placed in cardboard cartons with plastic liners. Each carton contained ten pieces of one primal type. One carton of striploin (*m. longissimus dorsi*) and two cartons of each of outside (*m. semitendinosus* ST) and knuckle (*m. rectus femoris*) were assigned to each of the following chilling treatments:

A. Placed directly in a chiller at 0°C
B. Placed directly in a chiller at -1.5°C
C. Placed directly in a chiller at -2.5°C
D. Placed directly in a chiller at -5°C
E. Placed directly in a freezer at -20°C

F. Chilled to 0°C for 24 hours then moved to -1.5°C

G. Chilled to 0°C for 24 hours then moved to -2.5°C

H. Chilled to 0°C for 24 hours, moved to -2.5°C for a further 24 hours, then moved to -5°C

I. Chilled to 0°C for 24 hours then moved to -5°C

The cartons were stored for 10 weeks and packs were opened at weeks 6, 8 and 10 for assessment.

A further small set of knuckle cuts (ranging 500-1000g) were treated with some potential protective agents prior to storage at -5°C. The food grade agents used were: salt; glycerol; sodium lactate and carrageenan. These were applied by rolling the cut in a container of the agent. Within the same subset of treated muscles, there was an untreated cut, designated 'coatings control', and one that had been subject to surface drying under cool air fans overnight.

All packs were evaluated for the proportion of packs freezing solid, drip loss during vacuum storage and 3 days retail display, and for visual appearance. Cuts were evaluated at weekly intervals for the presence of frozen packs. The evaluation simply involved the researcher squeezing each pack and recording if the cut was soft or hard. Hard was taken to indicate frozen, soft not frozen. A sensory panel of five participants assessed the visual appearance of each pack before opening. Each pack was then opened and assessed by the panel for confinement odour. The cuts were allowed to bloom for 30 minutes and then reassessed by the panel for visual appearance. Panel assessments were based on a 9-point scale, 9 being excellent, 0 being extremely poor. The volume of drip remaining in the vacuum bag was measured.

Subsequently, two samples of tissue representing a surface area of 5cm² each were removed from each cut for microbiological analysis, and a 1cm cube removed and fixed in 10% formalin for histological analysis. Each cut was then sliced into 1.5cm thick steaks and packaged in overwrap trays. The resulting retail packs were assessed using MINOLTA colourimetry, and displayed in a retail cabinet at 3°C, under fluorescent light for three days. At the end of the three day display, the packs were again assessed by MINOLTA colourimetry, and by a 3-member panel for visual appearance. Samples were taken for physico-chemical analysis (lipid oxidation, expressible juice and soluble protein concentration) and the volume of drip present in the retail tray measured.

Microbiological analysis

100 ml 0.85% saline was added to each stomacher bag containing both 5cm² surface samples. These were then stomached for 30s. Decimal dilution series were made from each stomacher bag, and plated according to the following procedures:

- Total Viable Count (TVC): plated onto Petrifilm aerobic plates and incubated aerobically at 35°C ± 1°C for 48 ± 3h
- *Brochothrix thermosphacta*: plated in duplicate onto STAA agar plates containing selective supplement and incubated aerobically at 22°C ± 1°C for 48 ± 3h
- Lactic Acid Bacteria (LAB): double diluted into MRS broth, plated onto Petrifilm aerobic plates and incubated anaerobically using Anaerogen W-Zip kits at 30-35°C for 48 ± 3h

Counts per square cm were calculated using an Excel spreadsheet.

Physico-chemical analysis

Lipid Oxidation

Lipid oxidation was assessed by the thiobarbituric acid-reactive substances (TBARS) method of Witte et al. (1970). All meat samples were heated at 75°C for 20 minutes in a water bath and then cooled in ice prior to determination. TBARS were calculated from a standard curve of malondialdehyde (MDA), freshly prepared by acidification of TEP (1,1,3,3-tetraethoxypropane), and calculated as mg MDA per kg sample.

Expressed Juice

Water-holding capacity (WHC) of raw and cooked meat has been related to organoleptic properties such as juiciness and tenderness. A change in WHC of muscle has been shown to be a sensitive indication of variations in the charges and structure of muscle proteins.

Determinations of WHC (in terms of expressed juice [EJ] values) were based on the high speed centrifugation method of Bouton et al. (1971). Whole, raw muscle samples weighing 3-4g were centrifuged at 100,000g (40,000rpm) for 1 hour in a Beckman Optima LE-80K ultracentrifuge. The samples were weighed before centrifuging and again after the expressed juice had been decanted to determine the liquid loss. Expressed juice from the samples was defined as the loss in weight after centrifuging expressed as a percentage of the initial weight of the sample.

The expressed juice was transferred into 1.5mL Eppendorf tubes and retained for protein concentration evaluation.

Protein Solubility

The concentration of protein in the expressed juice was measured by the Biuret method (Gornall et al. 1949) and was indicative of denaturation and subsequent solubility of the muscle proteins after storage.

5.0 RESULTS

This section summarises the significant outcomes of the study. A full set of results are presented in the appendix.

5.1 Proportion of packs frozen

The proportion of packs frozen under each chill storage treatment was calculated (Figure 1). Cuts chilled directly to -5°C and -20°C were all frozen after the first week of storage, while no cut at 0°C or -1.5°C froze. Cuts chilled directly to -2.5°C froze more slowly than those chilled directly to lower temperatures None were frozen after 1 week of storage, but 38% were frozen after 2 weeks of storage, 50% after 6 weeks of storage and 58% after 10 weeks storage. For the slow-chilled cuts, after 1 week of storage, 4% of cuts chilled under treatment G (0° for 24 hours then -2.5°C), 36% of treatment H (0° for 24 hours then -5°C) and 22% of treatment I (0° for 24 hours; -2.5°C for 24 hours then -5°C) were frozen. After 2 weeks of storage, those under treatment G plateaued at 12% frozen, while those under treatments H and I continued to freeze slowly, reaching 80% frozen by week 7.

In the cartons of coated cuts knuckle, none of the pieces coated in sodium lactate or rolled in salt froze under chilling treatments A, B, F or G, while a single piece froze under chilling treatment I after 6 weeks storage. All other coated cuts froze.

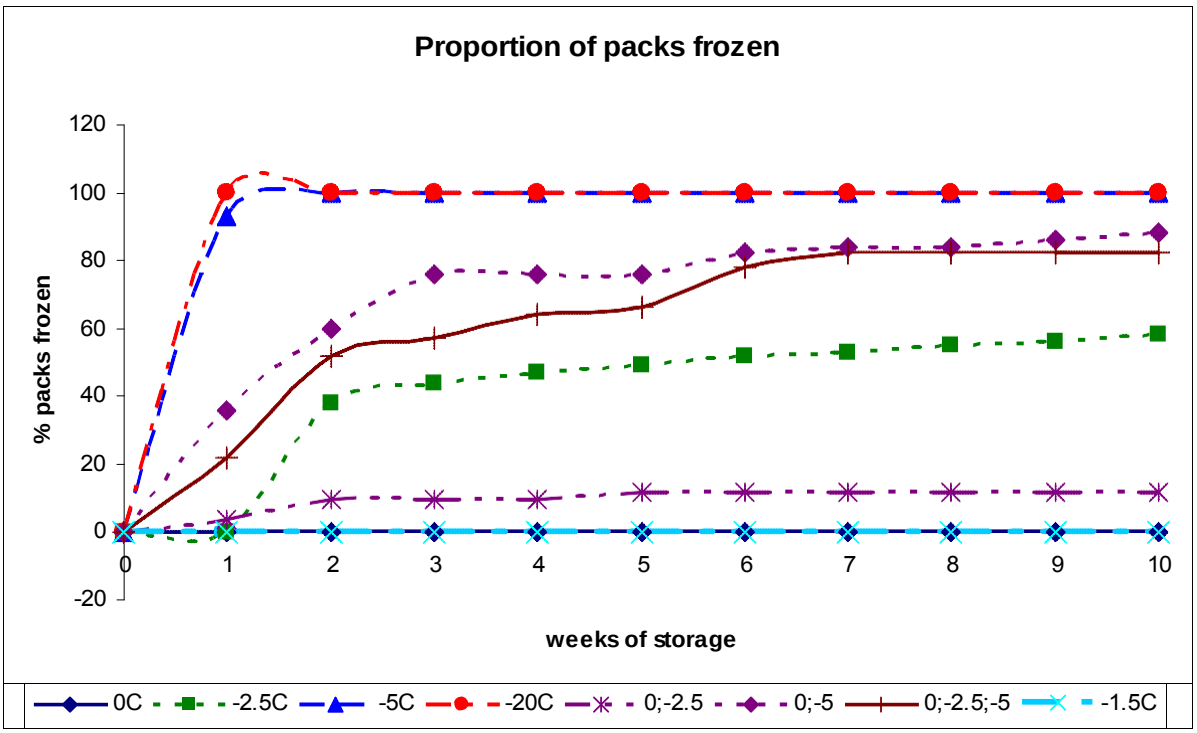

Figure 1: Proportion of packs frozen

5.2 Visual assessment of intact packs

Untreated cuts

There were no significant differences between the panel scores assigned to cuts from different temperatures of storage. In general, scores of 'normal' (4) to 'excellent' (8) were assigned. However, white dots were observed on the meat surface of cuts stored at -2.5°C and -5°C (figure 2). The white spots noted on cuts directly chilled to -2.5°C and -5°C were evident on all cuts that had frozen solid under the slow chilling treatments. They were largest and most prominent on those frozen solid under treatment H, and smaller and less distinct on these frozen solid under treatments G or I.

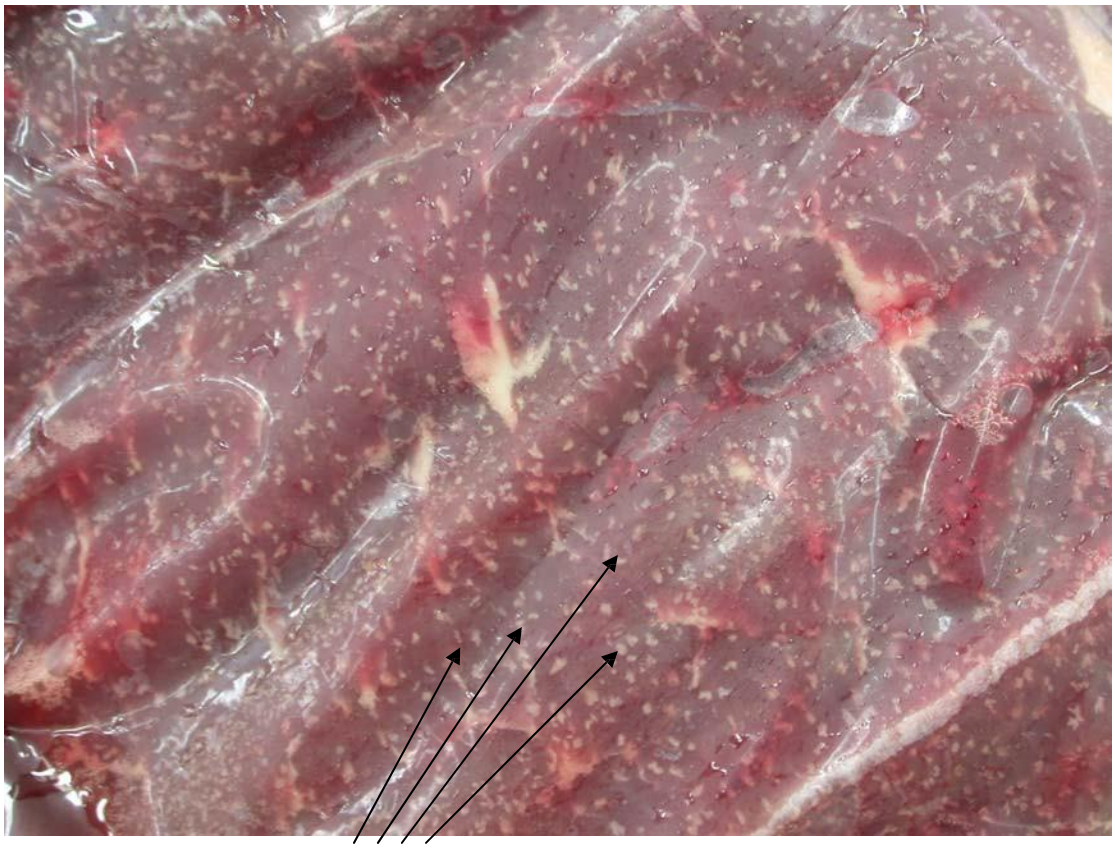

Figure 2: Dendritic white spots evident on meat surface

Cuts stored at -1.5°C received lower scores overall, particularly in weeks 8 and 10. This was due to browning (oxidation of myoglobin) of the surface of the primal, and was thought to be an artefact of the study, relating to the repacking that the cuts underwent.

At 26 weeks of storage, an outside (ST) pack was removed from each of 0°C and -2.5°C, and visual appearance compared. The 0°C pack had a distinct greenish colouration, while the -2.5°C pack maintained the normal purple of vacuum-packaged beef.

Treated cuts

After 8 weeks storage, the salted cuts were visually darker than other cuts, and were assigned scores of 2-3. All other cuts were assigned scores between 5 and 7 (figure 3).

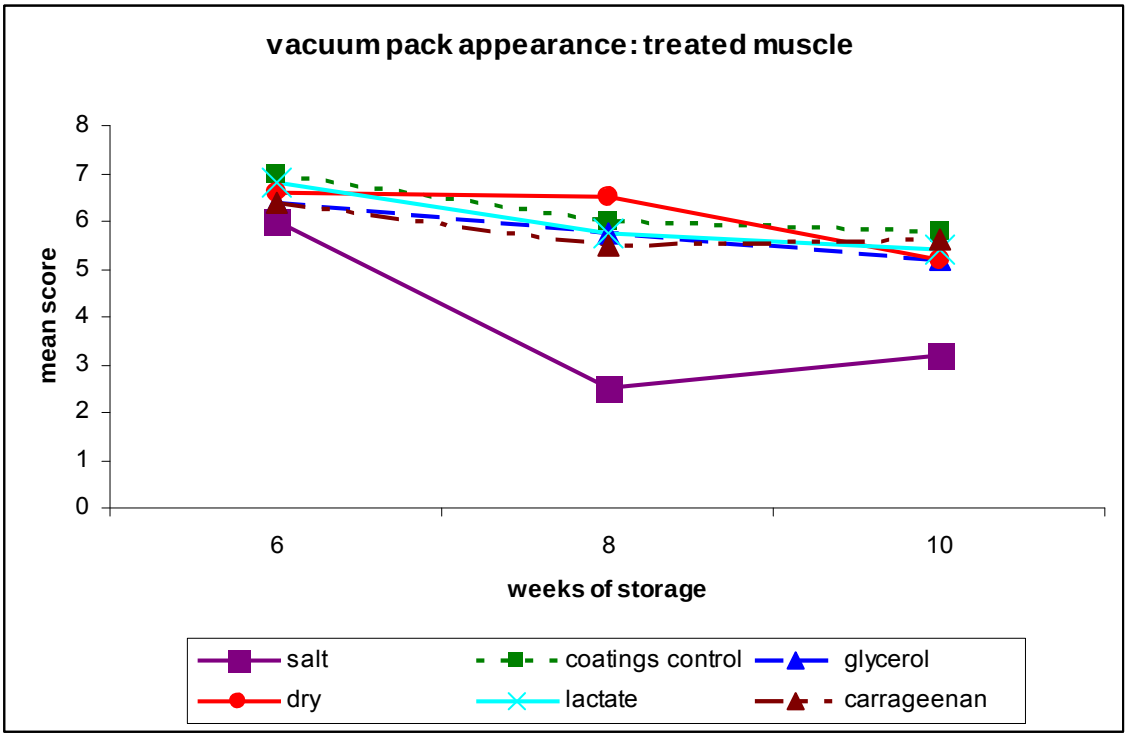

Figure 3: The proportion of packs frozen under each chilling treatment

5.3 Confinement odour assessment

Untreated cuts

In general, little odour was evident from cuts stored at -20°C and -5°C. The odour associated with cuts stored at -2.5°C began to become apparent after 8-10 weeks of storage, whereas a 'normal' confinement odour (panel score 4) was evident in 0°C packs from week 6, and this odour became more marked, particularly in knuckle and outside in subsequent weeks (figures 4-6). At -1.5°C, confinement odour was noticeable on outside (ST) at all sampling points.

Treated muscles

It was difficult to assess the treated group. The majority had little confinement odour, however, the salted cuts smelled salty, and those treated with sodium lactate began to develop a confinement-type odour by week 10 (figure 7).

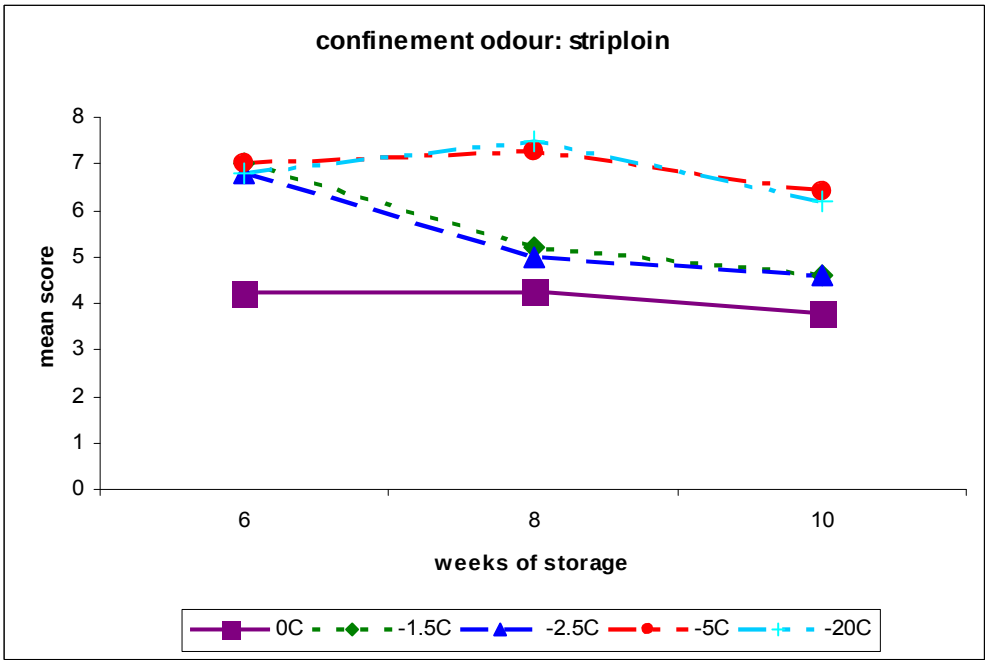

Figure 4

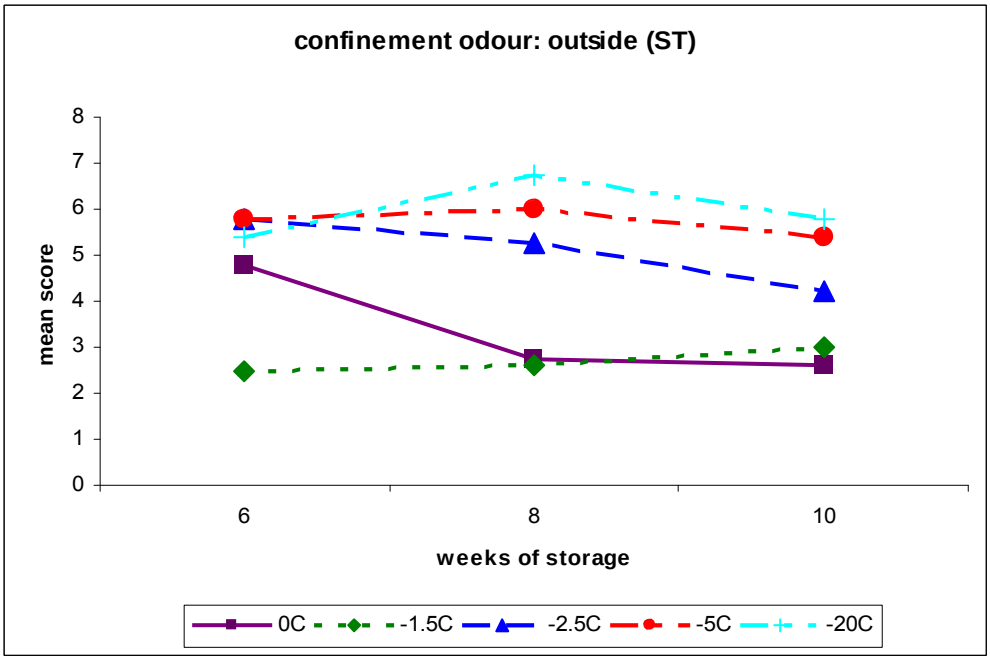

Figure 5

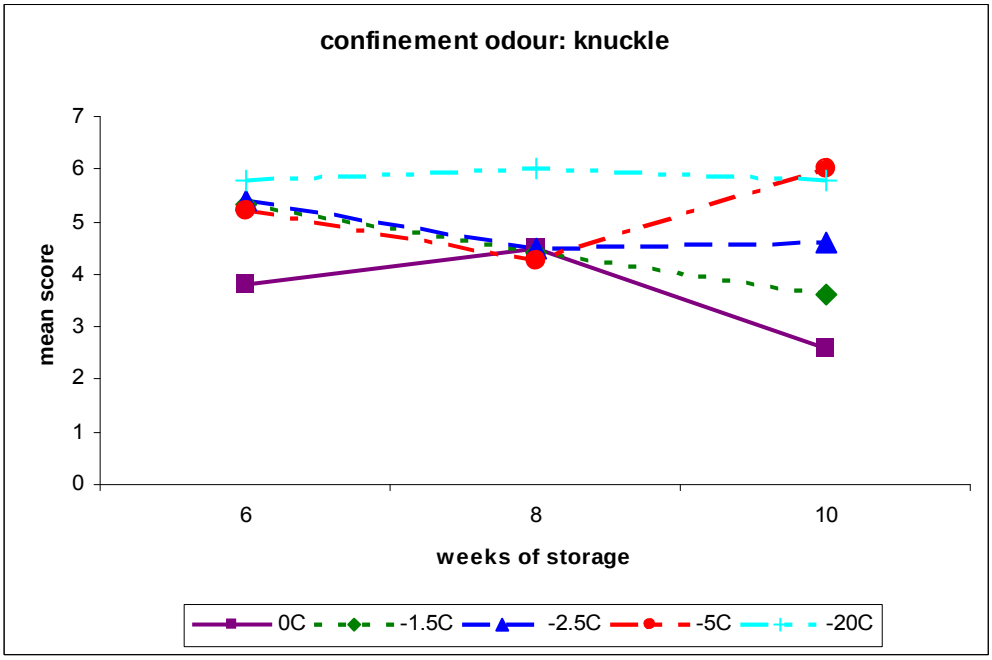

Figure 6

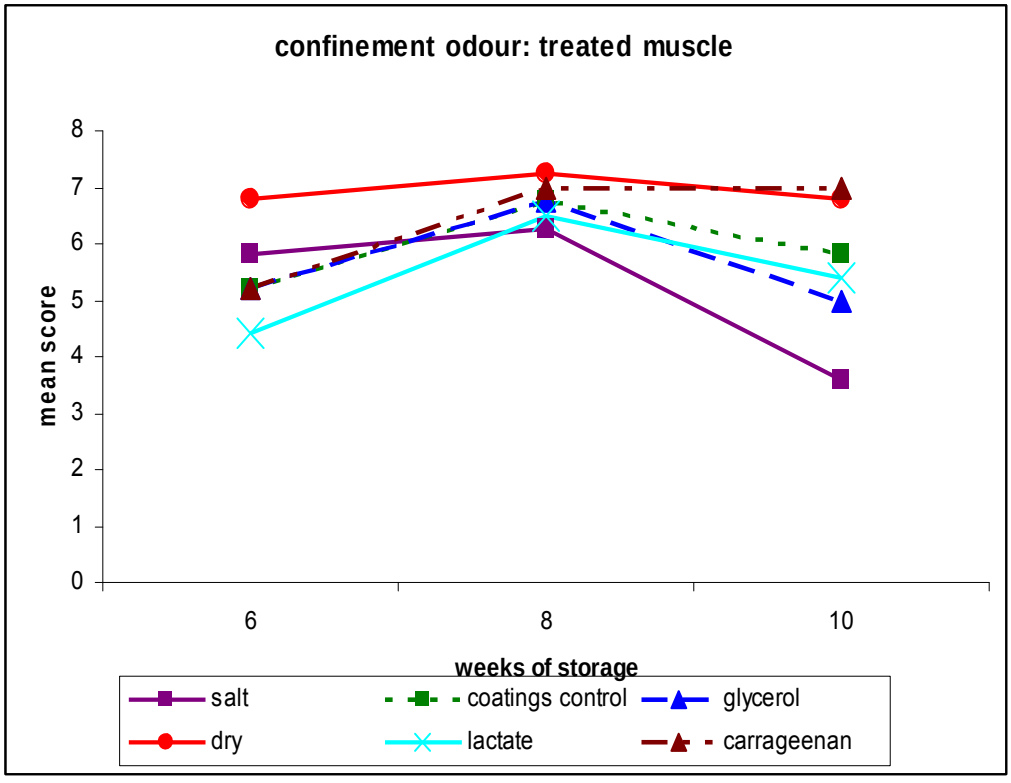

Figure 7

5.4 Post bloom visual appearance assessment

Untreated cuts

There were no significant differences between the panel scores assigned to cuts from different temperatures of storage. Scores of 'normal' (4) to 'excellent' (8) were assigned. Again, cuts stored at -1.5°C received lower scores overall, due to browning of the surface of the primal, and was thought to be an artefact of the study, relating to the repacking that the cuts underwent. The white dots as previously mentioned were visible on the meat surface of cuts stored at -2.5°C and -5°C (figure 8). These were confined to the surface of the cut. The underside of the steak looked completely normal.

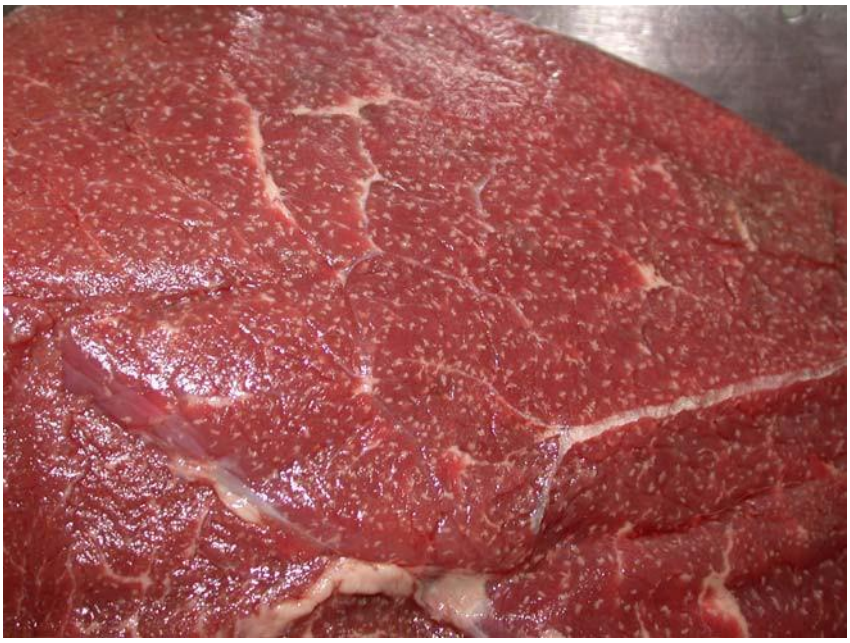

Figure 8: White dots on muscle stored at -2.5°C

Treated cuts

After 8 weeks storage, the salted cuts were visually darker than other cuts, and were assigned scores of 0-2. All other cuts were assigned scores between 4 and 7 (figure 9).

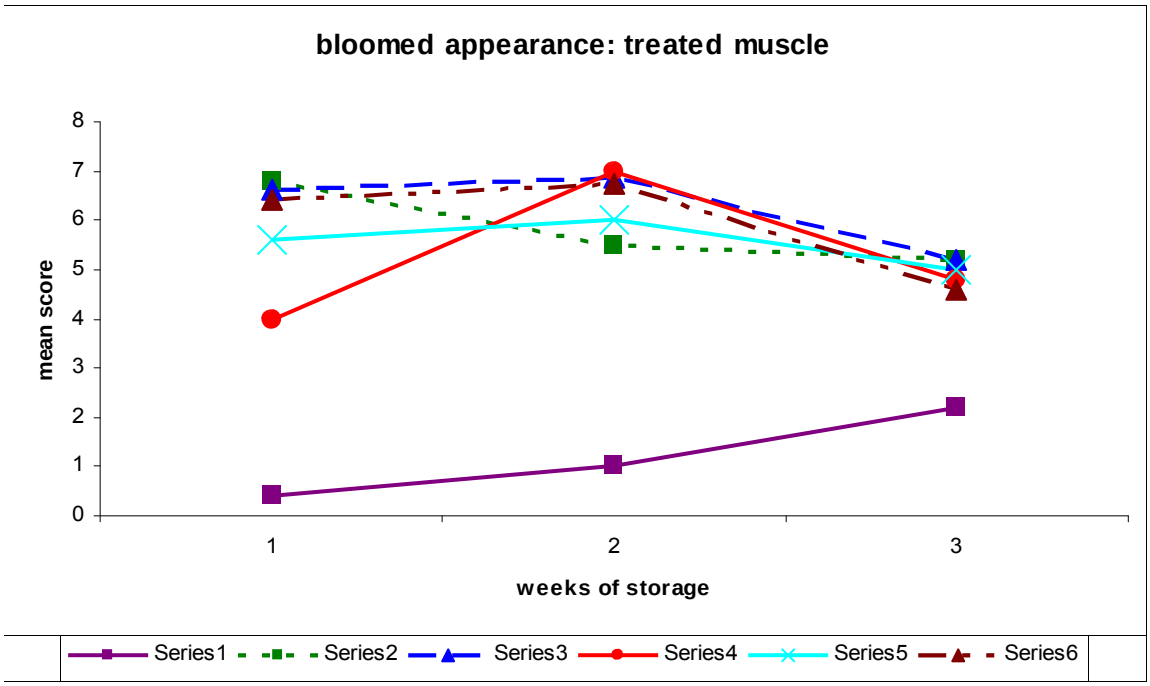

Figure 9

5.5 Retail pack visual assessment

Untreated cuts

There were no significant differences between the panel scores assigned to cuts from different temperatures of storage. Scores of 'normal' (4) to 'excellent' (8) were assigned. The white dots as previously mentioned remained visible on the meat surface after 3 days retail display (figure 10).

Treated cuts

After 8 weeks storage, the salted cuts were visually darker than other cuts, and were assigned scores of 2-4. All other cuts were assigned scores between 4 and 7 (figure 11).

Figure 10: -2.5 outside (ST) after 3 days retail display – white dots are evident on the left hand steak

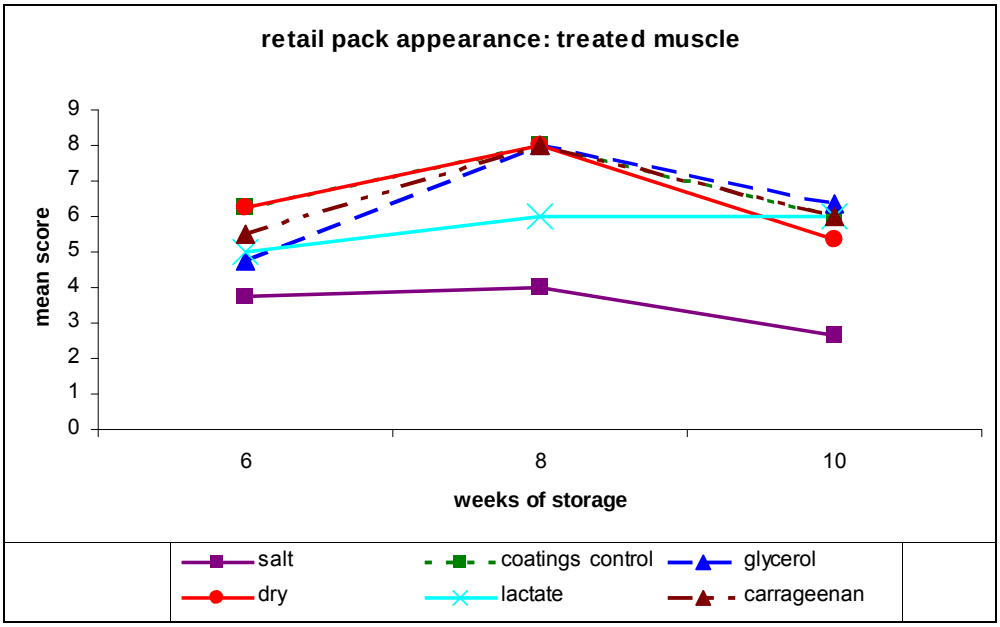

Figure 11

5.6 Drip loss

It was difficult to draw firm conclusions regarding drip loss based on the small numbers of samples tested. In general, overall drip loss during vacuum storage and retail display was lowest for cuts stored at 0°C and under treatment G (slow chilling to -2.5°C) as long as the pack was not frozen solid. Greatest overall drip loss was seen in the directly-chilled cuts, particularly at -1.5°C, cuts that had frozen solid and in cuts chilled under treatment H (0°C, then -5°C). Cuts chilled under treatment I (0°C, then -2.5°C, then -5°C) and directly chilled to -20°C showed an intermediate overall drip loss (figure 12). Storing meat at the sub-zero temperatures under trial resulted in excess drip production overall; salt and sodium lactate have a protective effect against this phenomenon (figure 13).

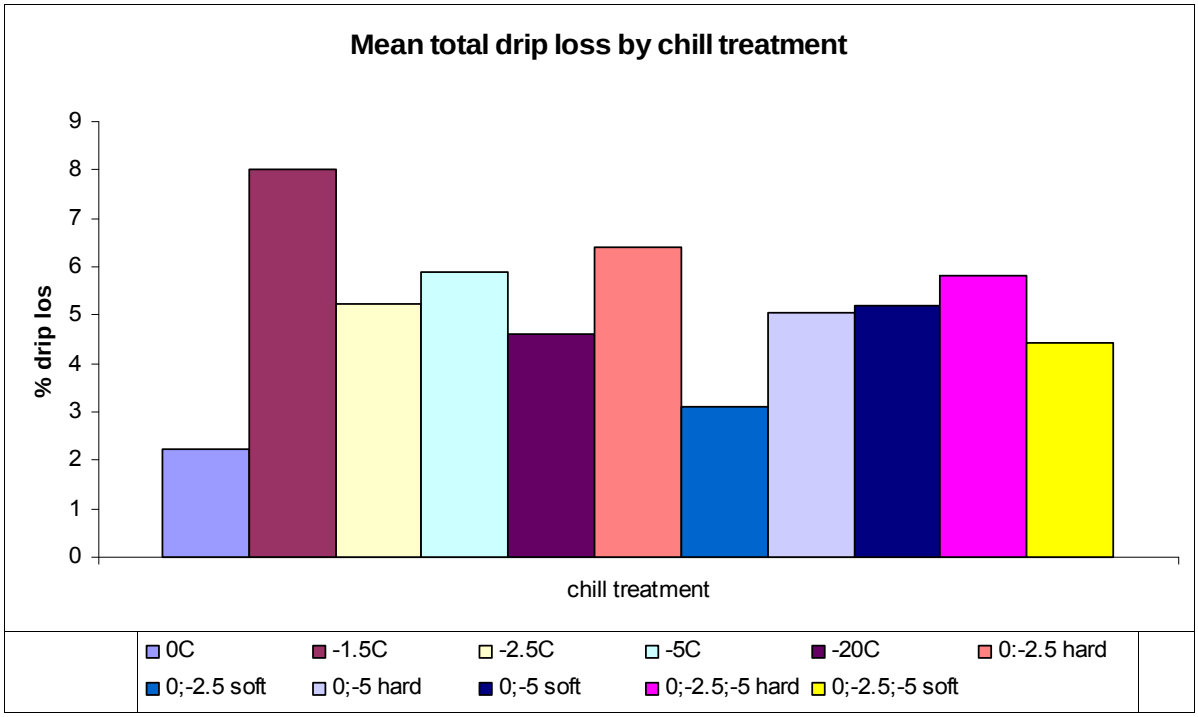

Figure 12: Overall drip loss by chilling treatment, averaged over sampling occasion and cut

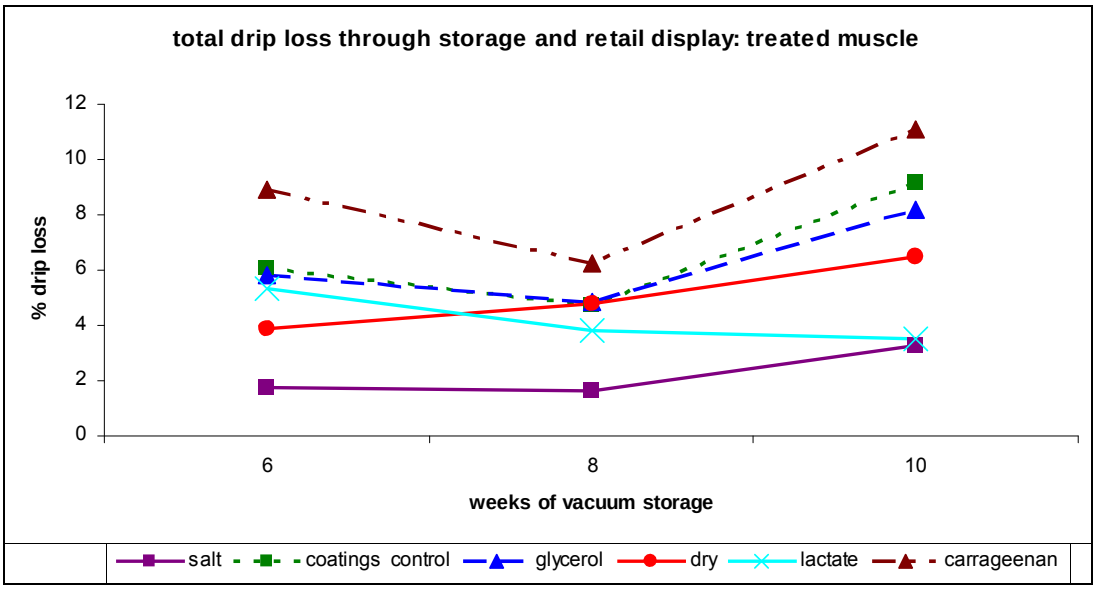

Figure 13

In general, the cuts stored at 0°C produced more drip during the vacuum storage period than did those stored at sub-zero temperatures. This may be due to the drip being bound as ice crystals within the meat tissue of those stored at sub-zero temperatures or to increased proteolysis, resulting in a looser structure and increased amount of water in the extracellular space. Bacterial enzymes may also contribute to the degradation and thereby affect the ability to retain water. However, once in the retail packs, excessive drip was produced by meat previously stored at sub-zero temperatures, compared with 0-0.13% loss in meat previously stored at 0°C (figures 14-17).

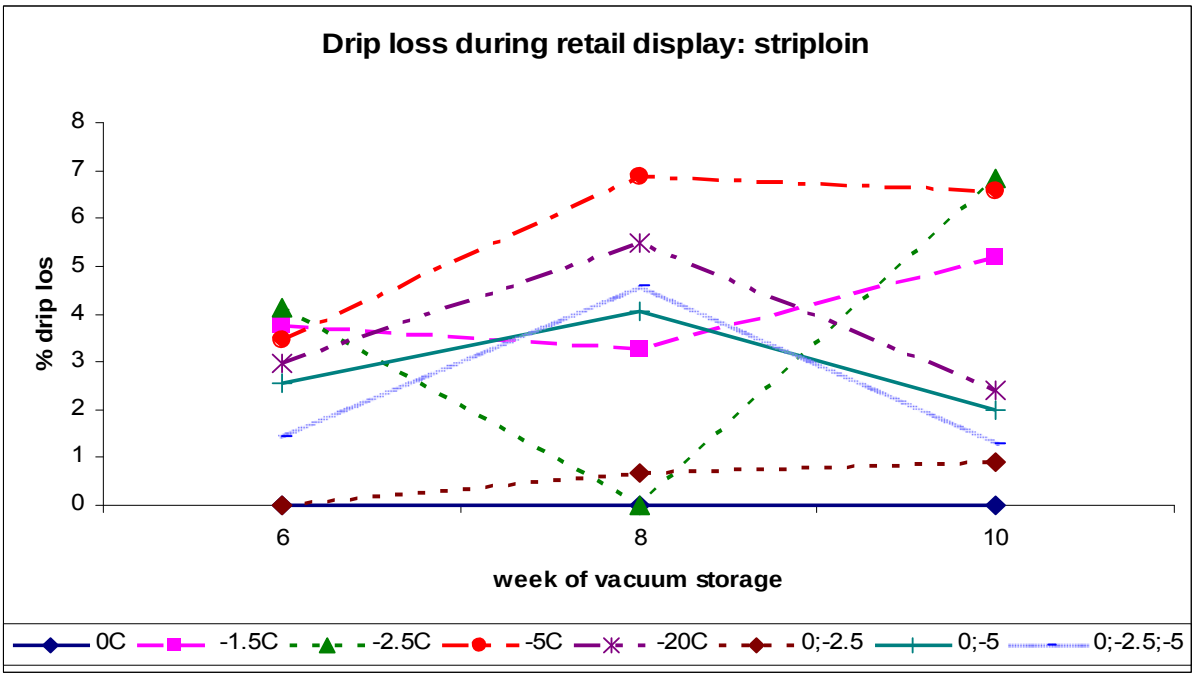

Figure 14

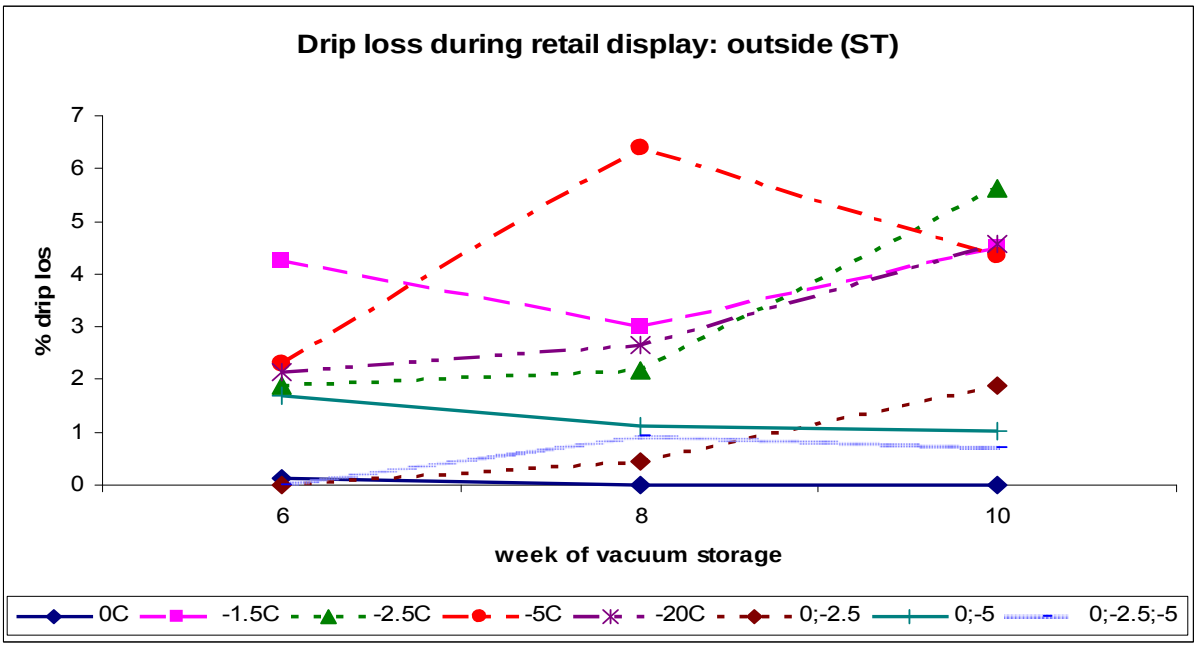

Figure 15

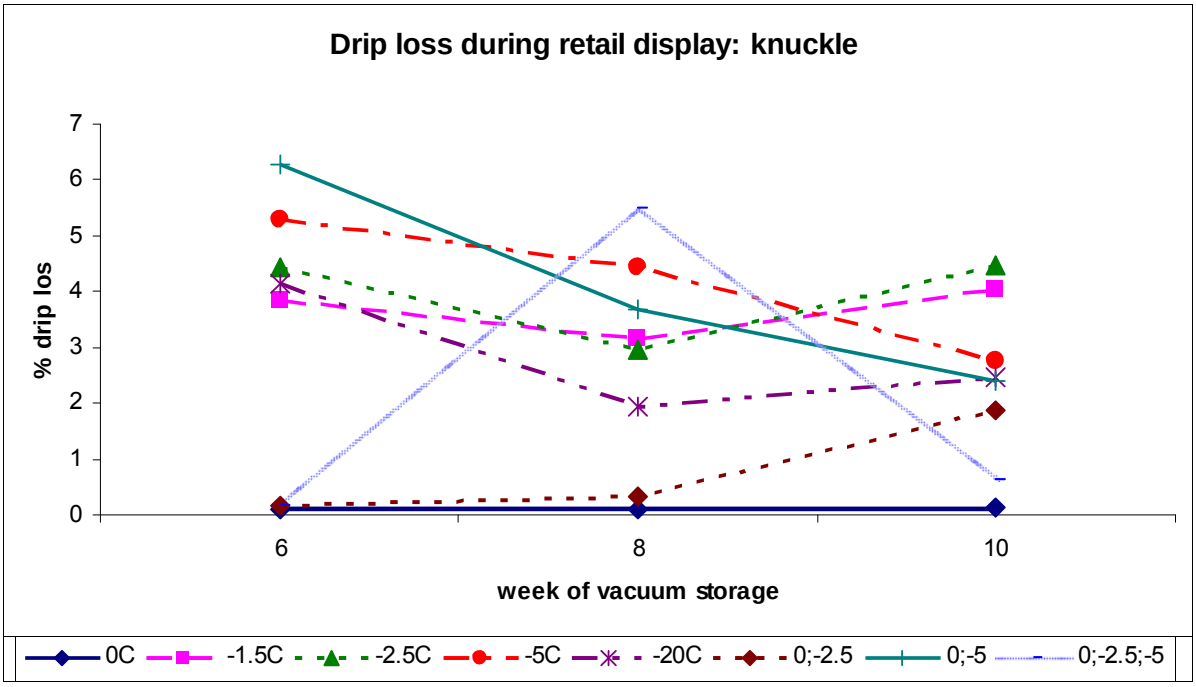

Figure 16

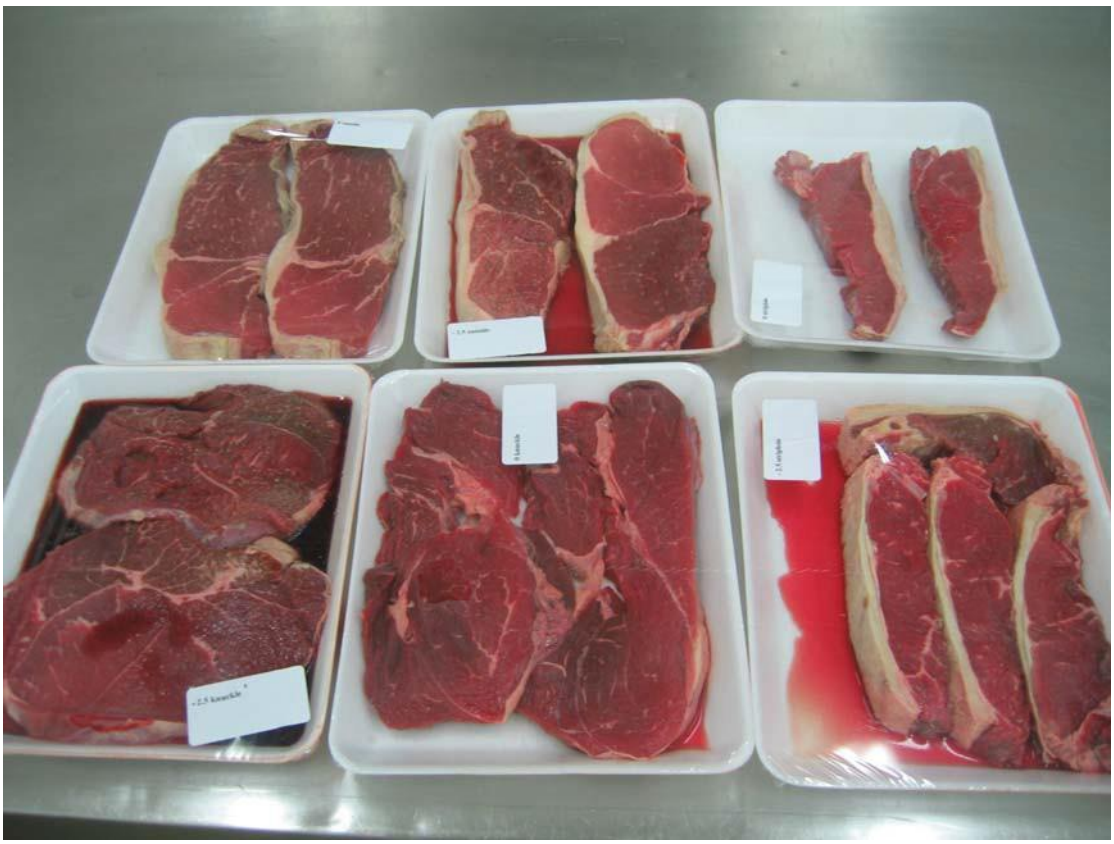

Figure 17: Untreated muscles after 3 days retail display. Top row l-r: 0 outside (ST), -2.5 outside (ST), 0 striploin; Bottom row l-r: -2.5 knuckle, 0 knuckle, -2.5 striploin; The white dots are evident on the left steak of the -2.5 outside (ST) and the top steak of the -2.5 knuckle.

Salting had a significant effect on the drip loss of the cut – no drip was evident in the retail tray. Sodium lactate also reduced the amount of drip produced during the retail display period (figures 18-19).

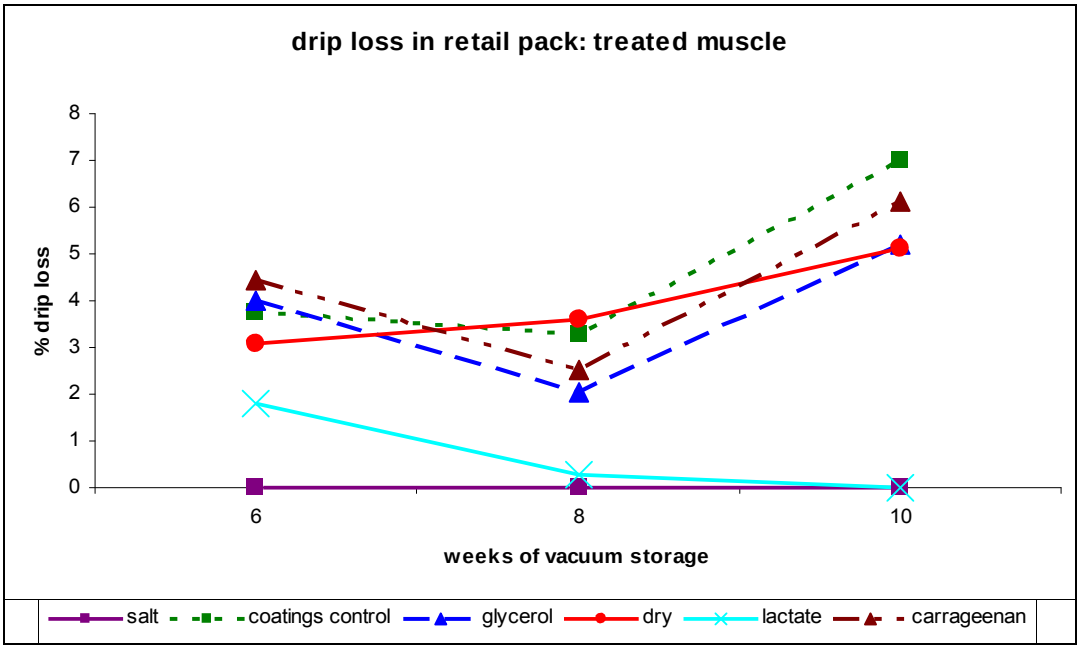

Figure 18

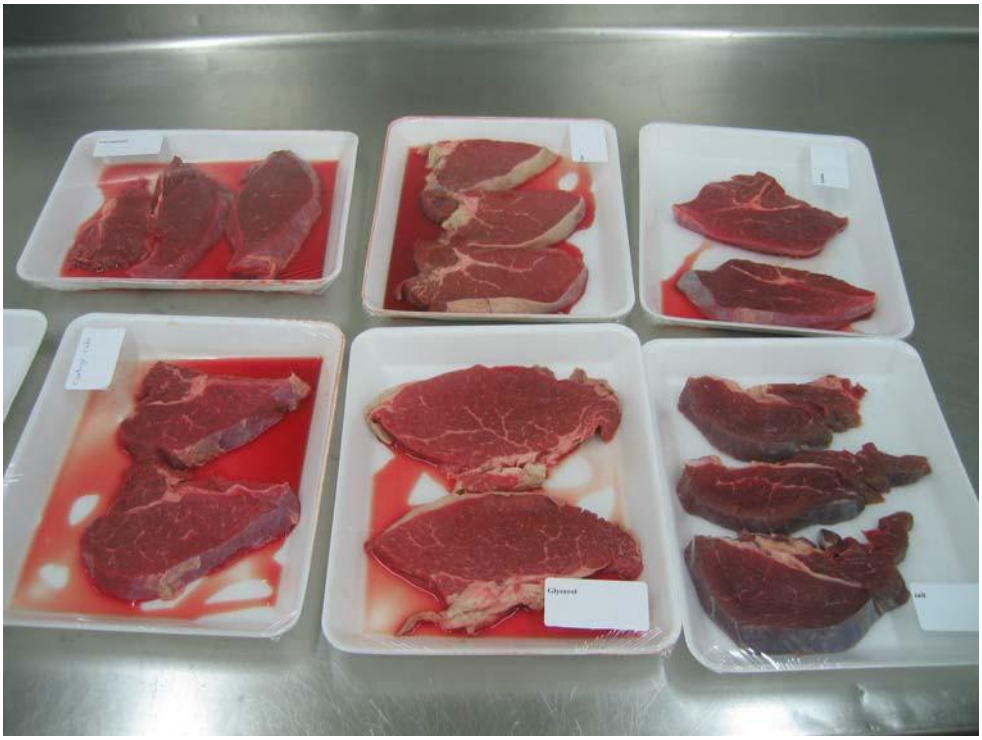

Figure 19: treated muscles after 3 days retail display. Top row l-r: Carrageenan, dry, lactate; Bottom row l-r: Coatings control, glycerol, salt; There was no drip in salted muscles, but they are dark and firm, and smell salty; There was limited drip in lactate treated muscle

There were no significant differences between the different storage temperatures in terms of water holding capacity (data not shown), and neither were there significant differences between treated muscles except salt. Salting muscle decreased the amount of juice that could be expressed through centrifugation (figure 20).

The water holding capacity data needs to be viewed in context with the drip loss results. Drip loss represents the most loosely bound water and samples with a high drip loss will therefore be able to hold on to a higher share of the remaining water during the centrifugal procedure. It would appear in salted muscle, the salt itself binds moisture preventing drip loss and expression of juice.

Low extractability of muscle proteins indicates freeze denaturation, especially of the myofibrillar proteins. The myofibrillar network retains most of the water in the muscle, therefore a high expressed juice percentage indicates protein denaturation.

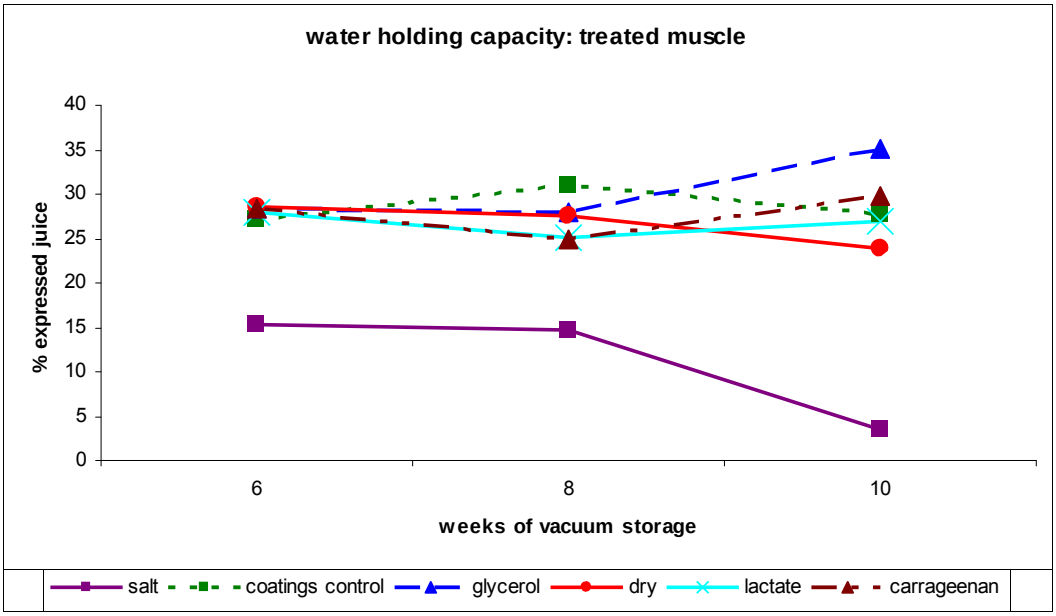

Figure 20

5.7 Soluble protein concentration

There were no significant differences between the different storage temperatures for any cut, including within the treated muscles group.

5.8 Lipid oxidation (TBARs)

There were no significant differences between the different storage temperatures in terms of lipid oxidation, and neither were there significant differences between treated muscles except for salt. Salting muscle increases the lipid oxidation (figure 21).

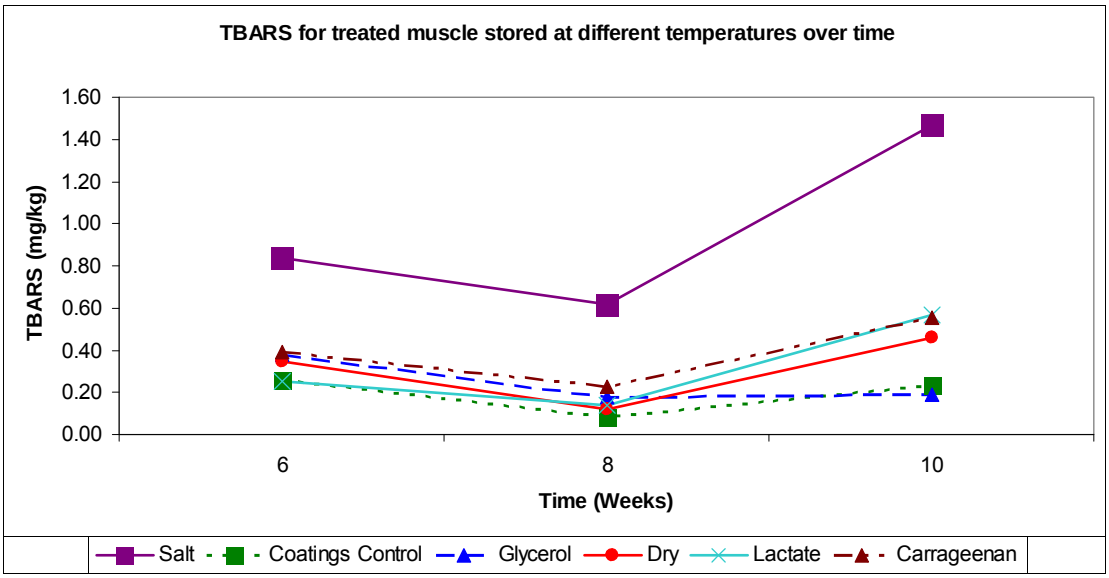

Figure 21

5.9 MINOLTA colourimetry

There were no consistent differences between the MINOLTA L, a, or b values for cuts stored at different temperatures, nor for cuts that had been treated with different coatings.

5.10 Microbiology

Untreated muscles

In general, the TVC of cuts stored at 0°C was around 4 $\log_{10}$ cfu/cm² greater than that of cuts stored at -1.5°C, -5°C or -20°C. The TVC of striploin stored at -2.5°C occupied an intermediate position, but that of outside (ST) and knuckle stored at -2.5°C was similar to those stored at -1.5°C, -5 °C and -20°C. The TVC of cuts stored at 0°C was in the range of 6-7 $\log_{10}$ cfu/cm², while for frozen cuts, in the range of 2-3 $\log_{10}$ cfu/cm² (fgures 22-24). In all cases, the TVC was predominantly made up of Lactic acid bacteria (LAB), which were again in the range of 6-7 $\log_{10}$ cfu/cm² in cuts held at 0°C and in the range of 1-4 $\log_{10}$ cfu/cm² in cuts held at -1.5°C, -2.5°C, -5°C and -20°C (figures 25-27). The high result at week 8 in knuckle stored at -5°C is likely to be an artefact of the study, as only a single pack of each cut and temperature was evaluated on each sampling occasion. There were no significant differences between the populations of homofermentative and heterofermentative LAB in any sample. *Brochothrix thermospacta* levels were also consistently higher in cuts stored at 0°C than in cuts stored at the other temperatures investigated (data not shown).

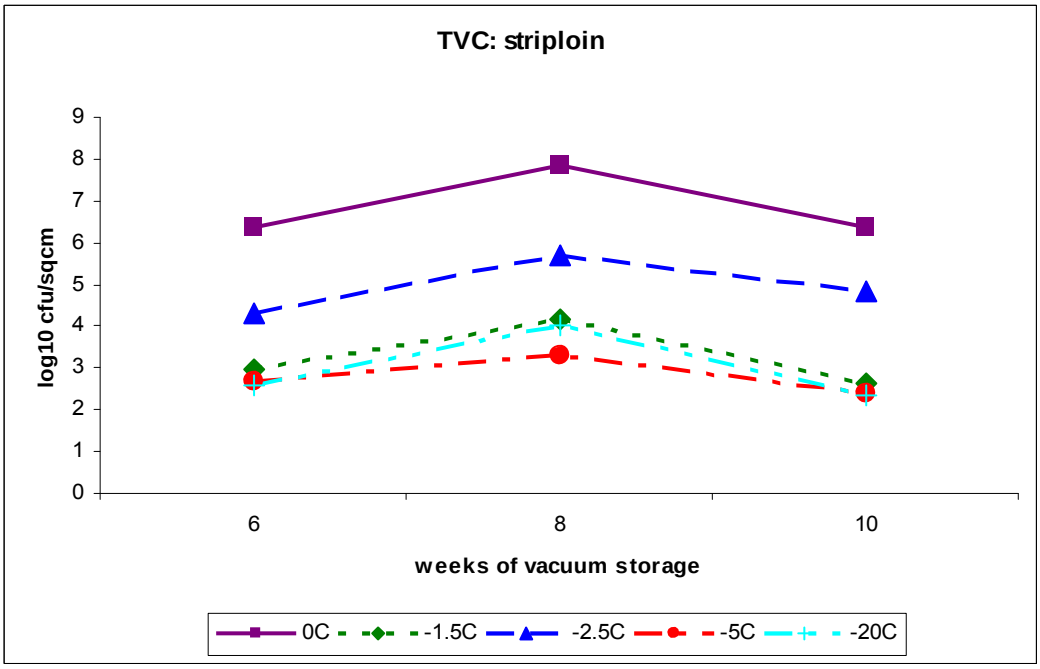

Figure 22

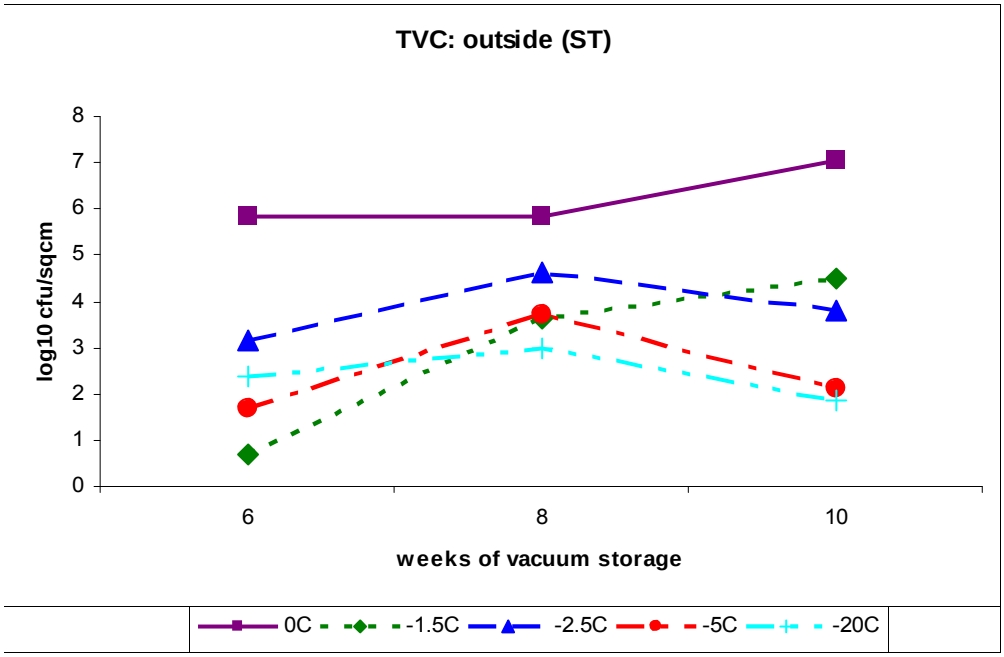

Figure 23

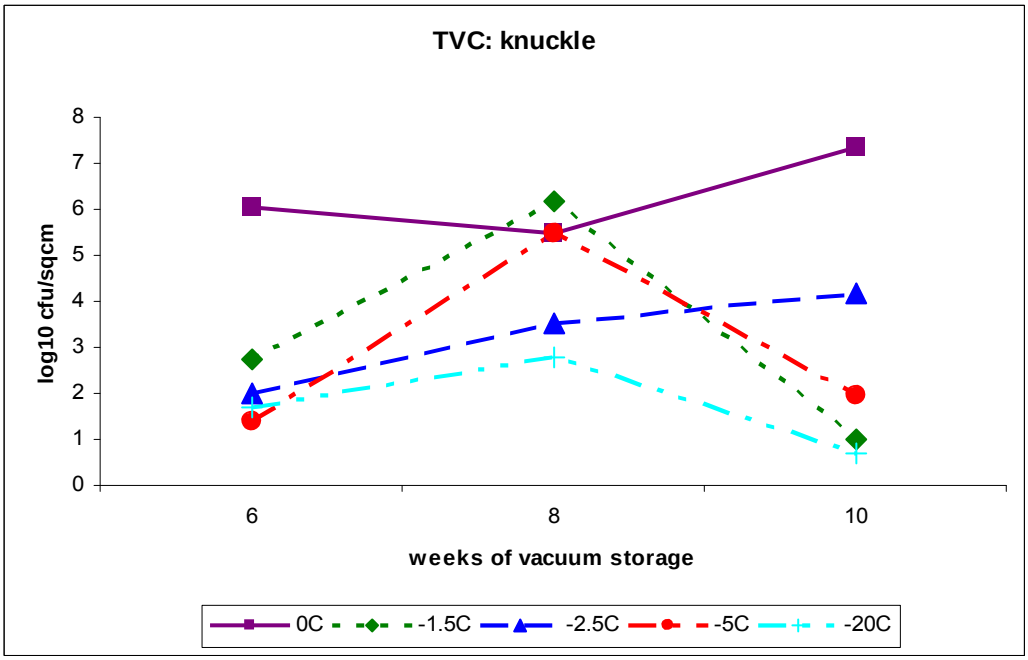

Figure 24

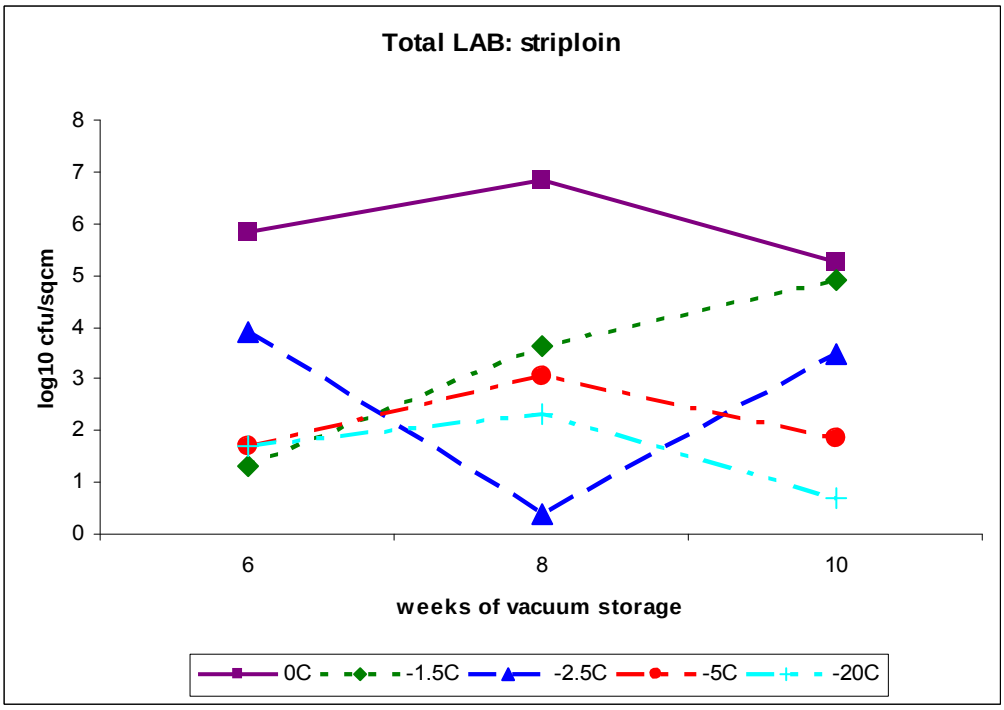

Figure 25

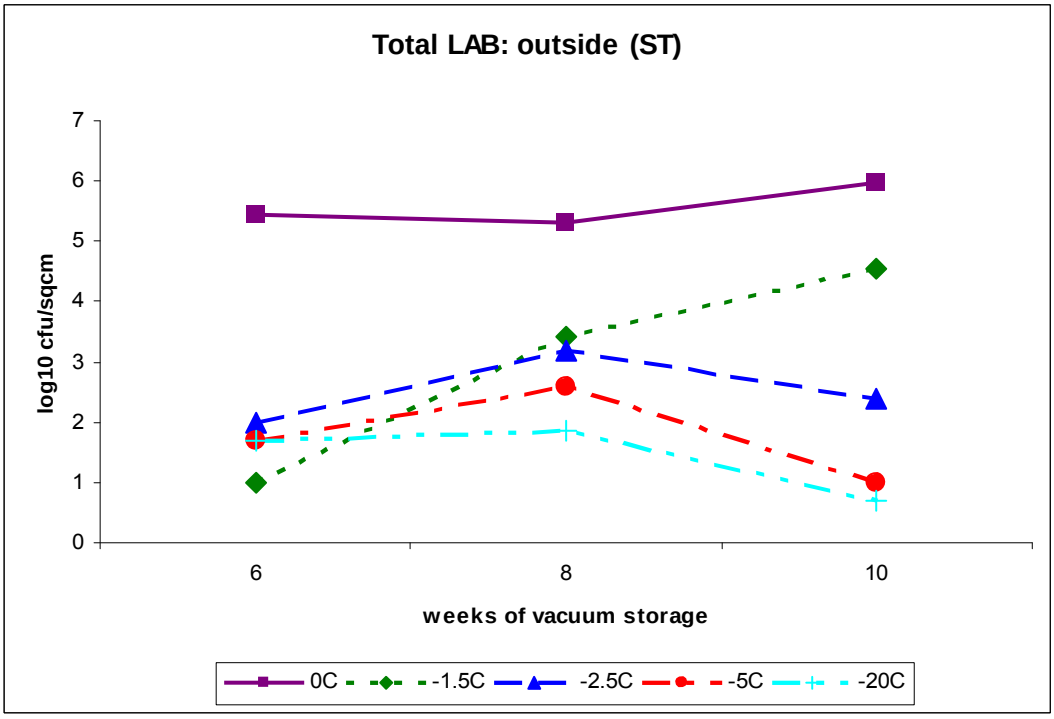

Figure 26

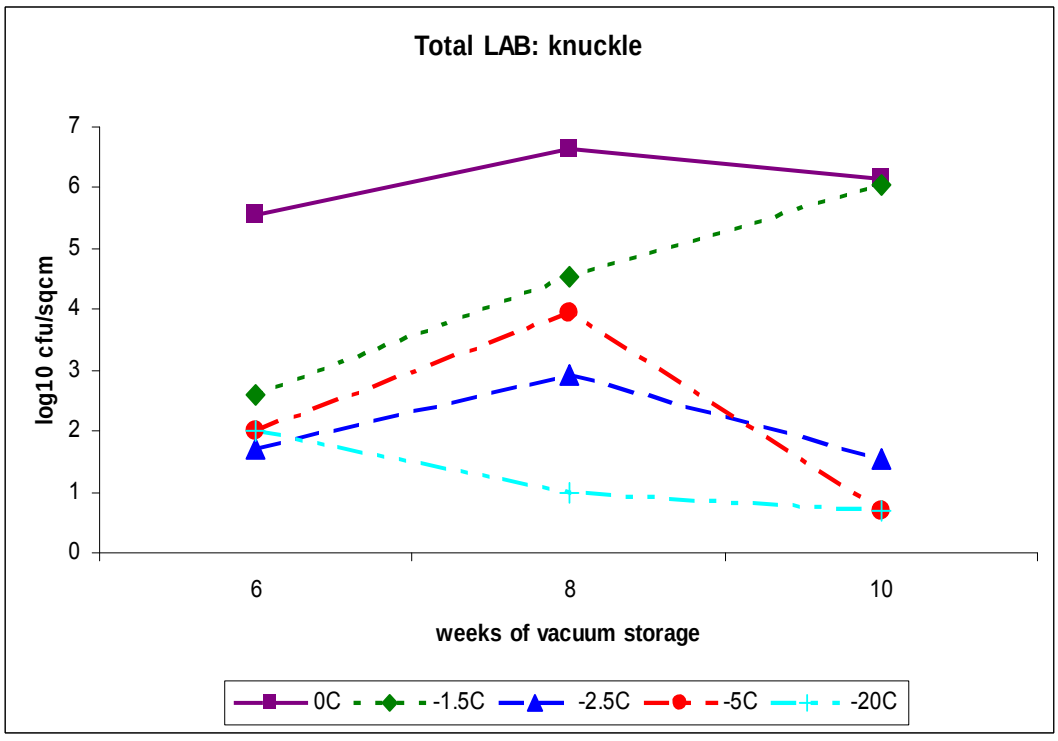

Figure 27

Treated muscles

There were no consistent differences between the microbial counts of the treated muscles stored at -5°C, although the TVC of salted cuts seemed to be decreasing during the trial

(figure 29). This was not mirrored in the LAB levels (figure 30), nor in *Brochothrix thermospacta* levels (figure 31).

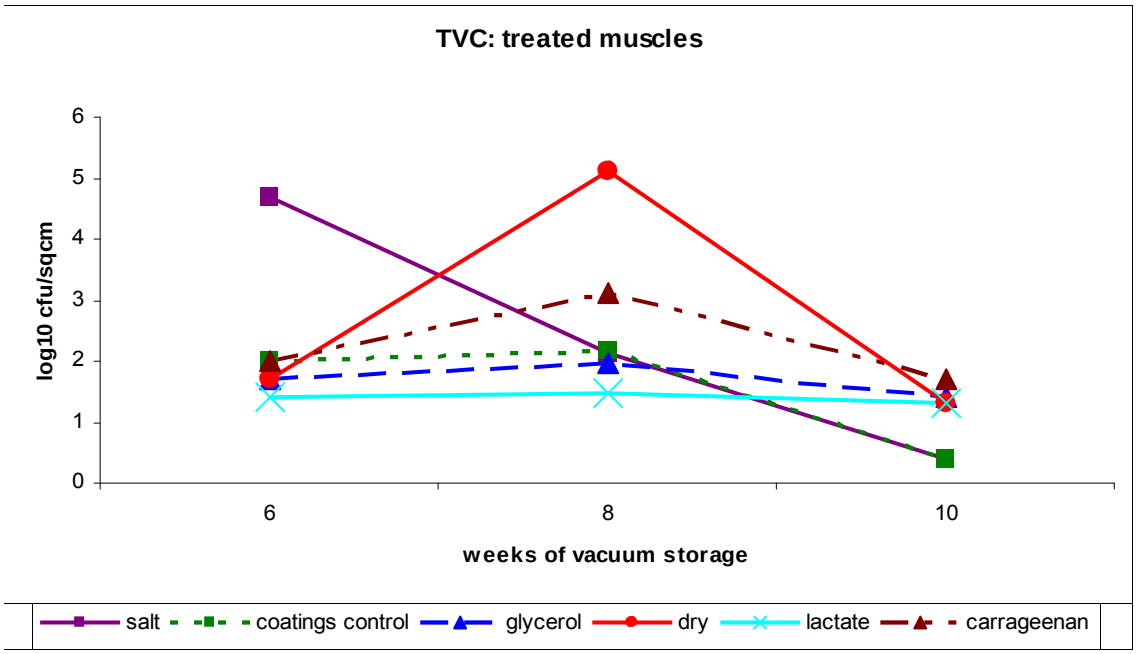

Figure 28

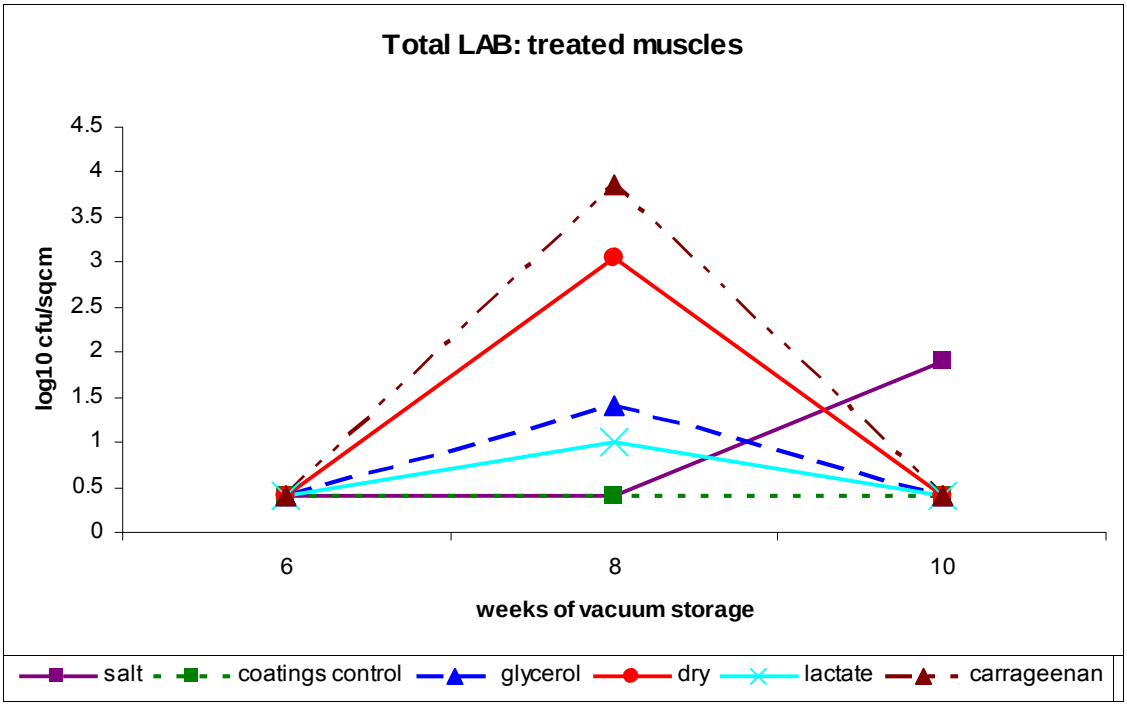

Figure 29

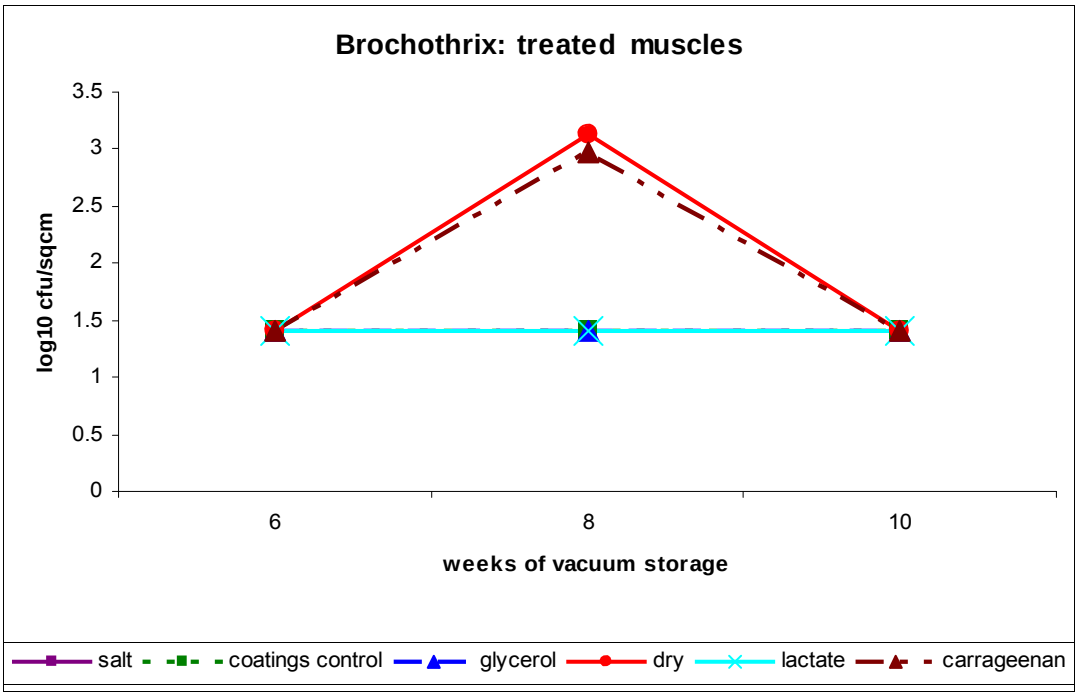

Figure 30

6.0 CONCLUSIONS

Beef stored at -2.5°C or -5°C demonstrate characteristics of frozen meat in terms of microbiology and drip loss. In addition, unsightly small white spots form on the surface of the cuts. Neither temperature would appear to be suitable for a deep chilling process aimed at maintaining the attributes of fresh meat.

When using deepchill temperatures, slow chilling helps to prevent freezing of beef cuts. This improves the visual quality of the product by reducing the incidence of white spots and reducing the drip loss, particularly during retail display. Slow chilling to -2.5°C resulted in only 12% of cuts being frozen solid, and thus displaying these undesirable attributes. Storage at such low temperatures, however, requires very strict control of the temperature in the chiller, to prevent ice crystal formation. It is unlikely in a commercial situation to be able to maintain the chiller temperature within the narrow range required to optimise deep chilling.

A minimum temperature of -1.5°C, as previously stipulated in legislation, would appear to be the lowest feasible storage temperature under commercial conditions, unless the beef was treated with a protective agent such as sodium lactate.

7.0 RECOMMENDATION

Deepchilling is feasible, but the product must be chilled slowly to its storage temperature. The temperature of -2.5°C is likely to be the absolute minimum temperature that could be commercially viable for vacuum packed beef, unless it has been treated with a freezing inhibitor such as sodium lactate.

8.0 REFERENCES

Allen, L., Nelson, A.I., Steinberg, M.P. and McGill, J.N. (1963) Edible corn-carbohydrate food coatings. II. Evaluation on fresh meat products. *Food Technology* 17: 1442-1446.

Anderson, T.R. (1960) US patent 2,948,623. August 9.

Anderson, T.R. (1961a) US patent 2,989,401. June 20.

Anderson, T.R. (1961b) US patent 2,989,402. June 20.

Anon (2006) AQIS Approved Arrangement Guidelines. http://www.daffa.gov.au/___data/assets/pdf_file/0019/113734/approved_arrange_guidelines.pdf. *Accessed* October 2007.

Anon (2007a) AS 4696:2007. Australian standard for the hygienic production and transportation of meat and meat products for human consumption. *CSIRO Publishing, Canberra, Australia.*

Anon (2007b) Export Control (Meat and Meat Products) Orders 2005 (as amended). http://www.comlaw.gov.au/ComLaw/Legislation/LegislativeInstrumentCompilation1.nsf/0/AD540313755F6D41CA25737200176433/$file/ExportControlMeatMeatProducts2005.pdf. *Accessed* October 2007.

Bouton, P.E., Harris, P.V. & Shorthose, W.R. (1971) Effect of ultimate pH upon the water-holding capacity and tenderness of mutton. J. Fd Sci. 36(3):435-439

Calvelo, A. (1981) *In*: Developments in Meat Science 2. Lawrie, R.A. (Ed.), Applied Science Publishers Ltd. London, New Jersey, 125.

Chang, K.L.B., Chang, J., Shiau, C-Y. and Pan, B.S. (1998) Biochemical, microbiological and sensory changes of sea bass (*Lateolabrax japonicus*) under partial freezing and refrigerated storage. *Journal of Agricultural and Food Chemistry* 46(2): 682-686.

Cutter, C.N. (2006) Opportunities for bio-based packaging technologies to improve the quality and safety of fresh and further processed muscle foods. *Meat Science* 74: 131-142.

Duun, A.S. and Rustad, T. (2007) Quality changes during superchilled storage of cod (*Gadus morhua*) fillets. *Food Chemistry* 105: 1067-1075.

Duy, B.H.N., Arason, S. and Porarinsdottir, K.A. (2007) Effects of dry ice and superchilling on quality and shelf life of arctic charr (*Salvelinus alpinus*) fillets. *International Journal of Food Engineering* 3(3): 29pages.

Earle, R.D. (1968) US patent 3,395,024. July 30.

Earle, R.D. and McKee, D.H. (1976) US patent 3,991,218. November 9

Earle, R.D. and Snyder, C.E. (1966) US patent 3,255,021. June 7

Eustace, I.J. and Bill, B.A. (1988) Investigation of temperature minima for the storage of chilled meat. *In* Proc 34th International Congress of Meat Science and Technology, pp 228-230.

Farouk, M.M., Wieliczko, K.J. and Merts, I. (2003) Ultra-fast freezing and low storage temperatures are not necessary to maintain the functional properties of manufacturing beef. *Meat Science* 66: 171-179.

Gallart-Jornet, L., Rustad, T., Barat, J.M., Fito, P. and Escriche, I. (2007) Effect of superchilled storage on the freshness and salting behaviour of Atlantic salmon (*Salmo salar*) fillets. *Food Chemistry* 103: 1268-1281.

Gennadios, A., Hanna, M.A. and Kurth, L.B. (1997) Application of edible coatings on meats, poultry and seafoods: a review. *Lebensmittel Wissenschaft und Technologie* 30: 337-350.

Gornall, A.G., Bardawill, C.J. & David, M.M. (1949) Determination of serum proteins by means of the Biuret reaction. J. Biol. Chem. 177:751-766

Grujic, R, Petrovic, L., Pikula, B & Amidzic, L. (1993). Definition of freezing rate – 1. Investigation of structure and ultrastructure of beef *M. longissimus dorsi* at different freezing rates. *Meat Science* **33** 301-318.

Guemez, J, Fiolhais, C. and Fiolhais, M. (2002) Reproducing Black's experiments: freezing point depression and supercooling of water. *European Journal of Physics* 23(1) 83-91.

James, C., Lejay, I., Tortosa, N., Aizpurua, X. and James, S.J. (2005) The effect of salt concentration on thefreezing point of meat simulants. *International Journal of Refrigeration* 28(6) 933-939.

Khwaldia, K., Perez, C., Banon, S., Desobry, S. and Hardy, J. (2004) Milk proteins for edible film and coatings. *Critical Reviews in Food Science and Nutrition* 44: 239-251.

Lanari, M.C. and Zaritzky, N.E. (1991) Effect of packaging and frozen storage temperature on beef pigments. *International Journal of Food Science and Technology* 26(6): 629-640.

Letney, L.R. (1958) US patent 2,819,975. January 24.

Meyer, R.C., Winter, A.R. and Weister, H.H. (1959) Edible protective coatings for extending the shelf-life of poultry. *Food Technology* 13: 146-148.

Olafsdottir, G., Lauzon, H.L., Martinsdottir, E., Oehlenschlager, J. and Kristbergsson, K. (2006) Evaluation of shelf life of superchilled cod (*Gadus morhua*) fillets and the influence of temperature fluctuations during storage on microbial and chemical quality indicators. *Journal of Food Science* 71(2): S97-S109.

Pearce, J.A. and Lavers, C.G. (1949) Frozen storage of poultry: effects of some processing factors on quality. *Canadian Journal of Research* 27: 253-256.

Petrovic, L., Grujic, R. & Petrovic, M. (1993) Definition of optimal freezing rate – 2. Investigation of the physico-chemical properties of beef *M. longissimus dorsi* at different freezing rates. *Meat Science* **33** 319-331.

Rahman, M.S., Kasapis, S., Guizani, N. and Al-Amri, O.S. (2003) State diagram of tuna meat: freezing curve and glass transition. *Journal of Food Engineering* 57(4) 321-326.

Reynolds, G. (2007) Superchilling keeps fish fresh longer, claim scientists. MeatProcess.com, http://meatprocess.com/news/printNewsBis.asp?id=74959, accessed.

Rosnes, J.T., Kleiberg, G.H., Sivertsvik, M., Lunestad, B.T. and Lorentzen, G. (2006) Effect of modified atmosphere packaging and superchilled storage on the shelf-life of farmed ready-to-cook spotted wolf fish (*Anarhichas minor*). *Packaging Technology and Scinece* 19(6): 325-333.

Schneide, J. (1972) US patent 3,667,970. June 6.

Shetty, C.S., Bhaskar, N., Bhandary, M.H. and Raghunath, B.S. (1996) Effect of film-forming gums in the preservation of salted and dried Indian mackerel (*Rastrelliger kanagurta* Cuvier). *Journal of the Science of Food and Agriculture* 70: 453-460.

Sivertsvik, M., Rosnes, J.T. and Kleiberg, G.H. (2003) Effect of modified atmosphere packaging and superchilled storage on the microbial and sensory quality of Atlantic salmon (*Salmo salar*) fillets. *Journal of Food Science* 68(4): 1467-1472.

Stemmler, M. and Stemmler, H. (1976) US patent 3,936,312. February 3.

Stoloff, L.S., Puncochar, J.F. and Crowther, H.E. (1948) Curb mackerel fillet rancidity. *Food Industry* 20: 1130-1132, 1258.

Sun, Da-Wen and Zheng, L. (2006) Innovations in freezing processes. *In* Handbook of frozen food processing and packaging, pp 175-195.

Weakley, D.F., McKeith, F.K., Bechtel, P.J., Martin, S.E. and Thomas, D.L. (1986) Effects of different chilling methods on hot processed vacuum packaged pork. *Journal of Food Science* 51(3): 757-760.

Witte, V.C., Krause, G.F. & Bailey, M.E. (1970) A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. J.Fd. Sci. 35:582-585

Wolfe, J and Bryant, G (2001) Cellular cryobiology: thermodynamic and mechanical effects. *International Journal of Refrigeration* 24(5) 438-450.

Wolfe, J (2007) Personal communication.

Alison Small

Anita Sikes

Danielle O'Callaghan

9.0 APPENDIX – DATA

9.1 Visual assessment of intact packs

Mean panel scores – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	-1.5	-2.5	-5	-20
Striploin	6	6.8	4.17	5	7.8	7
	8	7.25	3.6	6.75	5	7
	10	5.6	3	6	5	6.4
Outside (ST)	6	6.8	3.17	5.8	6	4.6
	8	4.25	2	5.25	5	5.75
	10	5.8	2.6	5.8	4.6	6
Knuckle	6	7.4	5.5	7	7.4	6.4
	8	6.25	4.2	6	5.75	6.75
	10	6.4	4.4	6	5.6	6.2

Mean panel scores – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	6	7	6.4	6.6	6.8	6.4
8	2.5	6	5.75	6.5	5.75	5.5
10	3.2	5.8	5.2	5.2	5.4	5.6

9.2 Confinement odour assessment

Mean panel scores – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	-1.5	-2.5	-5	-20
Striploin	6	4.2	7	6.8	7	6.8
	8	4.25	5.2	5	7.25	7.5
	10	3.8	4.6	4.6	6.4	6.2
Outside (ST)	6	4.8	2.5	5.8	5.8	5.4
	8	2.75	2.6	5.25	6	6.75
	10	2.6	3	4.2	5.4	5.8
Knuckle	6	3.8	5.33	5.4	5.2	5.8
	8	4.5	4.4	4.5	4.25	6
	10	2.6	3.6	4.6	6	5.8

Mean panel scores – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	5.8	5.2	5.2	6.8	4.4	5.2
8	6.25	6.75	6.75	7.25	6.5	7
10	3.6	5.8	5	6.8	5.4	7

9.3 Post bloom visual appearance assessment

Mean panel scores – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	-1.5	-2.5	-5	-20
Striploin	**6**	3.8	2.17	5.2	5.8	6.2
	8	8	3.33	8	4	7.75
	10	4.8	1.4	7	5.6	5.4
Outside (ST)	**6**	4.4	2.33	5.2	5.6	4.6
	8	3.25	3.5	7	5.75	6
	10	4.8	0.8	5.4	3	5
Knuckle	**6**	7	5.17	5	5.8	5.4
	8	4.75	3.33	5.5	5.75	5.88
	10	7.6	2.6	6	3.6	5.2

Mean panel scores – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	0.4	6.8	6.6	4	5.6	6.4
8	1	5.5	6.88	7	6	6.75
10	2.2	5.2	5.2	4.8	5	4.6

9.4 Retail pack visual assessment

Mean panel scores – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	-1.5	-2.5	-5	-20
Striploin	**6**	3.75	5.2	2.5	6.25	5
	8	4	5.8	6	6	8
	10	6	4.6	5	5.33	5.67
Outside (ST)	**6**	5.25	3.9	6.75	4.75	5
	8	4	3.6	8	8	8
	10	4.33	3.4	4	2.67	3.67
Knuckle	**6**	4.5	6.4	5.75	5.75	5
	8	6	6.9	6	8	8
	10	5	4.8	3	4.67	5

Mean panel scores – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	3.75	6.25	4.75	6.25	5	5.5
8	4	8	8	8	6	8
10	2.67	6	6.33	5.33	6	6

9.5 Drip loss

Mean percentage drip loss during vacuum storage – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	-1.5	-2.5	-5	-20
Striploin	**6**	1.24	6.14	1.59	0.69	1.11
	8	1	4.83	1.42	2.16	3.13
	10	3.57	5.92	2.12	1.71	4.98
Outside (ST)	**6**	1.3	2.4	1.19	0.39	0.58
	8	2.03	3.95	0.83	0.73	0.18
	10	2.38	4.88	0.45	0.59	0.15
Knuckle	**6**	3.17	2.86	1.96	2.24	0.39
	8	2.5	2.93	3.18	1.15	0.85
	10	2.3	3.15	1.97	0.75	1.41

Mean percentage drip loss during vacuum storage – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	1.74	2.27	1.82	0.79	3.56	4.47
8	1.65	1.45	2.8	1.16	3.52	3.68
10	3.28	2.14	2.96	1.36	3.5	5.01

Mean percentage drip loss during retail display – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	-1.5	-2.5	-5	-20
Striploin	**6**	0	3.76	4.13	3.46	2.98
	8	0	3.26	0	6.86	5.48
	10	0	5.17	6.82	6.59	2.41
Outside (ST)	**6**	0.13	4.26	1.87	2.29	2.13
	8	0	3.02	2.16	6.4	2.65
	10	0	4.52	5.61	4.36	4.56
Knuckle	**6**	0.09	3.83	4.44	5.3	4.13
	8	0.11	3.16	2.97	4.44	1.95
	10	0.12	4.05	4.46	2.75	2.46

Mean percentage drip loss during retail display – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	0	3.78	4	3.07	1.8	4.45
8	0	3.27	2.05	3.62	0.28	2.54
10	0	7	5.21	5.14	0	6.11

Mean overall percentage drip loss – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	1.74	6.04	5.82	3.86	5.36	8.92
8	1.65	4.72	4.85	4.78	3.8	6.22
10	3.28	9.14	8.17	6.5	3.5	11.11

Mean overall percentage drip loss – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	-1.5	-2.5	-5	-20
Striploin	**6**	1.24	9.91	5.72	4.15	4.09
	8	1	8.1	1.42	9.02	8.61
	10	3.57	11.5	8.94	8.3	7.39
Outside (ST)	**6**	1.43	6.66	3.06	2.69	2.71
	8	2.03	6.96	2.99	7.14	2.82
	10	2.38	9.4	6.06	4.95	4.71
Knuckle	**6**	3.26	6.68	6.4	7.54	4.53
	8	2.61	6.08	6.16	5.58	2.81
	10	2.42	7.2	6.43	3.51	3.88

Mean expressed juice – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature*			
		0	-2.5	-5	-20
Striploin	**6**	24.07	23.69	25.47	27.44
	8	22.38	24.92	29.92	31.13
	10	25.35	26.25	25.89	22.88
Outside (ST)	**6**	28.91	25.32	24.43	28.78
	8	25.99	30.79	27.29	29.41
	10	27.17	26.45	36.34	28.75
Knuckle	**6**	28.73	27.01	26.78	30.99
	8	25.45	24.92	27.95	30.68
	10	25.43	34.74	24.57	30.96

* Analysis not carried out on cuts stored at -1.5°C

Mean expressed juice – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	15.26	27.08	28.27	28.65	27.91	28.44
8	14.76	31.09	27.9	27.53	25.18	24.99
10	3.41	27.71	35.17	23.78	26.99	29.87

9.6 Soluble protein concentration

Mean protein concentration (mg/ml) – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature*			
		0	-2.5	-5	-20
Striploin	**6**	89.39	101.83	116.24	110.35
	8	113.39	80.25	77.97	88.69
	10	85.11	98.32	105.09	77.69
Outside (ST)	**6**	80.88	81.53	100.52	87.10
	8	72.45	72.45	79.60	58.80
	10	92.52	98.97	115.09	79.30
Knuckle	**6**	85.79	86.44	87.1	87.75
	8	99.42	97.47	90.64	75.05
	10	81.88	81.24	129.27	90.26

* Analysis not carried out on cuts stored at -1.5°C

Mean protein concentration (mg/ml) – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	84.48	70.40	70.73	81.20	77.28	75.31
8	58.8	63.68	91.94	77.32	75.37	97.36
10	84.78	99.61	80.92	88.33	97.36	81.88

9.7 Lipid oxidation (TBARs)

Mean TBARS (mg/kg) – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	-1.5	-2.5	-5	-20
Striploin	**6**	0.037	0.054	0.128	0.065	0.112
	8	0.303	0.16	0.055	0.055	0.029
	10	0.125	0.341	0.218	0.282	0.397
Outside (ST)	**6**	0.277	0.096	0.240	0.118	0.140
	8	0.885	0.278	0.202	0.095	0.084
	10	0.421	0.297	0.229	0.310	0.324
Knuckle	**6**	0.272	0.08	0.327	0.247	0.131
	8	0.484	0.251	0.126	0.068	0.131
	10	0.248	0.771	0.217	0.179	0.206

Mean TBARS (mg/kg) – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	0.841	0.256	0.379	0.345	0.255	0.394
8	0.619	0.080	0.174	0.119	0.136	0.229
10	1.470	0.230	0.187	0.458	0.568	0.552

9.8 MINOLTA colourimetry

MINOLTA L value

Mean L value – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	-1.5	-2.5	-5	-20
Striploin	**6**	45.81	39.39	40.86	40.84	40.15
	6 + 72 hr retail display	43.14	37.14	46.75	42.83	44.08
	8	39.1	43.04	40.22	44.14	41.28
	8 + 72 hr retail display	39.12	42.67	49.21	51.59	46.7
	10	42.92	39.79	40.06	36.23	41.06
	10 + 72hr retail display	43.19	39.31	45.05	43.37	43.87
Outside (ST)	**6**	42.44	40.24	44.05	42.2	45.92
	6 + 72 hr retail display	48.85	37.82	51.25	43.13	48.04
	8	45.8	39.30	41.96	42.13	46.04
	8 + 72 hr retail display	43.31	41.54	50.19	49.74	50.32
	10	44.29	42.49	42.23	43.67	44.39
	10 + 72hr retail display	44.71	36.91	44.68	43.58	46.4
Knuckle	**6**	41.91	37.94	43.16	45.08	42.41
	6 + 72 hr retail display	44.71	36.80	49.95	48.87	49.73
	8	46.45	39.88	40	41.33	40.47
	8 + 72 hr retail display	50.37	37.63	44.48	42.48	48.06
	10	41.91	39.80	43.16	45.08	42.41
	10 + 72hr retail display	44.71	39.80	49.95	48.87	49.73

Mean L value – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	43.35	40.9	40.89	42.22	40.95	42.78
6 + 72 hr retail display	45.37	52.69	51.97	49.22	50.55	48.13
8	43.03	40.82	42.87	46.06	41.64	43.84
8 + 72 hr retail display	42.2	48.25	47.16	47.43	43.42	46.73
10	40.92	40.78	40.52	44.46	42.42	40.44
10 + 72hr retail display	44.1	48.14	44.79	47.56	41.09	47.52

MINOLTA a value

Mean a value – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	12.02	14.11	9.53	13.32	12.9	8.19
6 + 72 hr retail display	4.09	7.9	5.53	7.82	4.82	7.96
8	6.9	10.66	9.02	11.79	12.59	9.73
8 + 72 hr retail display	9.08	8.77	9.49	10.08	10.4	6.67
10	10.47	12.67	12.05	10.82	9.85	11.06
10 + 72hr retail display	4.79	10.45	10.79	7.21	9	5.56

Mean a value – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	-1.5	-2.5	-5	-20
Striploin	**6**	10.93	22.48	11.01	8.75	9.6
	6 + 72 hr retail display	4.94	21.70	4.78	6.36	4.06
	8	9.25	19.62	12.8	12.24	8.14
	8 + 72 hr retail display	8.51	17.19	9.46	7.23	6.75
	10	11.61	17.93	11.06	8.5	11.37
	10 + 72hr retail display	12.87	16.99	7.36	9.35	9.22
Outside (ST)	**6**	15.77	24.71	13.56	11.44	14.34
	6 + 72 hr retail display	6.24	19.63	9.12	10.23	8.95
	8	16.16	15.41	12.08	13.72	14.87
	8 + 72 hr retail display	12.61	18.34	7.51	7.7	8.43
	10	15.48	19.70	10.85	9.8	12.22
	10 + 72hr retail display	11.03	16.49	9.89	9.03	7.47
Knuckle	**6**	15.99	22.53	15.25	14.09	16.25
	6 + 72 hr retail display	11.89	21.76	7.85	8.95	7.45
	8	16.34	18.50	13.76	11.83	14.98
	8 + 72 hr retail display	9.78	21.48	9.12	9.9	6.89
	10	12.02	17.21	11.69	12.45	13.07
	10 + 72hr retail display	9.81	17.21	6.23	8.87	7.79

MINOLTA b value

Mean b value – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	**-1.5**	**-2.5**	**-5**	**-20**
Striploin	**6**	5.35	5.76	-0.63	-4.76	-3.71
	6 + 72 hr retail display	4.94	4.95	2.28	0.58	0.88
	8	2.21	2.61	-0.45	-1.55	3.02
	8 + 72 hr retail display	2.2	3.62	1.32	1.5	0.77
	10	3.59	4.85	0.5	-3.14	-3.57
	10 + 72hr retail display	4.21	4.33	2.34	2.42	2.81
Outside (ST)	**6**	4.15	6.84	-1.4	-3.12	0.22
	6 + 72 hr retail display	4.7	4.69	4.18	-0.66	1.19
	8	4.67	-0.64	-1.9	-1.86	5.79
	8 + 72 hr retail display	3.27	2.04	1.47	1.71	3.31
	10	4.52	5.60	-0.56	-1.45	-2.73
	10 + 72hr retail display	4.13	3.76	3.9	2.59	4.54
Knuckle	**6**	4.66	4.72	-1.73	-0.63	-0.86
	6 + 72 hr retail display	2.23	5.16	1.06	-1.07	-0.42
	8	5.54	1.30	-2.23	-3.19	-0.14
	8 + 72 hr retail display	1.81	3.37	0.02	-0.64	1.08
	10	3.27	4.06	-2.93	-3.74	-2.42
	10 + 72hr retail display	3.27	4.06	2.78	1.39	4.96

Mean b value – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	Glycerol	dry	lactate	carrageenan
6	-2.69	-2.31	-4.22	-2.48	-2.98	-3.9
6 + 72 hr retail display	1.02	0.11	0.88	0.9	-0.2	3
8	-4.33	-2.68	-3.09	-1.33	-2.56	-2.67
8 + 72 hr retail display	-2.21	0.38	-1.37	0.22	-1.93	-2.2
10	-4.5	-3.09	-3.31	-2.84	-4.08	-4.52
10 + 72hr retail display	1.97	5.92	3.93	5.04	1.96	2.82

9.9 Microbiology

Total Viable Count (TVC)

Mean TVC (log_{10}cfu/cm²) – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	**-1.5**	**-2.5**	**-5**	**-20**
Striploin	**6**	6.38	2.95	4.31	2.7	2.6
	8	7.83	4.15	5.7	3.31	4.04
	10	6.35	2.61	4.85	2.38	2.34
Outside (ST)	**6**	5.84	0.70	3.15	1.7	2.4
	8	5.85	3.62	4.64	3.7	3
	10	7.07	4.49	3.81	2.1	1.88
Knuckle	**6**	6.04	2.76	2	1.4	1.7
	8	5.48	6.16	3.54	5.49	2.78
	10	7.35	1.00	4.17	1.95	0.7

Mean TVC (log_{10}cfu/cm²) – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	4.68	2	1.7	1.7	1.4	2
8	2.13	2.18	1.98	5.13	1.48	3.11
10	0.4	0.4	1.4	1.3	1.3	1.7

Brochothrix thermospacta count

Mean *Brochothrix thermospacta* (log_{10}cfu/cm²) – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	-1.5	-2.5	-5	-20
Striploin	**6**	6	0.40	4.17	3	1.4
	8	6.24	0.40	4.78	2.48	1.4
	10	5.57	0.40	4.83	1.4	1.4
Outside (ST)	**6**	4.4	0.40	2.65	1.4	1.4
	8	5.54	0.40	3.3	1.4	1.4
	10	4.77	0.40	1.4	1.4	1.4
Knuckle	**6**	4.33	0.40	1.4	1.4	1.4
	8	3.7	0.40	2	3.85	1.4
	10	4.56	0.40	1.7	1.4	1.4

Mean *Brochothrix thermospacta* (log_{10}cfu/cm²) – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	1.4	1.4	1.4	1.4	1.4	1.4
8	1.4	1.4	1.4	3.13	1.4	2.98
10	1.4	1.4	1.4	1.4	1.4	1.4

Lactic Acid bacteria (LAB) count

Mean total lactic acid bacteria (LAB) (log_{10}cfu/cm²) – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	-1.5	-2.5	-5	-20
Striploin	**6**	5.85	1.30	3.89	1.7	1.7
	8	6.85	3.65	0.4	3.06	2.31
	10	5.27	4.90	3.49	1.85	0.7
Outside (ST)	**6**	5.45	1.00	2	1.7	1.7
	8	5.31	3.41	3.18	2.6	1.85
	10	5.98	4.54	2.4	1	0.7
Knuckle	**6**	5.55	2.59	1.7	2	2
	8	6.65	4.55	2.93	3.93	1
	10	6.13	6.04	1.54	0.7	0.7

Mean total LAB (log_{10}cfu/cm²) – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	0.4	0.4	0.4	0.4	0.4	0.4
8	0.4	0.4	1.4	3.04	1	3.86
10	1.9	0.4	0.4	0.4	0.4	0.4

Mean homofermentative LAB (log_{10}cfu/cm²) – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	-1.5	-2.5	-5	-20
Striploin	**6**	5.65	0.70	3.76	1.4	1.4
	8	6.7	3.00	1.4	2.81	2.19
	10	5	4.48	3.28	1.3	1.4
Outside (ST)	**6**	5.24	0.70	1.7	1.7	1.7
	8	5.1	2.60	3	2	1.65
	10	5.9	3.40	2.34	1	1.4
Knuckle	**6**	5.46	1.93	1.4	1.4	1.7
	8	6.18	4.32	2.48	3.86	0.7
	10	6.06	5.54	1.18	0.7	0.7

Mean homofermentative LAB (log_{10}cfu/cm²) – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	1.4	1.4	1.4	1.4	1.4	1.4
8	1.4	1.4	1	2.4	0.7	3.63
10	1	1.4	1.4	1.4	1.4	1.4

Mean heterofermentative LAB (log_{10}cfu/cm²) – treated muscle stored at -5°C

Weeks of vacuum storage	Treatment					
	salt	control	glycerol	dry	lactate	carrageenan
6	0.4	0.4	0.4	0.4	0.4	0.4
8	0.4	0.4	0.7	2.93	0.7	3.48
10	1.85	0.4	0.4	0.4	0.4	0.4

Mean heterofermentative LAB (log_{10}cfu/cm²) – untreated muscle

Cut	Weeks of vacuum storage	Storage temperature				
		0	**-1.5**	**-2.5**	**-5**	**-20**
Striploin	**6**	5.4	1.18	3.32	0.4	1.7
	8	6.3	3.54	0.4	2.7	1.7
	10	4.93	4.70	3.08	1.7	0.7
Outside (ST)	**6**	5.04	0.70	0.4	0.4	0.4
	8	4.9	3.33	2.7	2.5	1.4
	10	5.18	4.54	1.48	0.4	0.7
Knuckle	**6**	4.81	2.48	1.7	0.4	1.7
	8	2.74	4.16	2.74	2.7	0.7
	10	5.3	5.88	1.3	0.4	0.4